ADVICE CONCERNING THE GENETIC LABORATORY TEST

1. **Description of the purpose of the proposed genetic laboratory assessment - TRISOMY test:**
TRISOMY test including all its variants is a **non-invasive prenatal screening test from the NIPT category** (**N**on-Invasive **P**renatal **T**esting), which is performed from a blood sample of a pregnant woman informed and instructed in accordance with the law and identified on page 1 of this request form ("pregnant woman") to determine the risk of complete trisomy of any of the chromosomes 21, 18 or 13, aneuploidy of sex chromosomes, presence of selected chromosomal microdeletions and/or determination of the predicted sex of the fetus ("**Test**" or "**Tests**"). The test is performed in the forms specified in items (a) to (d) below by **Medirex, a.s.**, with registered seat at Holubyho 35, 902 01 Pezinok, Company ID: 35 788 450, in its central laboratory at Galvaniho 17/C, 821 04 Bratislava ("**Laboratory**") and its cooperating suppliers.
The term "**trisomy**" means that instead of the usual 2 copies of chromosomes in the nuclei of fetal cells, one of the chromosomes examined is found in 3 copies, i.e. the total of 47 chromosomes are found in the cell nuclei (instead of 46 = 2 x 23 as is the case under common physiological circumstances). If chromosome 21 is present in 3 copies, this is the case of trisomy 21 that causes Down syndrome, trisomy 18 causes Edwards syndrome and trisomy 13 causes Patau syndrome.
The results of the Tests may or may not contain only information about the predicted or chromosomal sex of the fetus and about a pathological set of sex chromosomes, which are provided only within the scope of this Advice on Genetic Laboratory Testing ("**Advice**"), otherwise this information will not be included in the Test results. The sex of the fetus is determined on the basis of the presence and quantity of DNA segments corresponding to the X and Y sex chromosomes. The provision of information on the sex of the fetus shall be governed by the applicable legislation of the country where the sample was taken.
   a. **TRISOMY test** is a Test to assess the risk of complete trisomy of chromosome 21, 18 or 13 and the predicted sex of the fetus.
   b. **Trisomy test XY** is a test to determine the risk of complete trisomy of any of the chromosomes 21, 18 and 13, fetal chromosomal sex and genital chromosomal aneuploidies.

Table 1: TRISOMY test

Trisomy	Sex of the fetus
Down syndrome (trisomy 21)	yes, chromosomal sex
Edwards syndrome (trisomy 18)	
Patau syndrome (trisomy 13)	

Table 2: TRISOMY test XY, the tested chromosomal disorders

Trisomy	Sex of the fetus	Aneuploidies of sex chromosomes
Down syndrome (trisomy 21)	yes, chromosomal sex	Turner syndrome (45X)
Edwards syndrome (trisomy 18)		Kleinfelter syndrome (47, XXY)
Patau syndrome (trisomy 13)		XYY syndrome (47, XYY)
		XXX syndrome (47, XXX)

   c. **TRISOMY test +** is a Test that determines the risk of complete trisomy of any of chromosomes 21, 18 and 13, fetal chromosomal sex, aneuploidy of sex chromosomes and also the presence of selected chromosomal microdeletions where a part of a chromosome is missing.

Table 3: TRISOMY test +, the tested chromosomal disorders

Trisomy	Sex of the fetus	Aneuploidies of sex chromosomes	Microdeletion syndromes
Down syndrome (trisomy 21)	yes, chromosomal sex	Turner syndrome (45X)	DiGeorge syndrome (22q11)
Edwards syndrome (trisomy 18)		Kleinfelter syndrome (47, XXY)	Prader-Willi and Angelman syndrome (15q11)
Patau syndrome (trisomy 13)		XYY syndrome (47, XYY)	Cri-du-chat syndrome(5p15)
		XXX syndrome (47, XXX)	1p36 deletion syndrome
			Wolf-Hirschhorn syndrome (4p.16.3)

   d. **BabyGen** is a Test that provides information about the predicted sex of the fetus.

2. **Description of the proposed Test procedures:**
   a. The Test requires the collection of a small amount (10 ml) of blood obtained from a vein of the pregnant woman using a blood sampling kit. Sampling can be performed starting from the 11th week of pregnancy. After the first sample collection, in 3.9% of cases it is necessary to repeat the sampling process due to low proportion of the fetal DNA in the total isolated circulating DNA. This risk is somewhat higher for samples collected before completing the 12th week of pregnancy. Repeated sampling may also be required for non-compliance with the preanalytical conditions of the Test.
   b. Blood samples are collected into EDTA/Cell-Free DNA BCT (Streck) tubes or alternative tube types as recommended by the Laboratory. Samples of blood, plasma or DNA will be sent to the Laboratory that will ensure the test itself and issue an interpreted result of the Test in electronic form.
   c. The laboratory will process only the samples delivered together with a correctly completed request form, a signed referral by a specialist physician and this Advice signed by the pregnant woman. Collection of the samples, their dispatch to the Laboratory and delivery of the Test results to the indicating physician are provided by the contracted laboratory in the country of sampling.
   d. The subject of the Test is circulating DNA isolated from the plasma of the pregnant woman containing the so-called cell-free fetal DNA ("cff DNA") which is primarily found in placenta and circulates in the pregnant woman's blood. Isolated cffDNA to a great degree represents the total DNA of the fetus and it is analyzed by a specific genetic laboratory method - low coverage whole genome re-sequencing. Data obtained by whole genome re-sequencing analysis shall be evaluated using computer-assisted bioinformatic processes.
   e. Tests may be also performed in pregnancies using assisted reproduction techniques (ART), including cases with donated gametes.
   f. TRISOMY test can also be used in pregnancies with two fetuses; however, in the case of a pathological finding it is not possible to directly attribute the Test result to any particular fetus of the two.
3. **The risks of unexpected consequences of Tests for the pregnant woman and her relatives:**
   a. Risks related with blood sampling is minimal, bruising, or, in rare cases, inflammation may occur at the needle puncture site.
   b. The Test is referred to as non-invasive as no puncture of the uterus is required to obtain a sample for laboratory assessment. Therefore, there is no risk of miscarriage, bleeding, amniotic fluid leakage, intrauterine infection, or other gynecological and obstetric complications.
   c. The result of the Test may introduce serious health and ethical challenges for the pregnant woman, her partner, genetically related and socially related individuals, including the possibility of selecting abortion due to a medical indication in accordance with the applicable legislation of the country in which the sample was taken.
4. **The Tests have the following alternatives:**
   a. Other NIPT tests offered by other laboratory testing providers.
   b. Genetic laboratory testing from a sample obtained by invasive methods - sample collected using the so-called biopsy of chorionic villi ("**CVS**") or from a sample of amniotic fluid obtained by amniocentesis ("**AMC**").
5. **Test output:**
Assuming that it was possible for the Laboratory to process the sample in accordance with the sound laboratory practice, and it was possible to obtain a result that responds to the diagnostic question at the level of detection ability of the method used, the Laboratory shall issue the result of the Test (i) typically within 5 working days, or (ii) in about 10% of samples within 8 working days due to biological variability, starting from the next day following the delivery of the sample or the date of identification of the Test payment (whichever is later).
   a. **The result of the respective Tests is the information about the positive or negative result of the analysis with respect to**
      i. TRISOMY test - testing for trisomies and determination of the predicted fetal sex (see Tab.1);
      ii. TRISOMY test XY - testing of trisomies, determination of chromosomal fetal sex and the number of sex chromosomes (see Tab. 2);
      iii. TRISOMY test + -testing for trisomies, determination of fetal chromosomal sex and the number of sex chromosomes as well as other selected chromosomal disorders (see Tab.3).
   In cases when the Test fails to respond to the diagnostic question at the level of its distinction ability, the laboratory issues a partially informative Test result. The Laboratory will offer a repeat of the Test from a new sample free of charge. If, even after the repeated analysis, the Test result still fails to answer the diagnostic question, a partially informative result is issued repeatedly and conclusively and the Test is considered to have been properly performed in this case.
   b. **A negative result of the respective Tests means that the following has been determined:**
      i. TRISOMY test - low risk (less than 1:10 000) of over-representation of fetal DNA molecules attributable to the monitored chromosomes, which would indicate the presence of trisomy in chromosomes 21, 18 or 13 (listed in Tab.1)
      ii. TRISOMY test XY - low risk (less than 1:10 000) of over-representation/under-representation of fetal DNA molecules attributable to monitored chromosome or parts thereof, which would indicate the presence of trisomy in chromosome 21, 18 or 13, or sex chromosome aneuploidy (listed in Tab.2);
      iii. TRISOMY test + - low risk (less than 1:10 000) of overrepresentation/underrepresentation of fetal DNA molecules attributable to the monitored chromosome or parts thereof, which would indicate the presence of trisomy in chromosomes 21, 18 or 13, sex chromosome aneuploidies or other tested chromosomal disorder of those listed in Tab.3)
   c. **A positive result (standard findings) of the respective Tests means that the following has been determined:**
      i. TRISOMY test - high risk of over-representation of fetal DNA molecules attributable to the monitored chromosome, which indicates the presence of trisomy of one of the tested chromosomes 21, 18 or 13 (shown in Tab.1);
      ii. TRISOMY test XY - high risk of over-representation/under-representation of fetal DNA molecules attributable to the monitored chromosome or parts thereof, suggesting the presence of (i) trisomy in any of the tested chromosomes 21, 18 or 13 or (ii) aneuploidy of sex chromosomes (listed in Tab. 2);
      iii. TRISOMY test + - A high risk of over-representation/ under-representation of fetal DNA molecules attributable to the monitored chromosomes or parts thereof, suggesting the presence of (i) trisomy in one of the tested chromosomes 21, 18 or 13, (ii) aneuploidy of sex chromosomes, or (iii) other tested chromosomal disorder listed in Tab.3
   d. **Positive result (additional findings):**
   In the case of the Test, this means that the Laboratory has detected another aberration of genetic information, not targeted by the test, which is not a common part of the Test as listed in Tab. 1 to 3 (additional findings). For this purpose, the following shall be considered a significant change in genetic information:
      i. Trisomy or monosomy of any chromosome;
      ii. mosaic forms of trisomies or monosomies,
      iii. partial trisomy or partial monosomy of any of the chromosomes,
      iv. mosaic forms of partial trisomies or monosomies;
      v. other microdeletions and microduplications on any of the chromosomes that are not target subject of identification by TRISOMY test+ whereby a minimum of 10% fetal fraction is required, the excessive or missing segment must represent at least 10 million bases and the share of the pathological line must represent at least 50%. The Laboratory may report these findings in the Test result only under exceptional circumstances, namely (i) on the basis of a written consent granted by the pregnant woman to the procedure specified in this Advice, and (ii) if an interpretation of the Test result by a specialist in medical genetics is available.
   e. **Non-informative Test result** means that the supplied sample could not be processed in accordance with sound laboratory practice (e.g. the sample contained a low proportion of the fetal DNA, the pregnant woman is treated with **LMWH** (**L**ow **M**olecular **W**eight **H**eparin injections), the weight of the pregnant woman is over 90 kg), or the result of the Test does not answer the diagnostic question.
   In this case, the Laboratory
      i. shall automatically repeat the Test free of charge from the same sample (this applies to approx. 5% of the samples), or
      ii. shall offer a repetition of the Test from a new sample free of charge (applies to 3.9% of the samples). A new sampling is recommended to be carried out with an interval of 14 days from the first sampling. In this case, the date of delivery of the final Test result shall be delayed accordingly. If an informative Test result has not been obtained by repeated testing of the first sample and testing of the second sample, the Laboratory shall refund the full amount paid to the pregnant woman (with the exception of pregnant women who are treated with LMWH injections, when it is recommended to perform blood sampling shortly before the planned administration of the next dose of LMWH or in pregnant women with weight exceeding 90 kg).
   f. **Fetal sex determination:**
   All tests may predict fetal sex (male or female) or chromosomal sex of the fetus (XY-male, XX-female or another finding in relation to the aneuploidies of sex chromosomes listed in Tab.3), but (i) the results of the "**TRISOMY test**" and "**BabyGen**" Tests do not reveal any possible aneuploidies of sex chromosomes, while (ii) **TRISOMY test XY** and **TRISOMY test+** will also detect aneuploidies of sex chromosomes.
6. **Precision and predictive value of the Tests:**
   a. Precision of the Test represents a numerical expression of the probability that the Test result (negative or positive) is determined correctly. The probability of delivering a correct Test result (sensitivity) for trisomy 21, 18 and 13 is very high, comparable to the results of a large-scale meta-analysis published in 2015 summarizing the results of 37 studies from around the world (Gil MM, Quezada MS, Revello R, Jaklekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta- analysis. Ultrasound Obstet Gynecol. 2015 Mar;45(3):249-88). For trisomy 21, the Test precision is at least 99.9% and for trisomies 18 and 13 the precision is close to 99.9 %. The precision of the negative Test result alone (test specificity) is even higher, in the case of trisomy 21 it is more than 99.9%.
   b. The test also has a great predictive value, which is at least 10 times greater than the predictive value of standard biochemical or combined prenatal screening tests.
7. **Notification concerning Test limitations:**
   a. The Tests are considered highly effective screening tests, however they are not classified as diagnostic tests. Positive Test results, as findings of a non-invasivescreening test, must therefore be verified by diagnostic genetic laboratory assessment of a fetal sample obtained from the placenta via CVS (usually not later than the end of the 14th week of pregnancy) or from amniotic fluid sampled using AMC (usually not earlier than at the beginning of the 16th week of pregnancy).
   b. Despite the high precision of the Tests, it is possible that in isolated cases a sample may be evaluated as false positive, or even more rarely, as false negative, which could in both cases be caused by unknown or unpredictable biological properties of the sample (e.g. vanishing twin syndrome, placental mosaicism, maternal mosaicism) or by technological limits of the Tests.
   c. **The Tests do not enable provision of the result in cases where the detected fetal DNA fraction after analysis is lower than 5%. In this case, the Test is classified as non-informative and a repeated sampling is required 14 days after the first sample collection.**
   d. The Tests are only designed to detect chromosomal disorders that are listed in the Tab.1 to 3, and a minimum of 10% fetal fraction is required to obtain a fully informative result for detection of microdeletions, while the missing segment must represent at least 3 million bases. If the proportion of cffDNA detected in the sample is less than 10%, the Test result for microdeletion disorders should be considered as informative only to a limited extent. Its confidence level varies depending on the specific microdeletion and its size, as well as on the fetal fraction detected in the test sample. However, even in these cases, the result of the Test is considered to be performed properly and the provision of point 5 e.ii. shall not apply.
   e. Other changes of genetic information not subject to targeted detection shall not be further analyzed and reported in the Test result. If, in exceptional cases, these are listed in the Test result, they are marked as "additional findings" and require interpretation in a special mode (see item 5. c, d. of this Advice)
   f. The Tests are not primarily designed to detect:
      i. triploidies/or tetraploidies (multiple count of all chromosomes);
      ii. balanced chromosomal translocations (the exchange of sections of genetic material between chromosomes without any missing or excessive genetic information);
      iii. mosaic forms (the fetus contains cells with normal as well as pathological genetic characteristics);
      iv. chimerism (the fetus is composed of tissue of originally 2 different individuals).
8. **The Laboratory shall not accept any liability for incorrect Test result or incorrect interpretation of the Test result if:**
   a. the sampling was performed before the end of the 10th week of pregnancy;
   b. the preanalytical conditions of the Test were not complied with;
   c. the correct Test result could not be reached due to incorrect, incomplete, or confusing anamnestic data, e.g. concerning the duration of pregnancy, multiple pregnancy, and/or the vanishing twin syndrome, reported genetic pathology of any of the parents, concerning the treatment with LMWH;
   d. the Test result was influenced by the presence of other foreign DNA (other than fetal DNA) in the mother's organism, e.g. transfusion of donor blood, allogeneic organ or bone marrow transplant, foreign stem cell therapy, cancer;
   e. the fetus suffers from a disorder for the detection of which the Test is not intended (see item 7 f. of this Advice).
9. **The Laboratory undertakes to:**
   a. attempt to obtain an informative Test result in the event of an uninformative Test result
      i. by repeating the Test from the same sample (see item 5 e.i of this Advice);
      ii. by repeating the Test from a new sample (see item 5 e.ii of this Advice).
   b. after the Test has been completed, the rest of the original and/or processed blood sample will be stored in accordance with the relevant legislation in order to enable performing of further genetic laboratory tests to benefit the pregnant woman or her blood relatives. Any further genetic laboratory testing will be preceded by a genetic consultation in which a medical geneticist ensures that this Advice is updated.
   c. the unused part of the sample shall be disposed of/used in accordance with the consent of the pregnant woman granted in this Advice.
10. **Data on possible limitations in the way of life and capacity to work in connection with the Tests:**
The Test is a non-invasive assessment and does not endanger the pregnant women with abortion or other complications that may be associated with CVS or AMC.
11. **Instructions on the right of the pregnant woman to decide freely on the procedure for the provision of medical intervention:**
The pregnant woman is free to decide on the procedure for the provision of medical intervention or service.
12. **Information on the payment**
The Tests will be performed exclusively in the self-payer mode (not covered by the public health insurance) according to the applicable price list published at www.laboratomadlagnostika.sk. The price for the Tests can be paid to the Laboratory:
   a. **through the medical facility (clinic/physician)** on site when blood sample is collected for the Test, and after the physician confirms receipt of the payment on page 1 of the Request Form, no further activity is required from the pregnant woman;
   b. after taking of the blood sample for the Test through **the payment gateway at www.medirexplatby.sk** where the pregnant woman enters her identification data (provided on the Request Form), selects the type of the Test and the method of payment **(by card or bank transfer)** and can immediately make the payment through a secure connection interface. The identification data will be automatically assigned to the payment.
   c. after taking of the blood sample for the Test **on the basis of an invoice**, by bank transfer or deposit to the account. The invoice shall be generated by the system and sent to the pregnant woman after entering the identification data and sent to the specified e-mail address provided by the pregnant woman. When making a transfer based on an invoice, it is necessary to enter the correct bank account number (IBAN) of the Laboratory and the payment ID (also labelled as "VS" or "variable symbol"). Payments made without the VS or with a wrong VS cannot be correctly identified and assigned to the specific blood sample.

Payment process assistance shall be provided by the Laboratory client center hotline at 0800 400 800.