INFORMATION AND GUIDANCE FOR THE PATIENT CONCERNING GENETIC LABORATORY TESTING

1. **The purpose of TRISOMY test Complete, the genetic laboratory test:**
TRISOMY test Complete ("TTC test") is a non-invasive prenatal screening test from the NIPT category (Non-Invasive Prenatal Testing). It is based on analysis of blood sample collected from a pregnant woman who was instructed and informed in compliance with the statutory provisions and identified on the page 1 of this Request form (**„Pregnant woman"**). The aim is to determine the risk of presence of chromosomal aneuploidies and/or chromosomal aberrations at the whole genome level and to predict the sex of the fetus. TTC test is performed by **Medirex, a.s.,** registered at Holubyho 35, 902 01 Pezinok, company ID/registration: 35 766 450, in its central laboratory at Galvaniho 17/C, 821 04 **Bratislava ("Laboratory") and its cooperating suppliers.**
TTC test is designed to detect chromosomal aneuploidies and chromosomal aberrations. Chromosomal aneuploidy represents a change in the number of any chromosome, including sex chromosomes (gonosomes). Typically, it is a case of a trisomy or a monosomy of the whole chromosome. Chromosomal aberration is a sub-chromosomal change and represents an extra or a missing part of a single chromosome.Typically, it represents a duplication or deletion of a part of the chromosome.
The term 'trisomy'means that instead of standard 2 copies in the cell nuclei of the fetus, one of the tested chromosomes is found in 3 copies, i.e. there are 47 chromosomes in total in the cell nuclei (and not 46 = [2x23] as under standard physiological conditions). For example, if it is chromosome 21 that is found in 3 copies, we are looking at a case of trisomy 21, causing Down syndrome.
The term 'monosomy'means that instead of standard 2 copies in the cell nuclei of the fetus, one of the tested chromosomes is found in a single copy only, i.e. there are 45 chromosomes in total in the cell nuclei (and not 46 = [2x23] as under standard physiological conditions). For example, if it is chromosome X that is found in a single copy only, we are looking at a case of monosomy X, causing Turner syndrome.
The term 'deletion'means that the indicated chromosomal part is missing (it is deleted) on one of the tested chromosomes in the cell nuclei of the fetus. E.g., if the deletion has been indicated on the long arm of chromosome 22, on the location 11.2, we are looking at a case of 22q11.2 microdeletion, causing DiGeorge syndrome.
The term 'duplication'means that the indicated chromosomal part is doubled (duplicated) on one of the tested chromosomes in the cell nuclei of the fetus. For example, when the duplication is found on the short arm of chromosome 17, on the location 11.2, we are looking at a case of 17p11.2 microduplication, causing Potocki-Lupski syndrome.
Depending on the request of the Pregnant woman, the result of TTC test may also contain information about the predicted fetal sex. Fetal sex is determined based on the presence and quantity of DNA sequences corresponding to sex chromosomes X and Y. Provision of the information about fetal sex is governed by the appliable legislation of the country where the sample was collected.
Total circulating DNA is tested, isolated from the plasma of the Pregnant woman, which contains not only the maternal DNA but also the so-called cell free fetal DNA (cffDNA). The proportion of cffDNA in total circulating DNA is called fetal fraction. Free fetal DNA mainly comes from the placenta and to a large extent, it represents total DNA of the fetus. It is analyzed using the technology of low-coverage whole-genome sequencing. The obtained data is then assessed using a bioinformatic algorithm enabling detection of quantitative changes at chromosomal and sub-chromosomal level. Detection parameters, resolution and limitations of the test are indicated in sections 5 (Table 1), 6 and 7.
2. **Pre-analytical information:**
   a. TTC test requires sampling of a small volume (10 ml) of blood collected from the vein of the Pregnant woman using a sampling kit. The collection can be performed only after completing the 10th week of pregnancy. The results of testing of the first sample may sometimes be uninformative and therefore in less than 5% cases, the sampling of blood will have to be repeated. This may be caused by low fetal fraction or an ambiguous result from the technical perspective. For samples collected before completed 12th week of pregnancy, in pregnant women treated by low-molecular weight heparins (LMWH) and in pregnant women with weight > 90 kg, the likelihood of uninformative result is higher. In case of pregnant patients treated with LMWH, it is recommended to perform blood sampling just before the next dose of LMWH is administered. Repeated sampling may be required due to failure to comply with the pre-analytical conditions of TTC test.
   b. Blood samples are collected into EDTA/Cell-Free DNA BCT® (Streck) tubes or any alternative tubes following the recommendations by the Laboratory. Samples of blood, plasma, or DNA will be then sent to the Laboratory, which shall provide laboratory testing and issue an interpreted result of TTC test in electronic form.
   c. The Laboratory will only process samples delivered together with a correctly completed Request form with a signature and stamp by the indicating physician as well as Informed Consent signed by the Pregnant woman. Collection of the samples, their transport to the Laboratory and delivery of the results of TTC test to the indicating physician is arranged by the contracted Laboratory in the country where the sample is collected.
   d. TTC test can be also performed in pregnancies using the assisted reproduction techniques (ART) including the cases with donated gametes.
   e. TTC test cannot be used in multiple pregnancies (i.e. pregnancies with several fetuses).
3. **The risk of unexpected consequences ofTTC test for the Pregnant woman and her relatives:**
   a. The risk associated with sampling of blood is minimum, a bruise may develop in the location of needle prick or quite exceptionally, inflammation.
   b. TTC test is classified as non-invasive, since no incision into the uterus is necessary to collect the sample for laboratory testing. There is no risk of miscarriage involved, nor is there any risk of bleeding, leakage of amniotic fluid, intrauterine infection or another type of complication associated with invasive sampling of fetal DNA sample.
   c. The result of TTC test may lead to serious and substantial health-related and ethical challenges for the Pregnant woman, her partner, genetically and socially related persons, including the alternative to terminate pregnancy due to healthcare indication in compliance with the applicable legislation in the country where the sampling was performed.
4. **TTC test has the following alternatives:**
   a. Other available NIPT tests - TRISOMY test, TRISOMY test XY, TRISOMY test +; all of the above available from Medirex, a.s., or possibly other alternatives.
   b. Genetic diagnostic laboratory tests aimed at detection of chromosomal aneuploidies and/or aberrations from the sample collected by invasive sampling, using the so-called biopsy of chorionic villi (CVS) or from a sample of amniotic fluid collected using amniocentesis (AMC).
5. **The results ofTTC test:**
Provided that the sample can be processed by the laboratory in compliance with the good laboratory practice and a result can be obtained that answers the screening or diagnostic question at the level of resolution offered by the applied methodology, the Laboratory will issue the TTC test result usually within 5 working days, or possibly, in approx. 10% of samples within 8 working days (due to biological variability and unexpected technical factors associated with the analyzed sample). The deadline of 5 (or possibly 8) working days starts to run on the date following after the date of the delivery of the sample to the Laboratory or the date of identification of the payment for TCC test (whichever date occurs later).
   a. **TTC test result represents information about a positive or negative finding of the analysis with respect to the** detection of a high risk of chromosomal aneuploidy and/or aberration at the whole genome level, whereas the resolution of TTC test with regards to the size of the detected aberration depends on the fetal fraction determined in the analyzed sample (details below).
   b. **A negativeTCC test result implies that the following has been determined:**
      i. low risk (below 1:10000) of a trisomy/monosomy of any whole chromosome.
      ii. low risk (below 1:1000) of a duplication/deletion of parts of a chromosome/chromosomes.
   c. **A positive TTC test result means that** over-representation/under-representation of DNA molecules has been detected, which belong to whole chromosomes and/or their parts. In cases where fetal fraction > 5%, TTC test detects chromosomal aneuploidies with sensitivity > 99% and chromosomal aberrations with sensitivity > 80%, depending on fetal fractions and the size of aberration as indicated in Table 1.
Table 1: Detection parameters in chromosomal aberrations depending on the fetal fraction and the size of aberration. Sensitivity > 80% applies to every two values of detection parameters.

Fetal fraction	Size of aberration
5% - 6%	> 8 megabases
6% - 7%	> 7 megabases
7% - 8%	> 6 megabases
8% - 9%	> 5 megabases
9% - 10%	> 4 megabases
> 10%	> 3 megabases

The following results from the data in the table: to be able to declare a positive TTC test result which detected an aberration exceeding 3 megabases at the level of sensitivity > 80%, the fetal fraction must simultaneously represent > 10%. With the same level of sensitivity (> 80%), a larger aberration can be confirmed in case of testing a sample with a smaller fetal fraction.
In case of a positive TTC test result, the Laboratory recommends conducting a confirmatory analysis on a sample of amniotic fluid collected using amniocentesis (AMC). In order to keep the identical technical parameters of the screening and follow-up confirmatory test, the Laboratory will arrange free-of-charge testing of this sample using GenomeScreen Prenatal test as a standard component of TTC test. In case the consulting physician decides to send a sample of amniotic fluid for confirmatory analysis outside the Laboratory or in case he/she decides for diagnostic analysis using a different biological specimen (chorionic villi, amniotic cells), it is necessary to use a test with resolution that is sufficient to confirm or rule out the detected aneuploidy and/or aberration. In case the confirmatory analysis is conducted outside the Laboratory, we would like to ask you for information concerning the applied methodology and to provide the result of the confirmatory analysis to the Laboratory (see granting of consent below in the section Informed Consent).
   d. **Additional findings:**
In case of TTC test, detection of additional findings means that the Laboratory has identified a high risk of a significant change of genetic information which, however, does not meet the detection parameters (5.c., Tab. 1).
A significant change of the genetic information in this context can be any of the following:
      i. mosaic form of a chromosomal aneuploidy and/or aberration
      ii. any detected aberration which does not meet the above detection parameters of TTC test, but can be verified using the evaluating bioinformatic algorithms and is simultaneously biologically significant.
The Laboratory will disclose these findings in the result of TTC test only under exceptional circumstances based on a written consent of the Pregnant woman with the procedure indicated in this Information and Guidance and only if interpretation of TTC test results by a specialist in medical genetics is available. In case that the consulting physician does not have established cooperation with any regional center of medical genetics, the consultation could be arranged by the Laboratory.
   e. **Uninformative result of TTC test** means that the delivered sample could not be processed in compliance with the good laboratory practice (e.g. the laboratory method failed to give result, low fetal fraction in the sample was identified, the Pregnant woman is treated with LMWH, her weight is > 90 kg or the result of TTC test does not answer the diagnostic question. In such case, the Laboratory will:
      i. repeat TTC test free-of-charge from the same sample (this applies to approximately 5% of samples) in cases where the result is ambiguous from the technical perspective
      ii. offer free-of-charge repeating of TTC test from a new sample (this applies to approximately 5% of samples). The new collection is recommended as soon as possible in case of obtaining an ambiguous result of the analysis from the technical perspective or with a delay of 14 days after the first sampling in cases where low fetal fraction (< 5%) was identified in the sample after the initial analysis. In such case, the date of providing the final result of TTC test will be extended accordingly.
If an informative result of TTC test will not be obtained even after a repeated analysis of the first sample nor after analysis of the second sample due to test failure caused by technical factors, the Laboratory will return the whole paid amount to the Pregnant woman (except for those who are treated with injected LMWH, or Pregnant women weighing > 90 kg, or possibly those who failed to comply with the conditions in section 8).
If an informative result of TTC test is not obtained even after a repeated collection of blood sample from the Pregnant woman, the Laboratory recommends taking an alternative diagnostic test of a sample collected using invasive methods (see section 4 b.).
   f. **Prediction of fetal sex:**
TTC test predicts the sex of the fetus (male or female). The Laboratory will provide this information in the TTC test result based on the choice selected by the Pregnant woman in the Request form.
6. **The precision and predictive ability ofTTC test:**
   a. Precision of TTC test is a numerical representation of probability that the result (negative or positive) has been determined correctly.
      i. The probability of a correct result of TTC test for chromosomal aneuploidies, e.g. trisomy 21, 18 and 13 and aneuploidies of sex chromosomes X and Y is very high (> 99.9%), in compliance with the results of an extensive metanalysis published in 2017 summarizing results of studies from all around the world (Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017 Sep; 50(3):302-314).
      ii. The probability of a correct result of TTC test for chromosomal aberrations cannot be stated exactly due to size- and position-based heterogeneity of the possible findings and the variability of fetal fractions determined in the analyzed samples.
   b. TTC test also has high predictive ability, which is at least 10-times higher than the predictive ability of standard biochemical or combined prenatal screening tests in case of the most frequently detected chromosomal aneuploidies. For example, for trisomy 21, 18 and 13 the predictive ability of the test is evaluated using the positive predictive value and it exceeds 95% while the negative predictive value exceeds 99.9%. In case of chromosomal aberrations such as partial chromosomal deletions and duplications and based on the results of an extensive retrospective study conducted on more than 9500 samples using TRISOMY test, positive predictive value exceeding 60% was detected for positive findings detected using the detection parameters of TRISOMY test Complete (Sekelská M., Izsáková A., Kubošová K., at al., Detection and validation of sub-chromosomal aberrations detected as additional findings in routine noninvasive prenatal testing for common trisomies, Newslab, 2019; 10 (2): 69 – 71). Negative predictive value of detection of chromosomal aberrations at the whole genome level cannot be determined due to the above-mentioned extensive heterogeneity of the potential findings and the variability of fetal fractions.
7. **Warning with regards to limitations ofTTC test:**
   a. TTC test is regarded as a highly efficient screening test, however it is not a diagnostic test. A positive result of TTC test as a non-invasive screening test will therefore have to be confirmed by a genetic laboratory analysis of a sample collected using invasive methods (CVS, AMC).
   b. Despite the high precision of TTC test, it is possible that in rare cases the sample can be assessed as false positive or even less often as false negative, which is in both cases caused by unknown or unpredictable biological characteristics of the sample (e.g. vanishing twin syndrome, placental mosaicism, maternal mosaicism) or technological limits of TTC test.
   c. TTC test does not enable disclosing of the test result in cases where fetal fraction is < 5%. In such cases, the test is classified as uninformative and repeated sampling is required with a 14-day delay after the first collection.
   d. The results of TTC test aimed at detection of chromosomal aberrations (deletions and duplications) offer sensitivity > 80% in identification of aberrations with size determined by fetal fraction identified in the sample after its analysis; a more detailed information is provided in Tab. 1.
   e. Other changes in the genetical information identified thanks to non-targeted detection are not analyzed in detail and are not disclosed in the results of TTC test. If, in exceptional cases, they are disclosed in TTC test results, they are labelled as Additional Findings and require interpretation under a specific regime (see section 5 d. of this Information and Guidance)
   f. TTC test is not primarily designed to screen for:
      i. triploidy and/or tetraploidy (a higher number of all chromosomes)
      ii. balanced translocations of chromosomes and/or inversions (exchange of fragments of genetic material between chromosomes and within chromosomes with no genetic information extra or missing)
      iii. forms of mosaicism (the fetus contains simultaneously the cells with standard as well as pathological genetic markers)
      iv. chimerism (the fetus consists of tissue originating from two individuals)
      v. other genetically determined fetal pathologies which cannot be detected by this type of test from technical point of view (e.g. monogenic hereditary diseases).
8. **The Laboratory does not accept any liability for incorrect results of TTC test or incorrect interpretation of results in cases where:**
   a. blood sampling was conducted before completing the 10th week of pregnancy.
   b. pre-analytical conditions were not complied with.
   c. correct results could not be achieved due to incorrect, incomplete or confusing anamnestic data, e.g. information about duration of pregnancy, multiple pregnancy, and/or vanishing twin syndrome, any known genetic pathology in any of the parents of the fetus, treatment with LMWH.
   d. the result was affected by the presence of other foreign DNA (i.e. other DNA than that of the fetus) in the organism of the mother, e.g. resulting from transfusion of donated blood, allogenous transplant of organs or bone marrow, therapy by foreign stem cells, cancer disease.
   e. the fetus suffers a disorder that the TTC test is not designed to detect (see section 7 f. of this Information and Guidance).
9. **The Laboratory undertakes to:**
   a. upon obtaining an uninformative result of TTC test to make efforts to obtain an informative one
      i. by repeating TTC test from the same sample (see section 5 e. i of this Instruction and Guidance)
      ii. by repeating TTC test from a new sample (see section 5 e. ii of this Instruction and Guidance).
   b. after completion of TTC test, the remainder of the original and/or processed sample will be stored in compliance with statutory regulations so that to enable further genetic laboratory testing for the benefit of the Pregnant woman or her consanguineal relatives. Any further genetic laboratory test will be preceded by a genetic consultation, after which the geneticist will obtain a signed informed consent of the concerned individuals.
   c. unused part of the sample will be disposed with/discarded in compliance with the consent of the Pregnant Patient granted in this document.
10. **Information about the potential limitation in lifestyle and sickness absence in relation toTTC test:**
TTC test is a non-invasive test and it does not pose any danger to the Pregnant woman either in the form of miscarriage or any other complications associated with CVS or AMC.
11. **Information about the right of the Pregnant woman to freely decide about particular procedures or interventions applied in the provision of healthcare:**
The Pregnant Patient has the right to freely decide about any particular procedure or intervention applied in the provision of healthcare.