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Laboratory Manual - PGT

Validity of the document:	**22. 2. 2023**
Creation date:	20. 2. 2023
Approval date:	22. 2. 2023
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Document guarantor:	RNDr. Martina Hrubá, Ph.D.
Version:	**002** The changes compared to the previous version are highlighted in yellow according to the internal rules of the Genetika Plzeň, s.r.o. (highlighted title of a chapter signifies numerous changes though the whole chapter).
ID document:	**VD.GP 05**
Document type:	**Internal**
Printout No.:	
Other information:	

Before using a document, please verify that it is the actual version.

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1. Introduction

1.1 Purpose

The aim of this document is to inform in detail the applicants for **preimplantation genetic laboratory examination** about the scope of services provided by the Laboratory of Reproductive Genetics (RG), department of the Genetic Laboratory of Genetika Plzeň, s.r.o., in the field of embryonic genetic testing.

This Manual follows up on the Laboratory Manual of the Genetic Laboratory (GL) of Genetics Plzeň, s.r.o. and provides a complete overview of the offered preimplantation examinations, including medical indications for their conduction, instructions for requesting examination and proper collection and transport of samples and information about the way how the results of testing are provided.

The Manual is available in two language versions (CZ, EN).

1.2 Scope

The Manual serves as a source of information to all applicants for preimplantation examination at RG and is available to the applicants on the web site of the laboratory or it is provided on their request. Information about subsequent updated versions of the Manual and the related documents (forms of requisition, biopsy protocol and informed consents) are sent automatically to all applicants.

The Manual is a part of the controlled documentation of Genetic Laboratory of Genetika Plzen s.r.o. and as such it is binding for all laboratory employees.

1.3 Authorized persons

The head of the Laboratory of Reproductive Genetics, in cooperation with the head of Genetic Laboratory Genetika Plzen and the quality manager, is responsible for the elaboration and updating of this Manual.

Only a physician - a clinical geneticist or a specialist in gynaecology and reproduction medicine is entitled to request a preimplantation examination. If the examination is requested by a reproduction specialist and is planned to be covered by a Czech health insurance company, a genetic consultation with a recommendation (indication) of preimplantation testing must be preceding.

The persons performing sampling are healthcare professionals authorized to carry out these activities in accordance with the internal regulations of the applicant organization (respective laboratory or healthcare institution).

2. Definitions, terms and abbreviations

2.1 Definitions and terms

2PN	Successfully fertilized oocyte with two visible ProNuclei
Applicant	Applicant for the examination is the indicating physician
Biopsy protocol (BP)	The accompanying document of the primary embryonal samples intended for the examination in RG
Blastomere	A cell that develops by early mitotic division of the fertilized egg (zygote)
Chromosome	colorable structure in the cell nucleus, comprising spiralized nuclear DNA and histones
Client	Couple (a female-patient and her partner) undergoing therapy at the IVF centre, or only the female-patient
Collection system	Medium suitable for collection and subsequent transport of the primary collection sample
DynaMed	Internal clinical information database system of the IVF centers of Nextclinics (formerly IVF Zentren Prof. Zech and some others)
Employee	A person who works for an organization or single companies with the referral relationship closed either according to the Labour Code (employee) or other legal provisions.
Genetic Laboratory (GL)	Genetic Laboratory Genetika Plzeň s.r.o.
Informed consent (IC)	Consent of the examined person/client with genetic examination; obligatory part of the medical records of the client in the case of request for genetic examination
IVF centre	Centre of assisted reproduction
IVF laboratory	IVF (embryology) laboratory
Polar body	Haploid cell that is formed during meiotic division of an egg (oocyte); the 1. polar body is collected from the mature egg and the 2. polar body is collected from the fertilized egg (examination of the polar bodies examines only maternal chromosomes of the future embryo)
Requisition form	Obligatory accompanying document of each sample intended for the examination in the Genetic Laboratory
SET-UP	Examination of the partners, or other family members, before initiation of the PGD examination due to familial chromosomal aberration or a monogenic disease (PGT-M, PGT-SR)
Trophectoderm	Cells in the development stage of blastocyst (early embryo of 5–6 days of age) from which the extra-embryonal tissues later develop (placenta and umbilical cord)
Turnaround time (TAT)	Interval between receipt of the sample in laboratory and issue of the final report (in accumulation cycles the interval starts by receipt of final set of samples, in case of supplemental examination by receipt of the request)

2.2 Abbreviations

aCGH	Array Comparative Genomic Hybridization
AD	Autosomal dominant
AR	Autosomal recessive
BP	Biopsy protocol
ČIA	Czech Accreditation Institute, o.p.s.
CPB	Cryoprotective buffer
CZ	Czech
DE	German

ECA	European Cytogeneticists Association
EDTA	EthyleneDiamineTetraAcetic acid
EN	English
ESHRE	European Society of Human Reproduction and Embryology
F	Form
FISH	Fluorescence *in situ* hybridization
GL	Genetic Laboratory
HE	High-school employee
HIC	Health Insurance Company
IC	Informed consent (IC)
ICSI	IntraCytoplasmic Sperm Injection
ID	Identification number of the sample (or set of samples)
IMSI	Intracytoplasmic Morphologically selected Sperm Injection)
IT	Italian
IVF	*In vitro* fertilization
LH	Laboratory head
LLRG	Laboratory of medical and reproduction genetics (Repromeda)
LM	Laboratory Manual
MDA	Multiple Displacement Amplification (a special type of WGA suitable for Karyomapping)
MESA	Microsurgical Epidermal Sperm Aspiration
MoH	Ministry of Health
NGS	Next Generation Sequencing
NIN	National identification number (of the patient)
NZZ	Non-governmental organization
OPU	Ovarian puncture (egg collection)
PBS	Phosphate Buffered Saline
PGD	Preimplantation Genetic Diagnosis
PGS	Preimplantation Genetic Screening
PGT-A	Preimplantation Genetic Testing for Aneuploidies (new equivalent for PGS)
PGT-M	Preimplantation Genetic Testing for Monogenic disease (partial equivalent for PGD)
PGT-SR	Preimplantation Genetic Testing for Structural (chromosomal) Rearrangement (partial equivalent for PGD)
PVP	Polyvinylpyrrolidone
RG	Laboratory of Reproductive Genetics (Department of Genetic Laboratory Genetika Plzeň s.r.o.)
RT	Room temperature
SE	Secondary school employee
SLGG	Society of Medical Genetics and Genomics of Czech Medical Society of J. E. Purkyne
SNP	Single Nucleotide Polymorphism
SOPV	Standard operation procedure for laboratory examination
STR	Short Tandem Repeat
TAT	Turnaround Time
TESA	Testicular Sperm Aspiration
TESE	Testicular Sperm Extraction
WGA	Whole Genome Amplification
WGA DNA	Embryonal DNA after Whole Genome Amplification

3. Information on laboratory

3.1 Basic identification of the laboratory and contacts

Name of the organization	**Genetika Plzeň, s.r.o.**
CEO	MUDr. Petr Lošan
Type of organization	Non-governmental organization, limited liability company
Foundation of the organization	Registration in Commercial Registry in County Court Pilsen on 26. 5. 2003 under the file C 15141
ID	26357623
Tax ID	CZ699004286
Address	Parková 1254/11a, 326 00 Plzeň-Černice
Telephone	+420 377 241 529, +420 377 452 322
Email	recepce@genetika-plzen.cz
URL	www.genetika-plzen.cz

Name of the laboratory	**Genetic Laboratory (GL)**
Head of the laboratory	Mgr. Sabina Planetová
Telephone	+420 604 106 586
Email	sabina.planetova@next-clinics.com

Internal organization and competence of the employees of GL are clearly specified and described in the requirements of the implemented quality management system. All employees of GL are professionally competent for the work at a medical laboratory and the professional staff of the laboratory fulfils minimum criteria for a genetic laboratory specified by the Association of Medical Genetics and Genomics of the CzMA of JEP and the criteria of the Decree no. 99/2012 Coll. concerning the minimum requirements for the personal equipment of healthcare facilities.

The laboratory is accredited by ČIA according to the standard ČSN EN ISO 15189:2013. **For further information about Genetic laboratory of Genetika Plzeň s.r.o. see *VD.GP 04 Laboratory Manual.***

3.2 Information on the department Laboratory of Reproductive Genetics

Department	**Laboratory of Reproductive Genetics (RG)**	
Area of activity	The Laboratory of Reproductive Genetics performs specialized genetic examinations of embryonal samples and other related examinations	
Head of the department	RNDr. Martina Hrubá, Ph.D.	
Address of the laboratory	Parková 1254/11a, 326 00 Plzeň-Černice - 2. patro (3.NP)	
Working hours	Mo-Fri (07:00 a.m.-03:00 p.m.)	*Working hours or receipt of samples can be adjusted after agreement with the applicant/client.*
Receipt of samples		
Telephone	+420 605 179 548	
Email	martina.hruba@next-clinics.com	
URL	www.genetika-plzen.cz	

Laboratory of Reproductive Genetics (RG) of GL of Genetika Plzeň is the successor of the Genetic Laboratory of IVF Zentren Prof. Zech - Pilsen and as such is adapted to the requirements of centers of assisted reproduction for which it provides laboratory genetic examinations of embryonal tissues and ensures other specialized

examinations in the field of reproductive genetics. There is a long-term cooperation with IVF NextClinics both in the Czech Republic and abroad.

Laboratory of Reproductive Genetics is focused on preimplantation genetic examinations, i.e. specialized genetic examinations of the embryonal cells collected in the early embryonal development following *in vitro* fertilization (i.e. blastomeres, trophectoderm, or polar bodies). An integral part of the laboratory examination is also the appropriate interpretation of the examination results, related consultancy services and providing of the collection (transport) systems for collections of the primary samples for particular types of examinations.

At present, the molecular-genetic examinations of the embryonal tissues using the NGS and FISH methods is offered (PGT-A, PGT-SR, for more details see Section 4.3.1 and Section 4.3.2). Diagnosis of monogenic diseases using the molecular-genetic methods (PGT-M, for more details see Section 4.3.3, Karyomapping or OneGenePGT methods) is ensured by the examinations in the referral laboratory LLRG Repromeda.

Exceptionally, the examination or its part may be requested in another (external) laboratory. It concerns especially accomplishment of diagnostic/ confirmative examinations before PGT-M. For these examinations the laboratory offers the consultation services, related to the examination methods and selection of suitable examination, including providing of the information on the requirements for collection and transport of samples.

The examinations are carried out on the basis of the indication of a physician both for self-payers and insured persons of CZ HICs.

Laboratory has all necessary technical equipment for performing of the offered genetic examinations (microscopes, biohazard cabinets, thermocyclers, equipment for electrophoresis methods and others) in accordance with the requirements of the Decree no. 92/2012 Coll. on the technological and material equipment of healthcare facilities.

The certified diagnostic sets are preferentially used for the performed examinations.

3.3 Range of the offered services

3.3.1 Preimplantation genetic testing for aneuploidies (PGT-A)

Examination options	Method		Number of examined samples	Response time (TAT)
• **PGT-A of 24 chromosomes**	*NGS*	• **standard**	all samples	within **21 days**
		• **less than all samples**	as requested *	
		• **additional examination**	as requested *	
		• insured persons of a CZ HIC	**up to 8***	
• **PGT-A of 5 chromosomes (13, 18, 21, X, Y)**	*FISH*	• **standard**	všechny vzorky	within **21 days**

Information on examination

Preimplantation genetic screening (PGT-A) is a complex examination that can exclude sporadic (i.e. *de novo* arisen, non-inherited) deviations in the number of chromosomes (so-called aneuploidies) or their parts (segmental aneuploidies) and reduce the risk of abortion or birth of a child with genetic abnormality. Development of embryos with abnormal chromosomal complement is one of the main reasons of fertility disorders and reduced chance of pregnancy and birth of a healthy child. Therefore, inclusion of PGT-A into the IVF cycle increases markedly the chance of achieving pregnancy and birth of a healthy child.

The embryos with normal, i.e. euploid, chromosomal complement are fully recommended for the transfer into maternal uterus.

Mosaic findings, corresponding with the presence of the aneuploidy/ies only in a part of examined cells (relevant in trophectoderm samples), are reported and interpreted following these rules:

- **mosaic findings of numerical changes of chromosomes 13, 18, 21 and X, Y are not recommended for transfer due to the known risk of viable numerical changes of these chromosomes leading to the affected child's phenotype,**
- **mosaic findings of numerical changes of chromosomes 14 and 15 are not recommended for transfer due to the increased risk of viable uniparental disomy of these chromosomes leading to the affected child's phenotype,**
- **complex mosaic findings, including > 4 mosaic changes, are not recommended for transfer primarily due to the risk of reduced embryo implantation rate, increased pregnancy loss and higher risk of affecting the child's phenotype,**
- **for other mosaic findings, embryo transfer may be considered and performed after a genetic consultation and explanation of possible risks,**
- **in the report, distinction is made between segmental and whole-chromosomal mosaics, as well as between low (up to ~50 %) and high (over ~50 %) representation of aneuploid lineage in the examined sample.**

The rules for interpretation of mosaic findings are based on the expert recommendations of PGDIS and CoGen (see chapter 7.2 External related documentation).

Accreditated examination, currently on EmbryoMap™ platform (Vitrolife).
Examination cannot be performed in STATIM regimen.

Main indications for examination (however examination might be a part of any IVF cycle)

- Advanced maternal age (> 35 years);
- Recurrent miscarriages (twice and more)**;
- Delivery or abortion of a foetus with a chromosomal abnormality**;
- Repeated failure of previous (whole) IVF cycles (twice and more)**;
- Repeated failure of implantation after previous embryotransfers (twice and more);
- Significant worsened spermiogram parameters in the partner**;
- Using of sperm following TESE (collection of sperm directly from testes) or MESA (collection of sperm directly from epididymides) for IVF**;
- Status post oncology therapy using chemotherapy or radiation therapy in one or both partners**.

Examined embryonal material	
• **Trophectoderm** (day 5/6 of development)	• **Blastomere/es** (alternative option for FISH examination) (day 3 of development)
Examination methods	
PGT-A by new generation sequencing enables examination of larger (≥ ~ 10 Mbp) quantitative changes (gains or losses) of genetic material within the whole genome, i.e. in all 24 chromosomes. Compared to the previously used analysis by aCGH it is possible to assess and report mosaic form of aberrations. **Principle of the NGS method**: DNA is extracted from the collected cells with subsequent whole genome amplification and processing to so-called "library" (sample suitable for "reading" of DNA sequence). Each sample/library has its unique label that allows analysis of DNA from more individuals (embryos) at a time in one experiment. The samples/libraries are mixed together in the same ratio and the final library is subsequently prepared for the final sequencing. Reading of the sequence (sequencing) is performed by means of gradual synthesis of the new complementary chains of DNA to the read fragments. The read sequences are compared with the normal human genome using special software and their genomic position is determined. After assignment of the sequences to the single samples quantitative evaluation of all chromosomes is performed. **Limitation of the NGS method**: The used NGS method (low-pass whole genome sequencing) is limited by the size of the chromosomal rearrangements. Small chromosomal losses or gains cannot be detected and at the same time any other disease or development defects of the foetus which are not caused by the change of the number of examined chromosomes or their major parts cannot be excluded.	
PGT-A by fluorescent *in situ* hybridization (FISH) is performed usually only for 5 chromosomes and is used only rarely, based on the referral of a physician. **Principle of the FISH method**: The cell nuclei of the collected material are fixed on the micrometre slide. The pre-selected chromosomal sections are labelled with the specific fluorescent probe complementary to the respective section of DNA. Evaluation is performed using the fluorescent microscope. **Limitation of the FISH method**: The FISH method cannot exclude any other disease or development defects of the foetus which are not caused by change of the number of the examined chromosomal regions.	
Explanations: ***** *Number of samples that will be tested in one examination. The redundant samples will be processed and stored in RG for 1 year for possible additional examinations*. *** Indications with possible reimbursement from CZ HIC.*	

3.3.2 Preimplantation genetic testing for familial chromosomal aberrations (PGT-SR)

Examination options	Method		Number of examined samples	Response time (TAT)
▸ **PGT-SR** + **PGT-A of 24 chromosomes**	*NGS*	• **standard**	all samples	within **21 days**
		• **less than all samples**	as requested *	
		• **additional examination**	as requested *	
		• insured persons of a CZ HIC	**up to 8***	
▸ **PGT-SR** solely or **+ PGT-A 2 chrom. (13, 21)** **+ PGT-A 3 chrom. (18, X, Y)**	*FISH*	• **standard**	all samples	within **21 days**

Information on examination

Preimplantation genetic testing for familial chromosomal aberrations/rearrangements (PGT-SR) is a targeted diagnostics of unbalanced forms of particular familial chromosomal abnormalities which may cause birth of a disabled child or repeated pregnancy losses.

The embryos without an unbalanced form of familial aberration (i.e. normal/balanced) are recommended for transfer. When performing analysis using NGS method also sporadic aneuploidies of all chromosomes are excluded (PGT-A, see Section 3.3.1), when performing FISH only viable aneuploidies may be examined (see above; limited by technical feasibility).

Prior to the PGT-SR examination using the NGS method no repeated chromosomal examination of the parents (so called **SET-UP**) is needed.

On the contrary, in the PGT-SR examination performed using the FISH method the preliminary examination of the parents (SET-UP) is needed because of verification of accuracy of the proposed combination of the fluorescent probes. For the SET-UP examination new blood sample collection of both partners is needed (see Sections 4.7.5 and 4.8.3).

Each case is handled individually and therefore it must be consulted with RG in advance (the decision regarding SET-UP is performed depending on the extent of rearrangement, on the details in the documentation of previous diagnostic laboratory examinations of parents, etc., see also 4.3.1).

Accredited examination, currently on EmbryoMap™ platform (Vitrolife).
Examination cannot be performed in STATIM regimen.

Indication for examination

- Carriage of so called balanced chromosomal rearrangement (usually translocation) in one or both partners.
- The presence the numerical changes in sex chromosomes (gonosomes) including the mosaic form in one or both partners.

Examined embryonal material

- **Trophectoderm** (day 5/6 of development)
- **Blastomere/es** (alternative option for FISH examination) (day 3 of development)

Examination methods

PGT-SR + PGT-A using the NGS method enables examination of the unbalanced forms of familial chromosomal rearrangement of larger extent (with translocated segments ≥ ~10Mbp) and a screening of quantitative changes in other chromosomes at the same time.

PGT-SR using the FISH method is used only for the PGT-SR of unbalanced forms of familial structural rearrangements of a smaller extent in which the NGS method cannot be used.

Principle of the NGS and FISH methods / Limitation of the NGS and FISH methods: see Section. 3.3.1.

Explanations: ***** *Number of samples that will be tested in one examination. The redundant samples will be processed and stored in RG for 1 year for possible additional examinations*.

3.3.3 Preimplantation genetic testing for monogenic diseases (PGT-M)

<table>
<tr><th>Examination options</th><th colspan="2">Method</th><th>Number of examined samples</th><th>Response time (TAT)</th></tr>
<tr><td rowspan="3">• PGT-M
+ tentative PGT-A of 24 chromosomes
Any causative gene</td><td rowspan="3">Karyo mapping</td><td rowspan="2">standard</td><td>up to 5 *</td><td rowspan="3">within 30 days</td></tr>
<tr><td>up to 2
(NOT available for insured persons of a CZ HIC</td></tr>
<tr><td>more than 5 samples</td><td>as requested *</td></tr>
<tr><td rowspan="2">• PGT-M
+ PGT-A of 24 chromosomes
Only CFTR, FMR1, BRCA1, BRCA2, HBB, HTT genes</td><td rowspan="2">OneGene PGT (NGS)</td><td>standard</td><td>up to 5 *</td><td rowspan="2">within 30 days</td></tr>
<tr><td>more than 5 samples</td><td>as requested *</td></tr>
<tr><td colspan="5">Information on examination</td></tr>
<tr><td colspan="5">Preimplantation genetic testing for monogenic diseases (PGT-M) is a targeted diagnosis of particular serious familial monogenic diseases (autosomal recessive, autosomal dominant or X-linked) in which there is a high risk of birth of a disabled child.

The embryos with a genotype that does not cause the observed disease (depending on the type of heredity also the embryos with one parental mutation can be recommended for transfer) are recommended for transfer. The embryos with proven sporadic aneuploidy are also not recommended for transfer.

Before the preimplantation examination for a monogenic disease (PGT-M) a preliminary molecular genetic examination in several family members is needed (so called SET-UP; usually a new sample collection is needed, see Section 4.7.4 and 4.8.2) following the previous diagnostic DNA testing.

Each case is handled individually and therefore it must be consulted with RG in advance (see also 4.3.2)!

Accredited examination in referral lab. Examination cannot be performed in STATIM regimen.

Note: The own examination (for self-payers and the insured persons of the Czech healthcare insurance companies) is performed at the referral laboratory LLRG Repromeda.</td></tr>
<tr><td colspan="5">Indication for examination</td></tr>
<tr><td colspan="5">• Carriage of a predisposition (mutation) for a serious genetic disease caused by impairment of one gene in one or both partners that is associated with a high risk of birth of a child affected by the respective condition.</td></tr>
<tr><td colspan="5">Examined embryonal material</td></tr>
<tr><td colspan="5">• Trophectoderm (day 5/6 of development)
• Blastomere/es (less suitable material; only if trophectoderm cannot be obtained) (day 3 of development)</td></tr>
<tr><td colspan="5">Examination methods</td></tr>
<tr><td colspan="5">PGT-M by the Karyomapping method (Illumina) enables a robust examination of monogenic diseases on the principle of indirect diagnosis and at the same time the orientation screening of the quantitative changes of all chromosomes.

Principle of the Karyomapping method: The method is based on the comparison of DNA of the embryonal samples and DNA of the couple and a reference family member collected within SET-UP. DNA is extracted from the collected samples and in addition, the whole genome amplification (WGA of the MDA type) is performed in embryonal samples. The examination itself is performed on a special type of microchip (SNParray) that is able to detect large number of selected single-nucleotide polymorphisms of the whole genome at the same time. A particular combination of SNPs in the region of the gene causing a particular monogenic disease distinguishes the individuals (family members, embryos) and both their alleles of this gene (haplotypes). By comparing the found haplotypes with the family tree information of the examined family, the haplotypes associated with the presence of the respective mutation (i.e. carrying a risk for the condition) are then determined. In some cases, which however occur very rarely, it is necessary to use Karyomapping in combination with direct detection of the mutation. The method is universal and enables examination of any known monogenic disease. Another</td></tr>
</table>

advantage includes a significant reduction of complexity and time needed for the preparation phase of the examination (SET-UP).

Limitations of the Karyomapping method: PGT-M cannot exclude any other disease or congenital defect of the foetus which are not caused by mutation in the respective gene. Karyomapping cannot detect the *de novo* mutations in the embryonal DNA. Karyomapping, as a method primarily intended for elimination of a monogenic disease, does not provide detection of random aneuploidies to the level comparable with aCGH or NGS. Screening of aneuploidies using Karyomapping is only an additional examination that can increase success of therapy by means of selection of the most promising embryo and is offered charge-free.

PGT-M by the **OneGenePGT** method enables a robust examination of monogenic diseases on the principle of indirect diagnosis with combination of direct diagnostics of the mutation site and at the same time the screening of the quantitative changes of all chromosomes.

Principle of the OneGenePGT method: The method is based on the comparison of DNA of the embryonal samples and DNA of the couple and a reference family member collected within SET-UP. DNA is extracted from the collected samples and in addition, the whole genome amplification (WGA of the MDA type) is performed in embryonal samples. The examination itself is performed by NGS method that is able to detect at the same time large number of selected single-nucleotide polymorphisms (SNPs) in the targeted region of the genome. A particular combination of SNPs at the locus of the gene causing a particular monogenic disease distinguishes the individuals (family members, embryos) and both their alleles of this gene (haplotypes). By comparing the found haplotypes with the family pedigree information of the examined family the haplotypes associated with the presence of the respective mutation (i.e. carrying a risk for the condition) are then determined. NGS approach brings advantage of possible combination with direct diagnostics of the mutation site. Another advantage (as in Karyomapping) includes a significant reduction of complexity and time needed for the preparation phase of the examination (SET-UP). However, in contrast to Karyomapping, it is possible to perform a simultaneous PGT-A examination with a standard level of method resolution (see Chapter 3.3.1 above). PGT-A is performed at no extra charge.

Limitations of the OneGenePGT method: The method allow for examination of only selected monogenic diseases caused by the genes listed in the table above. HLA compatibility tests cannot be included. Any method of PGT-M cannot exclude any other disease or congenital defect of the foetus which are not caused by mutation in the respective gene. Karyomapping *De novo* mutations in the embryonal DNA cannot be detected.

Explanations:

***** *Number of samples following a successful whole-genome amplification that will be tested in one examination. In the case that RG receives more than the given number of samples, the redundant samples will also be sent to the referral laboratory, amplified, and stored for possible additional examinations.*

4. Manual for collections of the primary samples for PGT

4.1 Background information

This chapter includes specific instructions regarding request for PGT including the essentials of accompanying documents, correct collection of the primary samples and subsequent transport of the samples to laboratory.

At present, any type of PGT is offered in STATIM regimen.

4.2 PGT-A examination – SET-UP requirements

For PGT-A examination (Section 3.3.1) there is no need to perform preliminary examination of the parents and other family members (SET-UP). To ensure optimal schedule of activities of laboratory that is necessary for timely delivery of results it is appropriate that the applicant informs the laboratory on the planned examination in advance (preferably at the start of ovarian stimulation of the patient).

4.3 PGT-M and PGT-SR examination – SET-UP requirements

4.3.1 PGT for a familial chromosomal aberration (PGT-SR)

Any preimplantation examination in a couple with a familial chromosomal abnormality (PGT-SR, see Section 3.3.2) can be performed only **after previous agreement with the employees of laboratory**. Before starting IVF cycle with planned preimplantation diagnosis, it is necessary to contact the laboratory and provide following documents:

- original laboratory reports with results of cytogenetic examination of both partners,
- report on performed genetic counselling for the couple.

After evaluating the stated documents the laboratory will decide what method will be used for preimplantation diagnosis (NGS, FISH), whether preliminary tests are necessary (so called SET-UP) and in what extent (examination of karyotype and/or FISH). If SET-UP is necessary requesting clinician will also be informed about necessary collections of primary samples in particular family members or about addition of other medical records (final laboratory reports from genetic/cytogenetic examinations, or genetic consultations, of other family members).

Following termination of the SET-UP examination IVF cycle may be initiated.

4.3.2 PGT for a monogenic disease (PGT-M)

Any PGD examination in a couple with a monogenic disease (PGT-M) can be performed only **following previous agreement with the laboratory**. Before starting IVF cycle with planned preimplantation diagnosis it is necessary to contact the laboratory and provide following documents):

1) Original laboratory reports:

- **AR disease** – results of the molecular genetic examination of both partners or affected descendants,
- **AD disease** - results of the molecular genetic examination of the affected partner and one other affected family member,
- **X-linked disease** - results of the molecular genetic examination of the affected partner or results of the molecular genetic examination of the partner-carrier and one other affected family member,

2) Report on performed genetic counselling of the couple.

Following evaluation of the documentation the laboratory will decide whether PGT-M is possible for the particular type of the disease and the particular family. If yes, requesting clinician will also be informed about needed collections of primary samples of affected and healthy family members to prepare SET-UP examination.

Samples for the SET-UP examination before PGD for a monogenic disease (PGT-M) are received at RG that ensures their examination at referral laboratory LLRG Repromeda.

Following the successful SET-UP examination, it is possible to start IVF cycle.

4.4 Requirement for examination and accompanying documentation

All samples intended for receipt in RG (i.e. intended either for examination at RG or at referral laboratories or other diagnostic laboratories) **must be accompanied with the following**:

- Requisition form (see Section 4.4.1),
- Informed consent (see Section 4.4.2),
- Other required data specified e.g. in biopsy protocol or in protocol on successful whole-genome amplification if embryonal samples are concerned (see Section 4.4.3 and 4.4.4) or on protocol on isolation of DNA in the case of sending genomic DNA (see 4.4.5).

Forms for all required accompanying documentation are available at https://www.genetika-plzen.cz/en/documents-to-download.

All original documentation must be visibly completed and the required examination and further mandatory data must be clearly indicated, see Section 4.4.1–4.4.4. Accompanying documentation has to be enclosed to the sample as the printed and properly completed original documentation. In case of informed consent a scanned copy accessible electronically (in DynaMed or Medicalc system) is also accepted.

Verbal requirements for examination are not accepted.

4.4.1 Requisition for examination

To request for examination it is possible to use valid version of the form *Requisition for preimplantation genetic testing (F.GP 906)* that is available to applicants on the website (see above) or on the request. When another type of requisition form is used all required data must be included.

Set of embryonal samples from one patient has one requisition form. In the cases of uneven speed of development of the examined embryos it is possible, following previous agreement with the laboratory, to deliver a part of the samples to the laboratory later only with (further) biopsy protocol, within 30 days from delivery of the first set of samples with the requisition form.

The applicant (a physician requesting the examination) is responsible for correct and visible completion of the requisition form (except for data on collection of the primary samples) and by signing it he/she confirms accuracy of data in the requisition form.

Following mandatory data must be included in the requisition form:

1) Patient identification

- Name, surname, date of birth, NIN (if available), HIC (in insured person of a CZ HIC), sex,
- Address of residence,
- Identification of (male) partner in case of preimplantation examinations (name, surname, date of birth, NIN (if available), HIC (in insured person of a CZ HIC)).

2) Reason for examination (diagnosis)

3) Identification of the physician/institution

- Name, surname, and signature of the requesting physician,
- Identification of the institution - name, address.

4) Type and regimen of the requested examination

- Clear specification of the requested examination or a possible STATIM regimen,
- In case of additional examinations, it is necessary to indicate number and exact designation of the embryos which are to be examined.

5) Sample specification

- Type of biological material,
- Other information on the nature of the collected embryonal sample, i.e. specification whether it is a biopsy, re-biopsy, accumulation (i.e. samples are from the patient who has had planned more stimulation cycles before embryotransfer).

6) Records on sample collection

If the requisition form is intended for requesting the examination of primary sample of other origin than embryonal (e.g. peripheral blood, buccal swabs etc.):

- Date and time of collection of the primary sample,
- Name and signature of the person carrying out collection.

Records on collection of the primary sample of embryonal origin are usually stated in the biopsy protocol as further mandatory part of the accompanying documentation (for instructions for completion see Section 4.4.3).

If examination of the secondary sample (genomic DNA, embryonal WGA DNA, cytogenetic suspension, etc.) is requested, the records on collection of the primary sample have to be stated in an enclosed report of processing of the primary sample (isolation of genomic DNA, WGA, etc. see Sections 4.4.4-4.4.5). The person authorizing this report is responsible for proper aliquotation of the sample.

4.4.2 Informed consent with the genetic examination

Before each genetic examination the couple must be duly informed on the nature of the examination and must sign the corresponding informed consent (see Table 1) that is included in the patient medical records. Informed consent (IC) is a compulsory part of accompanying documentation enclosed to the sample, however it might be documented only electronically (in the DynaMed system). All valid informed consent forms are available to applicants on the website Genetika Plzen (https://www.genetika-plzen.cz/en/documents-to-download) or on the request.

The physician who is indicating the examination is responsible for correct and readable completion of the informed consent and by his signature confirms correctness of the stated data and he/she is a witness of patients´ consent.

Following mandatory data must be included in the informed consent:

1) Identification of patient (in case of preimplantation analysis of both reproductive partners)

- Name, surname, date of birth, NIN (if available), HIC (in insured person of a CZ HIC),
- Address of residence.

2) Reason for examination (diagnosis)

3) Consent with the examination and other stated conditions

- Date and signature of the patient (in case of preimplantation analysis of both partners),
- Date and signature of the physician.

Recommended is to specify the following:

- Required examination or methodology of the examination,
- Type of the primary sample.

Table 1: Summary of the used informed consent forms

Type of examination	Examining laboratory	Informed consent form
PGT-A (see 3.3.1)	**Genetika Plzeň s.r.o.**	***F.GP 907 Instructions and informed consent with PGT*** *(CZ, EN, DE, IT)**
SET-UP before PGT-SR (see 3.3.2 and 4.3.1)	**Genetika Plzeň s.r.o.**	***F.GP 901 Consent of the examined person (legal representative) with genetic laboratory examination*** *(CZ, EN, DE)*
PGT-SR (see 3.3.2)	**Genetika Plzeň s.r.o.**	***F.GP 907 Instructions and informed consent with PGT*** *(CZ, EN, DE, IT)**
SET-UP before PGT-M (see 3.3.3 and 4.3.2)	**LLRG Repromeda**	***F.GP 901 Consent of the examined person (legal representative) with genetic laboratory examination*** *(CZ, EN, DE)* *OR* **REPROMEDA** ***- SET-UP Instructions and inform consent with genetic testing*** *(CZ, EN, DE)*
PGT-M (see 3.3.3)	**LLRG Repromeda**	**REPROMEDA –** ***PGT-M Instructions and inform consent*** *(CZ, EN, DE)* *AND* ***F.GP 908 Informed consent to storage of samples (and to results management) after PGT*** *(CZ, EN, DE, IT)*
Other examination	**According to the instruction of laboratory (on the given request)**	

* consent F.GP 907 may be replaced by internal consent of applicant in combination with ***Informed consent to storage of samples (and to results management) after PGT***

4.4.3 Protocol on biopsy for PGT

Embryonal samples of the primary collection must always be accompanied by further detailed data, which can be stated e.g. on a biopsy protocol. RG provides form of *F.GP 909 Biopsy protocol for PGT* to the applicants on the website or on the request.

Following data regarding collection of embryonal samples are requested (**bold highlighted data are compulsory**):

1) Patient identification:

- **Name, surname, date of birth** (or NIN).

2) Records on collection of the primary sample:

- **Name, surname and signature of the employee who performed the biopsy,**
- Name, surname and signature of the employee who witnessed the biopsy,
- Day of development of the embryo when the biopsy is performed,
- Possible use of donor gametes,

- **Internal identification number (ID) of RG (if it has been assigned in advance by RG),**
- **Date and time of biopsy,**
- **Date and time of transfer of the sample into the collection medium** (i.e. transfer into the microtube with a suitable transport buffer for the required molecular biological examination or fixation on the slide),
- Further processing of the sample before the transport (centrifugation, storage etc.),
- Ordinal number (#) of the embryo,
- Quality of the embryo before the biopsy,
- **Confirmation that fertilization was performed using the ICSI** (/IMSI) method,
- **Information on success of fertilization** (2PN yes/no/missing info),
- Other notes relevant to the biopsy (**number of collected cells**, possibility of re-biopsy, etc.).

The embryologist performing the biopsy is responsible for providing the required data and their accuracy.

4.4.4 Protocol on successful whole-genome amplification (for examination of embryonal WGA DNA)

If the samples of embryonal WGA DNA are provided to the laboratory (in case of a requirement for further examination using the NGS method) they must be accompanied by the protocol showing previous successful whole-genome amplification.

The protocol must contain at least the following information:

- Date of collection of the primary sample,
- Specification of the primary sample (type of the biological material),
- Date of amplification,
- Specification (type) of the used kit for the whole-genome amplification,
- Image of the gel with a clear identification of the sample,
- Date and time of release of the protocol,
- Name and signature of the responsible person releasing the protocol.

4.4.5 Protocol on isolation of the genomic DNA

If the samples of genomic DNA are provided to the laboratory (in case of the SET-UP examination for a monogenic disease) they must be accompanied by the protocol showing successful DNA isolation.

The protocol must contain at least the following information:

- Date of collection of the primary sample,
- Specification of the primary sample (type of the biological material),
- Date of isolation,
- Specification (type) of the used isolation kit,
- Final concentration of the isolated genomic DNA,
- Date and time of release of the protocol,
- Name and signature of the responsible person releasing the protocol.

4.5 Requirements for urgent examination

At present, RG does not provide any PGT examination in the STATIM regimen (including examination in referral labs see also list of the examinations in Section 3.3).

4.6 Requirements for additional examination

The laboratory accepts requests for additional examinations only for the embryonal samples examined by the NGS or Karyomapping method which fulfil the following criteria:

- **Stored samples of embryonal DNA** (after successful whole-genome amplification or processing for NGS) **up to a maximum period of 1 year from the date of their receipt at RG**.

Additional examinations are performed on the basis of a new requisition form with the exact identification of the number and ID (#) of the embryonal samples. A new informed consent for the examination is not required.

4.7 Requirements for sample collection and collection sets

Compliance with the correct method of collection of the primary samples and subsequent handling are vital for the result of the examination.

Before the actual collection of embryonic samples, it is strongly recommended to contact RG and request a unique identification number (ID) for the patient, i.e. for a set of embryonic samples from the given IVF cycle. Alternatively, it is possible to agree with the sampling laboratory on the assignment of its own number serie.

During the collection, it is always necessary to verify the match **between the identification of the patient / embryo that undergo the sampling and the label of the collection container** to which the sample / biopsy is collected. The check by a second employee for rigorous prevention of sample mix-up during collection is recommended.

4.7.1 Sample identification

All samples intended for the examination in RG must be clearly identified, i.e. they must be labelled at least with the following data:

- Name and surname of the patient,
- Year of birth of the patient or his/her NIN number,
- Ordinal number of the embryo/embryos (in case of embryonal sample/s).

Alternatively, embryo samples taken in a 0.2ml microtube can be labelled by (recommended with respect to the microtube size):

- ID of the examined case (assigned in advance on the request by RG) on each tube,
- Ordinal number of the embryo on each tube,
- Full patient´s identification (i.e. name, surname, year of birth / NIN) alongside with case ID on the lid of rack.

Samples must always be labelled legibly and indelibly: **common sampling tubes** (e.g. blood, buccal swabs, etc.) on the side of the tube, **microtubes** labelled on the cap and horizontally on the side of the tube (see picture above) using a permanent alcohol-based marker (preferably black) or with a self-adhesive low-temperature label and the lid of the rack with a permanent alcohol-based marker, the **microscope slides** marked using a diamond tip and a regular pencil.

The laboratory accepts only properly labelled samples accompanied by the respective documentation according to the chapter 4.4.

4.7.2 Embryonal samples from the primary collection

The embryonal samples from the primary collections are obtained by means of biopsy of the embryos cultivated within the infertility treatment using assisted reproduction methods and therefore sample collection does not require any special preparation of the client.

However, the ICSI (or IMSI) method must be used for fertilization of the oocytes, the conventional IVF technique is not suitable!

Biopsy should be performed under sterile conditions by a specialized embryologist in the embryology laboratory of the IVF centre using a microscope equipped with laser and micromanipulators.

During collection of the samples intended for isolation and subsequent whole-genome DNA amplification (examination using the NGS and Karyomapping methods) it is always necessary **to pay attention to prevention of contamination** of the collected samples with extraneous DNA. The extraneous DNA could **cause incorrect result of the analysis and lead to serious undesirable consequences**. The course of examinations **can be negatively impacted also by using of unsuitable media or buffers**.

Contamination of an embryonal sample can be minimized by following the following rules:

- To use suitable (best disposable) personal protective aids (laboratory coat, gloves, cap, oral mask),
- To use clean sterile gloves without powder in the course of the whole procedure of biopsy and if a contamination is suspected they must be immediately replaced,
- To perform the whole procedure of biopsy, especially preparation of the cultivation dish and collection microtubes, in the clean and decontaminated laminar box,
- To use suitable sterile disposable consumables and media (DNase-free),
- To pay attention carefully that the "clean" end/tip of the pipette does not come into contact with any other object then the biopsy sample (it must not come into contact with the skin or any potential contaminant, with the outer side of the microtubes, working area of the laminar box, etc.),
- To handle the single collection tubes only in the clean decontaminated laminar box and to use only the originally provided frozen closeable (cryo)rack in the whole procedure of biopsy,
- To centrifuge the microtubes with collection medium always before opening/transport/storage,
- To minimalize the period of time during which the microtubes with collection medium are open.

The employees who perform collection of the primary samples are obliged to follow the instructions in this Manual.

Specifications of collected material are listed in Table 2.

Table 2: List of accepted embryonal samples of the primary collection

Sample type (tissue):	Day of biopsy (Day of cultivation of embryos)	Development stage	Number of collected cells
Blastomere	3	Blastomere	1-2
Trophectoderm	5/6	Hatching blastocyst	Approximately 5-10

The collection sets are identical for all types (tissues) of samples of the primary collection and vary according to the type of the examination method, see Table 3.

Table 3: Collection sets for embryonal samples of the primary collection

Required examination:	method	collection system		washing of the biopsy sample			collection of the control samples
		type	provided by	required	buffer	provided by	
▸ **PGT-A** (24 chromosomes)	**NGS**	0.2 ml microtubes with 2.0 µl 1xPBS	**RG** (on request)	YES	1x PBS /0.5% PVP	**RG** (on request)	YES
▸ **PGT-SR+PGT-A** (24 chromosomes)							
▸ **PGT-M**	**Karyo mapping / One GenePGT**	0.2 ml microtubes with 0.8 µl CPB/ 0.1%PVP			CPB /0.1%PVP		
▸ **PGT-A** (5 chromosomes)	**FISH**	microscopic slide type SuperFrost (with frosted one end)	-	NO	-	-	NO
▸ **PGT-SR**							

4.7.2.1 Special requirements for collection of samples for the examination by the NGS / Karyomapping / OneGenePGT methods

1) Washing of the biopsy sample

Before transfer of the biopsied sample into the collection tube it is recommended to wash it at least in 3 drops of the washing buffer (1x PBS/0.5% PVP, or CPB/0,1%PVP, see Table 3). If washing is not performed (e.g. because of highly viscous biopsied sample) it must be stated in the note on the biopsy protocol

2) Collection of the control samples

The following control samples are required:

- Control sample from the last washing drop (to each embryonal sample),
- Clean sample of the cultivation medium in which the biopsy was performed (always 1 control sample per each collected set of embryos per day of RG Genetic Laboratory is not used, at least one tube with a clean sampling medium must be attached to each set of samples.

3) Request and supply of the collection systems and washing buffer

The washing buffer and microtubes with the appropriate collection medium are provided on request to the embryology laboratory performing sample collection (see Tab. 5). The provided collection tubes and buffers are visibly marked with the expiry date which must be respected by the collecting laboratory.

Example of the collected samples and controls for one patient are shown in the attached scheme:

The patient samples with the laboratory ID 14/58 intended for the examination in RG or LLRG Repromeda									
⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	…etc.	⊗
23 **58/1**	23 **58/2**	23 **58/3**	23 **58/4**	23 **58/5**	23 **58/6**	23 **58/7**	23 **58/8**		***N***
○	○	○	○	○	○	○	○	…etc.	⊗
C58-1	***C58-2***	***C58-3***	***C58-4***	***C58-5***	***C58-6***	***C58-7***	***C58-8***		***NV-58 D5***

Explanations:	
⊙ 23 **58/1**	**Collected embryonal sample:** The microtube with the collection buffer and collected embryonal sample. The tube is labelled with the laboratory ID (if available) and the number of embryo or alternatively by the patient´s name, year of her birth and the number of embryo.
○ ***C58-1***	**Check of purity of the washing buffer (C) in each collected sample:** The microtube with the collection buffer in which the sample of the washing buffer from the surrounding environment of the last washing drop of a particular sample is collected. The tube is labelled with letter „C“ in combination with the laboratory ID (if available) and the number of embryo or alternatively with the patient name, year of her birth and the number of embryo.
⊗ ***NV-58 D5***	**Check of purity of the cultivation medium (NV):** The microtube containing the collection buffer in which the sample of the clear cultivation medium where biopsy was performed is collected (without the collected embryonal sample). The tube is labelled with letter „NV“ and the laboratory ID (if available) or alternatively with the patient name and year of her birth. Day of sampling (i.e. day of embryo development) is also stated).
⊗ ***N-58 D5***	**Check of the collection buffer (N) – ONLY when collection b. provided by GR is NOT used:** The microtube containing the clear collection buffer in which neither the collected embryonal sample, buffer from the last washing drop nor cultivation medium is added. It is used to check the integrity of the sampling system - to check its purity (= to exclude contamination with extraneous DNA). The tube is labelled with letter „N“ and the laboratory ID (if available) or alternatively with the patient name and year of her birth. Day of sampling (i.e. day of embryo development) is also stated).

4) Preparation of the sampling set before sampling

The collecting laboratory will prepare the sampling set for collection of the given set of embryos on the day of collection according to the following rules:

- One collection system is always provided for one patient (set of embryos).
- The number of tubes with the collection medium in the set corresponds to the sum of the announced embryos suitable for biopsy, corresponding number of negative controls of the washing buffer and cultivation medium and 2–3 back-up tubes.
- The set of tubes is provided **in the frozen closeable cryoracks** at the maximum temperature of 0 °C (so called “on ice”) in the thermo bag equipped with frozen pads.
- Microtubes are labelled according to the rules described above. The sides of the tubes have to be **labelled horizontally**, vertical marks might be damaged by rubbing against the rack (see picture in the chapter 4.7.1)!

- During preparation of collection set the relevant anti-contamination measures must be followed (see 4.7.2), particularly the microtubes with collection medium have to be **centrifuged** before their use (i.e. before their opening and tubing of the embryonal samples or controls).

5) Correct handling with the collection system during collection

The collection system must be handled in accordance with the anti-contamination measures (see above) and with a particular emphasis on the following rules:

- During the collection it is necessary to minimize the time for which the tubes are outside the cryoracks and the cooled environment of the transport thermo bag,
- Immediately after the collection the microtubes with the collected samples must be centrifuged for at least 3 minutes.

4.7.2.2 Special requirements for collection of the samples for the FISH examination

If a delayed transport of the transport is performed (more than 60 minutes from the collection, see Section 4.8 and Table 5) the slide with the fixed sample must be treated by immersion to the solution of methanol for approximately 15 minutes at room temperature (“additional fixing“).

4.7.3 Samples of embryonal WGA DNA

The laboratory accepts for all offered PGT types only the primary embryonal samples (from the primary collection, see 4.7.2).

4.7.4 Samples for the SET-UP examination for a monogenic disease

For the preparation examinations of specified family members before PGD for a monogenic disease (SET-UP before PGT-M) collection of peripheral blood must be performed for each examined person:

- **5 ml of nonclottable peripheral blood into the tubes containing EDTA (K_2EDTA či K_3EDTA)**

  Samples of peripheral blood should be always collected by a physician or appointed healthcare staff (nurse) in the specified premises (out-patient department, collection room). Before collection of peripheral blood for the genetic examination no special preparation of the patient is needed (it does not have to be performed on an empty stomach or at a specific daily time, etc.). In the case of imminent contamination with other DNA (for example following organ transplantations or transfusions) the laboratory employees must be informed and further steps must be discussed. Integrity of the collection system must not be affected following the collection (the sample must not be aliquoted, etc.). After collection, the sample is mixed by repeating inverting of the tube and then stored in the refrigerator at temperature 2-8°C till the transport.

If a sample of peripheral blood cannot be obtained it may be exceptionally replaced with a sample from buccal mucous membrane. However, this collection is not recommended for the Karyomapping examination because the quality of the acquired DNA may not to be sufficient for the successful SET-UP examination:

- **5x buccal swabs**

  Collection of buccal swabs can be performed by the patient or healthcare staff trained according to the instructions in the Instructions for buccal swab sample collection (Appendix 1). **The collection sets may be requested from RG** (special collection tubes with the active desiccating system ensuring better the sample integrity are provided)

According to the requirements of the examining laboratory and evaluation of the respective individual case it is possible to request also another type of samples (sperm, tissue/DNA of the aborted foetus, etc.).

For further information see *VD.GP 04 Laboratory Manual GL Genetika Plzeň*.

4.7.5 Samples for SET-UP examination for a chromosomal aberration

For the set-up cytogenetic examination (SET-UP) before PGD for a chromosomal familial aberration (PGT-SR) a collection from specified family members is required for the following samples:

- **5 ml of non-clottable peripheral blood into the tubes containing heparin (Na-heparin or Li-heparin)**

  The samples of peripheral blood should be always collected by a physician or appointed healthcare staff (nurse) in the specified premises (out-patient department, collection room). Before collection of peripheral blood for the genetic examination no special preparation of the patient is needed (it does not have to be performed on an empty stomach or at a specific daily time, etc.). After collection, the sample is mixed by repeating inverting of the tube, left approx. 30 min. at room temperature and then stored in the refrigerator at temperature 2-8°C till the transport.

For further information see *VD. GP 04 Laboratory Manual* GL Genetika Plzeň.

4.8 Transport and storage of the samples

The transported samples must be handled as potentially infectious material. All samples must be transported in the closed packs (closed box for the microscopic slides, closed microtubes in the rack/cryorack with the cap or in the closeable plastic sachet, tubes of the closed collection system for peripheral blood, etc.).

Furthermore, if the samples are transported via regular mail or courier, each sample (or a set of embryonal samples) must be in the given closeable pack wrapped individually in the further plastic sachet, optimally in the double pack.

The original documentation for the samples must be stored separately from the samples so as to avoid its spoilage or staining with biological material (best by storing in the single plastic sachet).

4.8.1 Embryonal samples

Embryonal samples can be delivered to RG by:

- **Immediate delivery** (the method of transport of the embryonal samples of the primary collection from the embryology laboratory close to RG), for transport conditions see Table 4,
- **Postponed transport,** for transport and storage conditions see Table 5. For transport within the Czech Republic, the laboratory provides transport of samples by the Nextclinics collection service (upon a request of sampling IVF laboratory).

Table 4: Immediate transport of the embryonal samples of the primary collection

Collection system	**Immediate transport to RG**		
	Time	**Temperature**	**Storage of the samples during transport**
0.2ml collection microtube (PGT-A/ PGT-SR by NGS, Karyomapping/ OneGenePGT)	within **60 minutes**	below **8 °C**	Frozen cryorack* + bag with the cooling packs
Microscopic slide (PGT-A/ PGT-SR by FISH)		**RT**	Closeable box for the preparations + bag

Explanatory notes: * **Increase of temperature above approximately 4°C is indicated for guidance by the cryorack by gradual change of colour** (from green to yellow or purple to pink). This change of colour starts at RT gradually from the edges of the rack after approximately 20 minutes and a complete change of colour of the rack occurs after approximately 180 minutes. **Therefore, to ensure the required temperature during collection and transport (i.e. up to 8°C) the tubes/samples must always be placed at the site of the rack where the change of colour has not occurred** yet. A rack with complete change of colour cannot be used both for transport and for temporary storage of the samples during collection.

The cryorack with the samples can therefore be left outside the transport bag only for the period of time that is absolutely necessary in the course of sample collection!

Table 5: Postponed transport of the embryonal samples according to the collection system

Collection system	Delayed transport to RG			
	Time	**Tempera-ture**	**Storage of the samples before transport**	**Storage of the samples during transport**
EMBRYONAL SAMPLES FROM THE PRIMARY COLLECTION				
0.2ml collection microtube (PGT-A/ PGT-SR by NGS, Karyomapping/ OneGenePGT)	within **30 days**	**Storage < -15 °C** **Transport < 0 °C**	**1)** Place the set of microtubes for single patient into the rack/cryorack with the lid or into the closeable plastic sachet. **2)** Store the set in the freezer.	**1)** Place the set of microtubes for single patient (in the rack/cryorack with the cap or in closeable plastic sachet) into a bigger closeable plastic sachet. **2) Transport:** **a) transport within 48h.**: Place into the polystyrene box with abundance of freezing pads (always use cryorack!!); freezing pads and cryorack have to stay, before the transport, overnight in the freezer (< -15°C) **OR** **b) transport duration 48-96h.**: Place into the polystyrene box with at least 6kg of dry ice (cryorack is not necessary).
Microscopic slide (PGT-A/PGT-SR by FISH)	within **7 days**		**1)** Perform fixation according to 4.7.2.2. **2)** Store the slides in the closeable slide box. **3)** Store the box in the freezer.	**1)** Place the closeable box with the slides into a bigger closeable plastic sachet. **2)** Place it into the polystyrene box with the freezing pads; freezing pads have to stay, before the transport, overnight in the freezer (< -15°C).

4.8.2 Samples for the SET-UP examination for a monogenic disease

The following rules apply for correct transport and storage of the samples for the SET-UP for a monogenic disease:

- **Samples of peripheral blood for the SET-UP examination**

  Samples of peripheral blood for molecular genetic examination (collection systems K_2EDTA, K_3EDTA) can be stored in the refrigerator at temperature of 2–8°C. The sample/s must be delivered to the laboratory no later than 14 days after collection (**optimally within 7 days**). The transport is performed either at room temperature (if it takes up to approximately 12 hours) or at 2–8 °C (prolonged transport). The samples should not be frozen!

- **Buccal swabs samples**

  Properly dried samples of the buccal swabs can be stored for maximum period of 1 week at room temperature or 1 month in the refrigerator at 2–8 °C or for 1 year at a temperature below -15 °C from the collection. Transport is performed at room temperature.

For further information see *VD.GP 04 Laboratory Manual* GL Genetika Plzeň.

4.8.3 Samples for the SET-UP examination for a chromosomal aberration

The peripheral blood samples for the set-up cytogenetic examination (SET-UP) using the karyotype analysis and/or FISH (Na-heparin, Li-heparin collection systems) can be stored in the refrigerator at temperature 2–8 °C. The sample/s must be delivered to the laboratory within 96 hours after collection. The transport is performed either at room temperature (if it takes up to approximately 12 hours) or at 2–8 °C (prolonged transport).

For further information see *VD. GP 04 Laboratory Manual GL Genetika Plzeň*.

4.9 Background information on safe handling with the samples

According to the valid legislation (or the Decree of the MoH no. 306/2012 Sb. on prevention of spreading of infectious diseases and hygiene requirements on operation of the healthcare establishments and social care institutions) these principles for safety of work with biological material where prepared which must be followed by all employees of IVF centre and collaborating subjects:

- Each sample must be considered as potentially infectious,
- Avoid direct contact with potentially infectious material during sampling and use personal protective aids,
- Use disposable sterile aids for sampling, if possible,
- Dispose of the used material for sample collection safely in accordance with the principles for disposal of hazardous (infectious) waste,
- Secure the biological material during transport (see also the rules in Section 7.8) so as the following is avoided:
    - Spoilage of the samples by the external physical effects,
    - Contamination of the accompanying documentation or outer side of the tubes or other packs with biological material and threat of a person who handles the sample; this is the reason for rejection of a sample (see Section 5.2 and Tab. 7),
- If the sample is not transported immediately, it must be stored in a dedicated area.

5. Preanalytical processes in the laboratory

5.1 Receipt of the samples in RG

The samples fulfilling all requirements specified in Section 5 are accepted for processing. The sample receipt record is performed on the requisition form with the date and time and signature of the employee receiving the sample. All employees of RD are trained for receipt of the samples.

During receipt of the sample to the laboratory the following parameters are especially checked:

- Number of samples and respective controls (or consistency with data in the biopsy protocol a/or requisition form),
- Integrity of the collection system (slide, microtube with transport medium) (see Section 4.9),
- Presence and readability of the sample labelling (see Section 4.7.1),
- Presence of correctly and legibly completed mandatory accompanying documentation (see Section 4.4),
- Match of sample identification with the accompanying documentation,
- Quality and amount of the sample (see Section 4.7.2 - 4.7.5),
- Compliance with the conditions for correct transport of the samples (see Section 4.8).

5.2 Solving of non-conforming situations during sample receipt and criteria for rejection of the sample

The samples which do not comply with all the requirements for proper receipt to the laboratory are classified into three groups based on the severity of the situation and possibility of its solving - see Table 6.

Table 6: Method of solving the non-conforming situations during receipt of the samples

Non-conforming situation	Solving of the situation
• Completely missing sample/samples • Unidentified sample (unidentified or illegibly/incorrectly labelled sample) • The samples transported to the laboratory cannot be paired with the original documentation • The used collection system precludes processing of the sample via the required method • The collection system is damaged • The collection system or accompanying documentation is stained with biological material/sample	**The sample is ALWAYS REFUSED.** RG employee must immediately inform the applicant about the situation and ask for repeated collection. The original documentation is archived, the sample is destroyed. Note: Stained documentation is photographed and the original is destroyed.
• Collection of unsuitable biological material • Using of unsuitable collection system • Insufficient amount of the sample for the required examination • Non-compliance with the time interval for sample delivery to the laboratory • Non-compliance with required temperature / method of transport to the laboratory • Non-compliance with the conditions of previous storage of the sample (time or method of previous storage)	**The sample is RECEIVED and, if possible, further processed**. If it is suitable/necessary the applicant should be informed in the case of receipt of a non-conforming sample. The non-conforming situation during receipt of the sample and its possible impact on the result are, if suitable/necessary, stated in the final report (if the examination was not completely cancelled after agreement with the referring clinician).

Non-conforming situation	Solving of the situation
• Missing requisition form • Incomplete data on the requisition form • Missing informed consent (IC) • Incomplete data in the IC • Missing biopsy protocol (BP) for PGT or a record on sampling on the requisition • Incomplete data in the biopsy protocol or in the record on sampling • Other missing documentation, if required	**The sample is ACCEPTED and its processing is initiated** **but the final report is released after addition of the missing documents/data.** RG employee must immediately inform the applicant about the situation and ask for providing the missing data.

6. Providing of the results and communication with the laboratory

6.1 General principles

Results (final reports, result sheets) can be provided and handed over only to the requesting physician (applicant) or other healthcare personnel who is taking part in the care of the respective patient.

Law enforcement authorities (police, courts) are provided with the results only on the basis of a written request that is submitted by a statutory representative as an order to the head of laboratory. In this case the results or findings are provided in the written form via a statutory representative.

The results of each laboratory examination are precise, clear and unambiguous. The result sheets contain all data required by the standard ČSN EN ISO 15189:2013 Medical laboratories - Requirements for quality and qualification and they also respect the relevant international recommendations of the ECA. The final report always includes either direct interpretation of the results or at least the information needed for their correct interpretation. Recent literature sources should be used for interpretation of the results.

The final reports are prepared in the text editor in the Czech or English language, protected against editions and issued after review by authorized responsible persons.

The results are released electronically by uploading them to the internal Medicalc information system. For external applicants, the results are then either placed in the DynaMed information system (IVF centers Prof. Zech, ProCrea Swiss IVF Center s.r.o.) or sent via secure MediDAT transmission. The requesting physician is always immediately notified of the result release (DynaMed users at ProCrea Swiss IVF Center in Pilsen via the internal ToDo application, other requesting physicians via email).

6.2 Intervals from receipt of the sample to providing of the result

Standard time of delivery of the final report (turnaround time, TAT) are provided in each offered examination in Section 3.3. Delivery time of the final report is a time period from receipt of the sample or request for examination (in additional examinations) in the laboratory to the release of the examination result. The laboratory undertakes to comply with the TAT for 90 % of the results. The remaining 10 % is reserved for the situations when more demanding processing of the sample and/or repeating of the analysis is required.

The samples are processed in the order in which they are received by the laboratory with the exception of a preferred processing of the samples in the STATIM mode.

In PGT examination no critical intervals are stated.

6.3 Changes of the results and findings

In case of need of change of already published final report the indicating physician is always immediately informed and the new corrected final report clearly marked as revised is issued.

The revised report always contains reference to the original report (date of the original report and patient identity) and date and time of its release. The revised report is authorized by the head of the laboratory or his/her deputy.

In such case, only the head of the laboratory or his/her deputy communicates with the indicating physician.

6.4 Information about the results via phone

The laboratory (RG) does not provide the results by phone.

6.5 Consultation activities of the laboratory

Interpretation of the results is a part of the text of the result sheet. Further consultation in relation to the examination results is provided only by a physician or an employee with university degree with a specialized qualification.

The laboratory continually informs all applicants on all significant changes related to the offered laboratory services.

6.6 Ensuring the needs for collection of biological material

The laboratory ensures preparation of the collection system for examination using the NGS and Karyomapping method (see also Section 4.7.2.).

RG provides the collection sets for collection of samples of buccal swabs including the instructions for collection of buccal swabs (Appendix 1 - Instructions for buccal swab sample collection).

6.7 Complaint handling

To possible complaints from the applicants or patients is paid special attention, they are always thoroughly investigated and in the case of legitimate complaints they should trigger improvement of the laboratory services.

A complaint can be filed by means of a written form (standard mail, fax, email) or verbally (by phone, personally). Small complaints are solved immediately by the employee who received the complaint and then he/she informs the laboratory management. Other complaints are handled by the laboratory management at least within 30 working days.

6.8 Personal data protection

The Genetic Laboratory has established the Ethics Code that is mandatory for all employees. Each employee of the Genetic Laboratory is obliged to protect information about patients regardless of how this information has been acquired, collected, and stored. This information is considered as confidential and the employees are aware of their duties to maintain confidentiality regarding the facts they got to know in relation to their profession.

Personal data are processed in accordance with the GDPR and Act No. 110/2019. Specific processing policies are available on the website.

7. Related documentation

7.1 Internal documentation

VD.GP 04 Laboratory Manual GL Genetika Plzeň

F.GP 901 Consent of the examined person (legal representative) with genetic laboratory examination (CZ, EN, DE)

F.GP 906 Requisition for PGT (CZ, EN)

F.GP 907 Instructions and informed consent with PGT (CZ, EN, DE, IT)

F.GP 908 Informed consent to storage of samples (and to results management) after PGT (CZ, EN, DE, IT)

F.GP 909 Biopsy protocol for PGT (CZ, EN)

7.2 External documentation

Laws, ISO standards, guidelines

- *ČSN EN ISO 15189:2013 Medical laboratories - Requirements for quality and competence*
- *Act on: 373/2011 Sb. on Specific health services, as amended*
- *Decree no. 99/2012 Coll. on minimum requirements for the staffing of health services.*
- *Decree no. 306/2012 Coll. specifying conditions for prevention of origin and transmission of infectious diseases and hygienic requirements on management of HCF and social care*
- *Vyhláška č. 92/2012 Sb. o věcném a technickém vybavení zdravotnických zařízení*
- *Unsurpassable specialty limits 816 – Laboratory of medical genetics (revised and approved by the SLGG committee on 21. 10. 2018)*
- *General Guidelines and Quality Assurance for Cytogenetics. ECA Newsletter 2012;29:7-25.*
- *Specific Constitutional Cytogenetic Guidelines. ECA Newsletter 2012;30:11-19.*
- *European guidelines for constitutional cytogenomic analysis. Eur J Hum Genet. 2019 Jan;27(1):1-16*
- *ESHRE PGD consortium best practice guidelines for fluorescence in situ hybridization-based PGD. Human Reproduction, Vol.26, No.1 pp. 25–32, 2011*
- *ESHRE PGD consortium best practice guidelines for amplification-based PGD. Human Reproduction, Vol.26, No.1 pp. 33–40, 2011*
- *ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. Human Reproduction, Vol.26, No.1 pp. 14–24, 2011*
- *ESHRE PGD Consortium/Embryology Special Interest Group best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Human Reproduction, Vol.26, No.1 pp. 41–46, 2011*
- *PGDIS Newsletter-PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage, http://www.pgdis.org/docs/newsletter_071816.html*
- *PGDIS Newsletter, May 27, 2019: PGDIS position statement on on the Transfer of Mosaic Embryos in PGT-A*
- *Report of PGDIS Expert Cosultation on Mosaic Embryo Transfer, August 19, 2021: PGDIS position statement on the Transfer of Mosaic Embryos 2021*
- *COGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies, http://www.ivf-worldwide.com/cogen/oep/publications/cogen-position-statement-on-chromosomal-mosaicism-detected-in-preimplantation-blastocyst-biopsies.html*

LLRG Repromeda

- *Laboratory Manual LLRG Repromeda*
- *REPROMEDA - Instruction and Informed Consent for Couples for Pre-Implantation Genetic Testing for Monogenic Diseases (PGT-M) (CZ, EN, DE)*
- *REPROMEDA - Instructions and informed consent of the examined patient (legal representative) with genetic laboratory examination (SET-UP) (CZ, EN, DE)*

7.3 Appendix

Appendix 1: Instructions for buccal swab sample collection

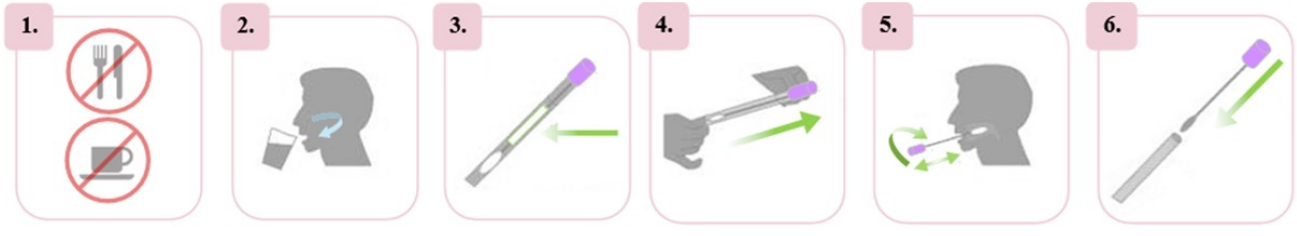
Appendix 1: Instruction for buccal swab sample collection using kit supplied by Genetic Laboratory

In order to obtain successful results of the genetic analysis, it is necessary to perform thorough sample collection of the buccal cells using sterile buccal swab collection kit. The sampling procedure described below is crucial for obtaining sufficient DNA quantity, therefore please read the manual carefully just before sampling initiation:

1. **Subject being swabbed (examined client/patient) is not allowed to smoke, eat and drink except still unsweetened water, chew chewing gum or make any type of oral hygiene at least 60 minutes before sampling. Subject's mouth must not be contaminated with any biological material of another person (avoid kissing, breast-feeding etc.) at least 60 minutes before sampling. Before handling the sampling kit, wash (or disinfect) and dry your hands thoroughly. Sampling kit for 1 person usually contains 5 buccal swabs.**
2. If possible, make subject rinse her/his mouth out with still unsweetened water just before the sampling procedure.
3. Take the first flocked swab out of the original package **and label the receptacle (tube) legibly with the subject's name, surname, date of birth** and **the time** and **date of sample collection**. For sample labelling always use permanent marker.
4. Remove the swab from the tube carefully (always hold only the unsterile part of the swab - purple or white cap). **Avoid touching sterile part of swab (stick and tip of a swab) any other material than subject's oral cavity during the sampling!**
5. Place the actual swab tip to subject's oral cavity (between inner surface of a cheek and gums) and gently rub and rotate swab along the inside of the cheek (similar movements like during tooth brushing), ensuring that the entire swab-tip has made contact with the cheek. It is recommended to exert adequate pressure on the mucosal surface to separate sufficient number of buccal cells. **Try to do swabbing both cheeks with one tip for at least 1 minute**. If excessive amount of saliva forms in the mouth during sampling, it is recommended to discontinue swabbing and resume after swallowing.
6. After sample collection, place the swab directly in the receptacle (original tube) and close it tightly. **Every single supplied buccal swab contains a desiccant located in the cap which absorbs moisture to promote timely and proper drying of the swab. Therefore, no further manipulation with the swab is required!** Place all buccal swabs collected from one subject into one zipper plastic bag.

Follow these instructions for the collection of all samples

(always use all buccal swabs in one sampling kit per 1 person).



The buccal swab samples can be stored **in dark place at room temperature up to 1 week after collection**, or **in the fridge (2-8 °C) up to 1 month after collection**, or **in the freezer (below -15 °C) up to 1 year after collection**.

Room temperature is suitable for sample transport.