



Univerzita Komenského v Bratislave
Lekárska fakulta
Comenius University In Bratislava
Faculty of Medicine

Sasinkova 4
811 08 Bratislava

Ústav lekárskej biológie, genetiky a
klinickej genetiky
Institute of Medical Biology, Genetics
and Clinical Genetics

Diagnostic Algorithm for Genetic Analyses of Reproductive Losses

Project: GIFD — Genetical Spectrum of Intrauterine Fetal Death Causes in Slovakia

Ethical Committee of the University Hospital Bratislava: Approval EK 1/92003

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Purpose and Scope

1.1 Purpose

This document defines the stepwise diagnostic algorithm used to select, sequence and interpret genetic analyses performed on samples from reproductive losses and their parents. It is issued in two layers. Part I records the testing pathway actually applied within the GIFD project between M1 and M24, translating the laboratory optimisation of WP2 (BIOLMAT) and the analytical experience of WP3 (BIOLEXP) into a documented pathway. Part II sets out the revised algorithm recommended for routine prospective use, derived from the register data reported in D15. Where the two differ, Part II supersedes Part I for prospective use.

1.2 Scope

Applies to fetal, placental and umbilical cord or blood tissue and to parental blood samples collected under the GIFD Sampling and Storage Guideline (D04), for reproductive losses from 13+0 completed gestational weeks onwards. Within this scope the algorithm distinguishes two groups:

- Group A — reproductive loss between 13+0 and 19+6 gestational weeks.
- Group B — intrauterine fetal death from 20+0 gestational weeks onwards.

The 20+0 boundary is used as a decision point governing the order in which methods are applied, and not only as the definitional threshold of intrauterine fetal death. Group membership is determined by gestational age alone, because the type of pregnancy termination is frequently not known to the laboratory at the time the analysis is indicated. Losses below 13+0 weeks fall outside this document: numerical chromosomal abnormalities dominate that period and QF-PCR retains a high yield there.





1.3 Intended users

Laboratory staff and clinical geneticists at the coordinating institute performing the analyses; pathologists at the Institute of Pathological Anatomy for the histology-correlation step; referring obstetricians and gynaecologists for sample submission and result interpretation. Step B4 is indicated and interpreted by the clinical geneticist together with the obstetrician and, where relevant, the haematologist.

2. Background and Rationale

Chromosomal abnormalities dominate reproductive losses in the first trimester, but the spectrum of genetic causes shifts in later gestation to include aneuploidies, microdeletion syndromes and monogenic disorders. After the 20th week an abnormal karyotype has been reported in up to 20 % of stillborn fetuses with anatomical abnormalities or growth restriction and in around 5 % of fetuses without anatomical abnormalities, with trisomies of chromosomes 13, 18 and 21 and X monosomy the most frequent findings (Korteweg et al., 2008).

In the GIFD register no chromosomal aberration was detected above 20+0 weeks (0 of 43 evaluable analyses; 95 % CI 0–8.2 %), whereas every such aberration occurred below that boundary (3 of 24; Fisher's exact test, $p = 0.042$). The upper confidence limit does not exclude the approximately 5 % reported in the literature, so the result is stated throughout as not detected in this cohort and never as does not occur. Its consequence is one of order and priority of methods, not of omitting a method.

Because product-of-conception samples frequently fail in cell culture, conventional karyotyping is largely replaced in this setting by molecular methods, as QF-PCR, MLPA, array CGH and sequencing, that do not require viable cells and remain interpretable on suboptimal or macerated material. The relative yield of these methods, and the order in which they should be applied, depends on local screening practice and case mix, which is why the GIFD project generates a Slovakia-specific algorithm rather than importing one from another population. A substantial





proportion of reproductive losses remains unexplained even after autopsy; the purpose of this algorithm is to make the best use of a limited biobank and testing budget by combining structured clinical documentation with a staged laboratory workflow.

3. Target Population, Samples and Prerequisites

3.1 Case selection

Reproductive losses from 13+0 gestational weeks onwards. Excluded from etiological denominators are terminations of pregnancy for fetal anomaly, postnatal deaths and losses below 13+0 weeks; a termination for anomaly is indicated precisely because a finding has already been established, and a postnatal death is not an intrauterine loss. Excluded categories are examined by the same laboratory pathway but are reported under a separate denominator. In multiple pregnancy each fetus is sampled and analysed separately, zygosity is recorded, and the case is reported as one case with two fetuses; every table states whether its denominator is cases or fetuses.

3.2 Preferred sample material

Musculus quadriceps femoris (preferred, when available); placental tissue or chorionic villi, always labelled as placental material and never as fetal tissue; umbilical cord segment or umbilical blood; maternal and paternal blood for trio analysis and haplotype-based causality assessment.

3.3 Prerequisites

- Signed informed consent (PIF);
 - sample received and coded per the biobank procedure (D03);
 - sample processed per the Sampling and Storage Guideline (D04), including quality triage of degraded or macerated material.
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4. Step-by-Step Proposed Diagnostic Algorithm (Figure 1)

Step 0 - Sampling and documentation (common to both groups before or after 20. gestational week)

- Preferred material: fetal skeletal muscle (musculus quadriceps femoris), carries the lowest risk of maternal DNA contamination and yields the best nucleic acid quality in a macerated fetus.
- Additional material: umbilical cord segment, placental tissue section (always labelled as placental material), cord blood where available.
- Parental blood is drawn at the index event, concurrently with fetal sampling. Later collection is logistically demanding and is in practice rarely achieved; without parental samples the origin of submicroscopic findings cannot be assessed and a proportion of variants will remain classified as variants of uncertain significance.
- Record the source of gestational age dating (ultrasound/CRL, last menstrual period, autopsy biometry) and report every available value separately. The difference between clinical dating and autopsy biometry indicates fetal retention and must not be lost by merging the values.
- Complete the structured reproductive history field P / Ab / TOP / EP together with a separate field for previous intrauterine fetal death from 20+0 weeks. A previous loss from 20+0 weeks is never recorded as spontaneous abortion. Gravidity is counted including the index pregnancy; parity is the number of previous deliveries from 22+0 weeks.

Step 1 - Clinical risk-factor documentation (parallel modifier)

Five anamnestic risk factors are recorded in every case: a positive result on first- or second-trimester prenatal screening; maternal age of 40 years or over; a previous pregnancy affected by aneuploidy; hydrops fetalis; and a congenital anomaly detected on prenatal ultrasound. This layer records and prioritises; it does not gate access to any method.





- Group A: a risk-factor-positive case proceeds from step A1 to step A2 automatically, without a further indication being required.
- Group B: risk-factor status does not change the order of methods, since array CGH is the first diagnostic method in every case. Hydrops fetalis and a prenatally detected congenital anomaly move the case preferentially to step B3.

Limitation. This layer derives from a single-centre cohort of 55 cases, in which 21 cases (38 %) presented at least one factor and the yield in the risk-factor-positive subgroup was 14.3 % (3/21; 95 % CI 3.0–36.3 %), against 0 % among risk-factor-negative cases. That cohort is a subset of the GIFD register and its three positive cases are the same three chromosomal findings that underlie the difference between the groups, so the two observations are not independent. The confidence interval is wide and the layer has not been validated prospectively. It is therefore secondary to gestational age and must not be used to withhold testing.

Group A — reproductive loss between 13+0 and 19+6 gestational weeks

Step	Method	Evidence from the GIFD register	If negative
A1	QF-PCR — chromosomes 13, 15, 16, 18, 21, 22, X, Y and triploidy (Devyser Extend v2, capillary electrophoresis on ABI 3130xl, GeneMapper)	Yield 3/24 (12.5 %); all chromosomal aberrations of the register fall in this group	Proceed to A2
A2	Array CGH (chromosomal microarray; Agilent SurePrint G3 8×60K or an equivalent SNP array)	Yield 1/5 performed; detects submicroscopic changes beyond the reach of QF-PCR	Proceed to A3 where the phenotype is suggestive
A3	Targeted panel or exome sequencing according to phenotype, preferably as a trio	Applied once in the register; literature adds approximately 8.5 % after a normal microarray	Proceed to case closure (Section 9)





Group B — intrauterine fetal death from 20+0 gestational weeks

Step	Method	Evidence from the GIFD register	If negative
B1	QF-PCR — as a sample identity check, exclusion of maternal DNA contamination and determination of the gonosomal complement; not as a diagnostic step	Yield 0/43. Retains value as a rapid, inexpensive control of the preanalytical phase: the register XY : XX ratio was 34 : 32, i.e. 1.06 : 1, excluding systematic maternal contamination, which would shift the ratio toward XX	May be skipped; proceed to B2 without waiting
B2	Array CGH — indicated as the first diagnostic method, concurrently with B1 or immediately after it	Both findings of this group came from array CGH; yield 2/11 performed (18.2 %). Under universal application the detection rate is expected to be higher than the register shows	Proceed to B3
B3	Exome sequencing or a targeted panel according to phenotype, preferably as a trio. Panel focus: cardiac electrophysiology including long QT syndrome, neuromuscular disorders and fetal akinesia, metabolic disorders, disorders of early developmental pathways and of placental function	Not deployed in the register — an identified gap. Literature adds approximately 8.5 % after a normal microarray	Proceed to B4
B4	Investigation of maternal mechanisms: obstetric antiphospholipid syndrome, and inherited thrombophilias where the history indicates	A clinically actionable cause of placental failure and late fetal death, and the only category with a direct preventive effect on a subsequent pregnancy	Proceed to case closure (Section 9)

Case closure

No case is closed as unexplained without fetal and parental DNA being retained in the biobank. Variants of uncertain significance are re-evaluated at an interval of three to five years, because databases of genes essential for early development are systematically under-populated and





reclassification is to be expected in this group of disorders. The family is offered re-evaluation, and the laboratory report states that DNA has been stored.

5. Causality Categorisation

Once genetic testing is complete each case is assigned to one of three categories: (a) a genetic change identified that is a direct cause of the reproductive loss; (b) a genetic change identified that is not considered a direct cause, assessed against parental haplotypes and inheritance pattern; (c) no genetic change identified.

The assignment is made in two stages. First, every sequence variant or copy-number finding is classified for pathogenicity using the ACMG/AMP framework for sequence variants (Richards et al., 2015) and, for MLPA- and array CGH-derived findings, the ACMG/ClinGen technical standards for constitutional copy-number variants (Riggs et al., 2020), against the standard evidence criteria of population frequency, functional data, gene or region dosage sensitivity, segregation with parental samples and de novo status. Findings classified as benign or likely benign are non-contributory and the case defaults toward category (c) if no other finding is present. Variants of uncertain significance are tracked separately, are not automatically assigned to category (b), and remain pending reclassification.

Second, for findings classified as pathogenic or likely pathogenic, case-level causal attribution follows the probable-cause and possible-cause logic of the INCODE framework (Dudley et al., 2010). A finding is assigned to category (a) where it meets an INCODE-equivalent probable-cause threshold, judged against gestational timing, phenotype and autopsy findings, placental findings and the known natural history of the condition. A pathogenic or likely pathogenic finding that does not meet this threshold – for example through incomplete penetrance, a phenotype inconsistent with the observed case, or a competing placental or obstetric explanation — is assigned to category (b), analogous to the INCODE possible-cause tier.





6. Reporting Standards

1. Explicit denominator. Every reported diagnostic yield states its explicit denominator and the type of pregnancy loss included. Intrauterine fetal deaths, spontaneous second-trimester losses, terminations for fetal anomaly and postnatal deaths must not share a denominator. Where cases and fetuses differ in number, state which of the two is the denominator.
2. Placental material. Findings obtained from chorionic villi or placental tissue are never reported as the fetal genotype without confirmation in fetal tissue. Confined placental mosaicism is reported separately and excluded from denominators of fetal findings. This is the most frequent single source of overstated yield in reproductive loss.
3. Reporting a negative result. An unexplained loss is not the exclusion of a genetic cause; it is the exclusion of those causes detectable by the methods applied. This wording forms part of both the laboratory report and the counselling interview, together with the information that DNA has been stored and that re-evaluation will be possible as diagnostic options expand.
4. Variants of uncertain significance are reported as such, tracked separately and re-evaluated at three to five years.
5. Limits of interpretation. Where parental samples are unavailable, the resulting interpretative limitation is stated explicitly in the laboratory report.



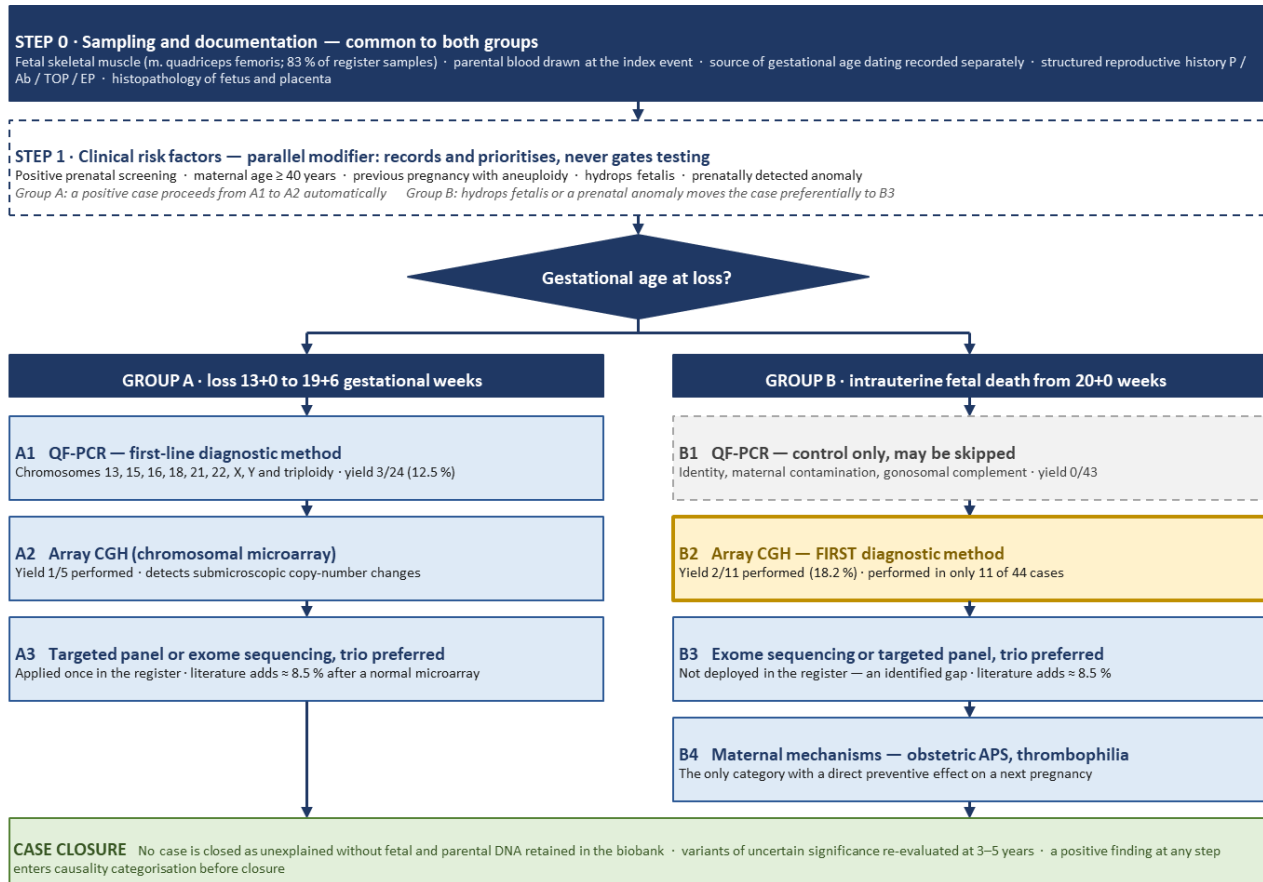


Figure 1 GIFD Diagnostic Algorithm – Genetic Analysis of Reproductive Losses





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References

Dudley DJ, Goldenberg R, Conway D, Silver RM, Saade GR, Varner MW, Pinar H, Coustan D, Bukowski R, Stoll B, Koch MA, Parker CB, Reddy UM; Stillbirth Collaborative Research Network. A new system for determining the causes of stillbirth. *Obstet Gynecol.* 2010;116(2 Pt 1):254–260.

GIFD project. Deliverable 15 — Final Report, WP4, M24. Comenius University Bratislava, Faculty of Medicine, 2026. Data cut-off 25 August 2026. Source of all register figures cited in this document.

Korteweg FJ, Bouman K, Erwich JJ, Timmer A, Veeger NJ, Ravisé JM, Nijman TH, Holm JP. Cytogenetic analysis after evaluation of 750 fetal deaths: proposal for diagnostic workup. *Obstet Gynecol.* 2008;111(4):865–874.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015;17(5):405–424.

Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, Pineda-Alvarez D, Aradhya S, Martin CL; ACMG. Technical standards for the interpretation and reporting of constitutional copy-number variants. *Genet Med.* 2020;22(2):245–257.

