Institute of Hematology and Blood Transfusion
NRL for DNA Diagnostics
Department of cell chimerism
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The Final Report of the External Proficiency Testing (EPT) Program in Quantitative Analysis of Cell Chimerism for the Year 2025

Program Variants:

1. **Basic** – includes recipient DNA, donor DNA, and 5 quantification samples
2. **Extended** – includes recipient DNA, donor DNA, and 10 quantification samples

Material:

DNA samples were isolated from buffy coats in accordance with the standard operating procedure (NRL_01_SOP_14_01, Addendum 1).
Recipient – X270
Donor – X260

Regular Round:

1_2025 – X260/X270, expected recipient genotype: 0%
2_2025 – X260/X270, expected recipient genotype: 4%
3_2025 – X260/X270, expected recipient genotype: 5%
4_2025 – X260/X270, expected recipient genotype: 84%
5_2025 – X260/X270, expected recipient genotype: 83%
6_2025 – X260/X270, expected recipient genotype: 39%
7_2025 – X260/X270, expected recipient genotype: 27%
8_2025 – X260/X270, expected recipient genotype: 1%
9_2025 – X260/X270, expected recipient genotype: 2%
10_2025 – X260/X270, expected recipient genotype: 2%

An additional round was not organized in 2025.

EPT Assignment:

Quantitative examination of cell chimerism:

Basic variant - 5 samples
Extended variant - 10 samples

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Participating Laboratories – Regular Round:

Domestic Participants:

Institute of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Czech Republic

Department of Hematology and Oncology, University Hospital Pilsen, Czech Republic

Laboratory of Molecular Biology, Department of Hematology and Oncology, University Hospital Olomouc, Czech Republic

Center for Molecular Biology and Genetics, Department of Hemato-Oncology, University Hospital Brno, Czech Republic

DNA Diagnostics Laboratory, Department of Medical Genetics, Institute of Clinical and Molecular Pathology and Laboratory Genetics, University Hospital Ostrava, Czech Republic

Foreign participants:

NZOZ Medigen Diagnostyka Molekularna, Wieliszew, Poland

Laboratory of Molecular Biology, Department of Hematooncology Diagnostics, Lower Silesian Oncology Center, Wrocław, Poland

Laboratory of Hematology and Oncology Diagnostics, Department of Clinical Immunology, University Children´s Hospital, Cracow, Poland

Laboratory of Immunogenetics, University Center of Laboratory Medicine, University Clinical Center of the Medical University of Warsaw, Warsaw, Poland

Laboratory of Molecular Genetics, Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Budapest, Hungary

Bone Marrow Transplant Unit Laboratory, Aghia Sophia Children´s Hospital, Athens, Greece

Tissue Typing & Immunology Laboratory, Gayrettepe Florence Nightingale Hospital, Istanbul, Turkey

Tissue Typing Laboratory, Faculty of Medicine, Istanbul University, Turkey

Department for Transfusion Medicine and Cellular Therapy, AKH, HLA Laboratory, Medical University Vienna, Austria

Medirex a.s., Bratislava, Slovakia

A total of 15 laboratories participated (designated A to O): five in the basic variant, ten in the extended variant, plus the organizer.

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Results:

Statistical evaluation of the results was performed using median values and standard deviation. The standard deviation was determined based on statistical analysis of results from previous years of EPT (including value variance and regression), and recalculated into a Z-score value (the closer the value is to zero, the more accurate the result is). An overview is provided in the table ***Comparison of All Participants in EPT 2025***, which is part of the appendix.

Category Rating:

- **Excellent** ($[z] \leq 1$)
- **Good** ($1 < [z] \leq 2$)
- **Acceptable** ($2 < [z] \leq 3$)

correct results

- **Under the detection limit of the laboratory** (the sensitivity of the participant's method is insufficient to detect the minor genotype. Example: The expected value of the minor genotype is 0.2%, but the participant's method has a sensitivity of 1%. In this case, only the majority genotype is detected, and the result is considered correct. However, if the participant detects both genotypes and quantifies them, the result is evaluated using the Z score.
- **critical** ($[z] > 3$) – **incorrect result**

To achieve a successful performance in the EPT, a minimum success rate of 80% is required (i.e., 8 out of 10 samples in the extended variant, or 4 out of 5 samples in the basic variant).

In the regular round, 82.8% of the results fell into the Excellent category, 7.6% into the Good category, 3.4% into the Acceptable category, 0.7% into the Critical category, and 5.5% into the category Under the Detection Limit of the Laboratory.

All participants met the criteria for successful participation (achieving a minimum success rate of 80%). One participant achieved a success rate of 90%, while the remaining participants achieved 100%.

The results of individual participants were also evaluated based on percentiles. The percentile chart illustrates the performance of all participants in relation to each other. Given the availability of two variants (basic and extended), the participant in the basic variant received only one chart, while the participant in the extended variant received two charts. The first chart compares the participants' performance in the analysis of the first five quantification samples (1_2025 to 5_2025), while the second chart compares the performance of laboratories participating in the extended EPT variant, those analyzing all ten quantification samples (1_2025 to 10_2025).

The lower the participant's percentile, the more successful they are in comparison with other laboratories. The results are presented in the charts ***EPT 2025 – Basic variant*** and ***EPT 2025 – Extended variant***.

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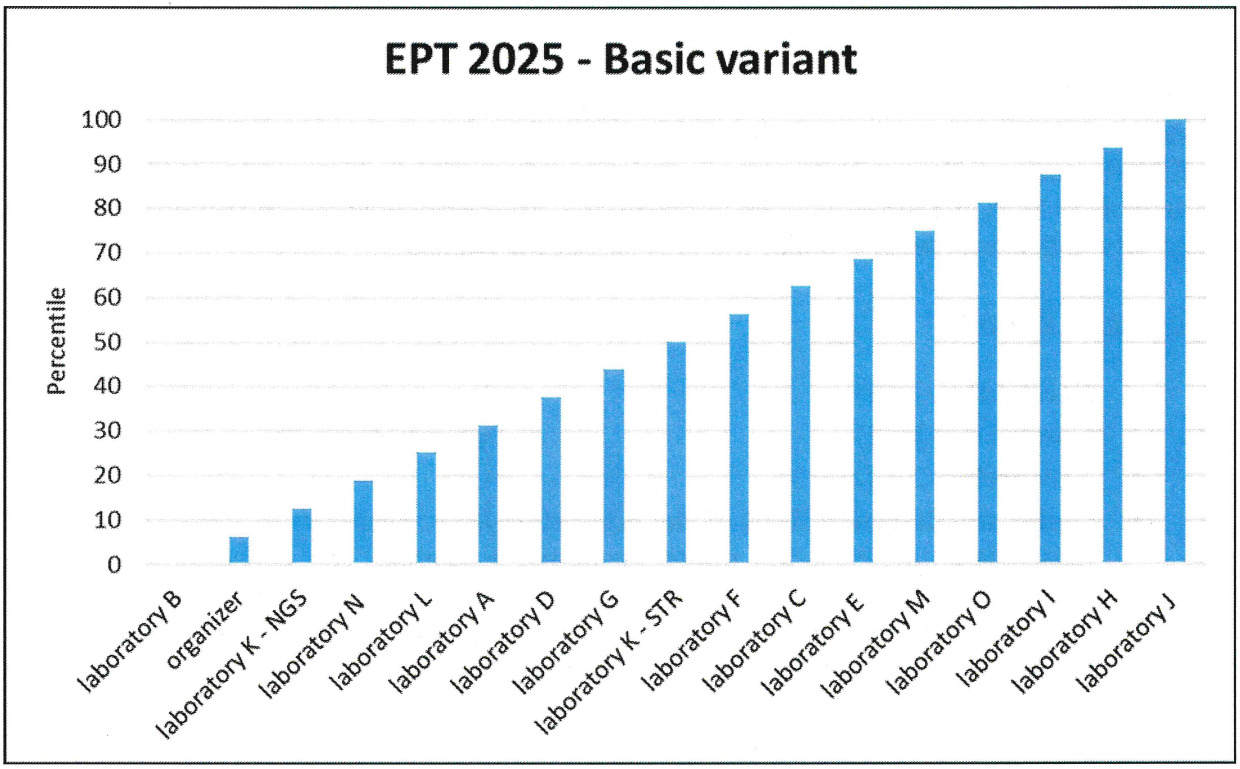

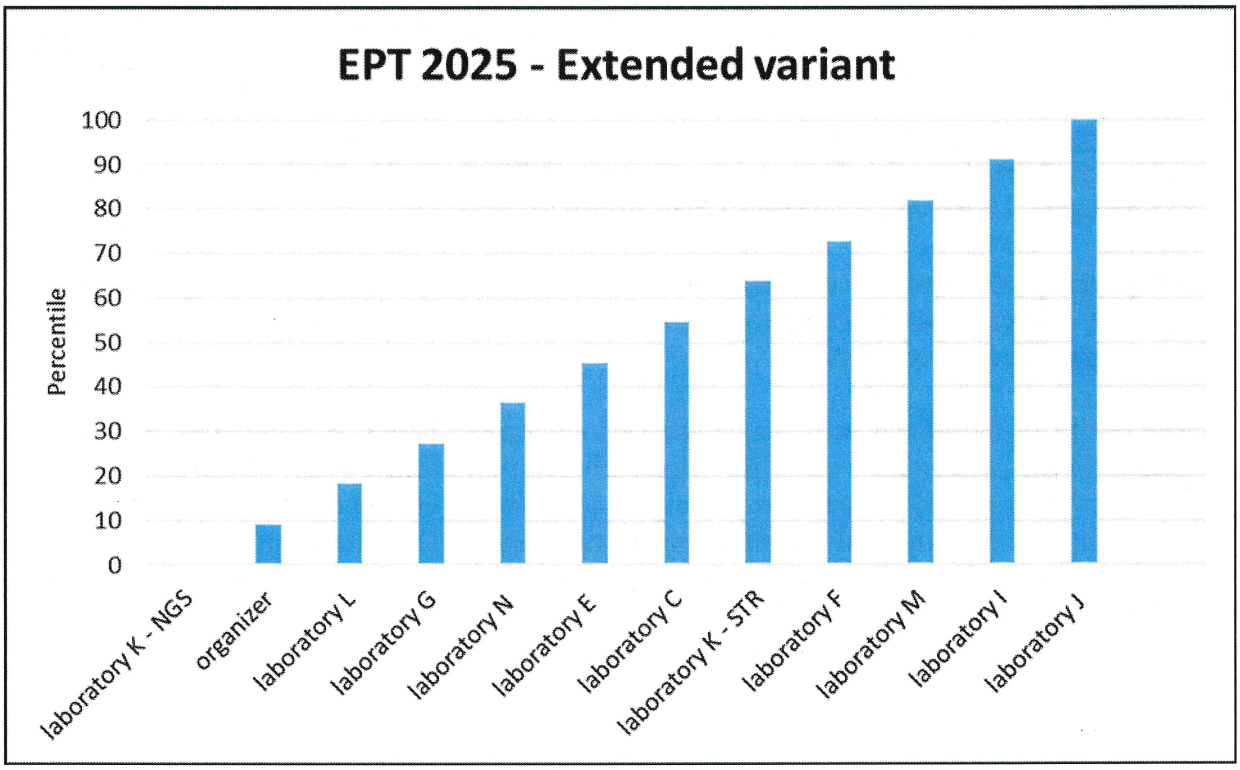

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Reproducibility Testing:

This year's round once again provided participants the opportunity to test the reproducibility of their methods. In the extended variant, samples 9_2025 and 10_2025 were prepared as two aliquots from a single original sample. The results of all participants in the extended EPT variant are summarized in the table titled ***Summary of Reproducibility of the Kits Used***. The testing yielded very positive results: most laboratories reported a difference of 1% or less between the two identical samples. The maximum reported difference in the percentage representation of the recipient genotype in the identical sample was 2%. Two laboratories did not detect the minor genotype at all due to the 5% detection limit of their methods.

Summary of Reproducibility of the Kits Used

Percentage difference in the result between samples 9_2025 and 10_2025	Number of laboratories	Used kits
0	4	in-house STR, in-house qPCR; Mentype Chimera; PowerPlex 16HS; NGStrack
< 0.2	2	KMRtrack (2x)
< 0.5	1	in-house STR, VNTR
< 1	1	in-house STR
< 2	1	AmpFLSTR Identifiler Plus
2	1	in-house VNTR
Under the detection limit of the laboratory	2	AmpFLSTR Identifiler; Qiagen InvestigatorID Plex Plus Kit

An overview of the methods used by participating laboratories for quantitative analysis of cell chimerism, including their sensitivity, is provided in the table titled ***Summary of Methods Used by All Participants – Quantitative Analysis of Cell Chimerism, 2025***. A list of commercial kits used in the participating laboratories is presented in the table ***Overview of Utilized Kits in 2025***. Both overviews are included in the appendices of this report.

Conclusion:

This year's round of External Proficiency Testing in the field of quantitative analysis of cell chimerism was successfully completed. All participants received a certificate confirming their successful participation.

Processed by: Mgr. Lucie Stefflová

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