Institute of Hematology and Blood Transfusion
NRL for DNA Diagnostics
Department of cell chimerism
Head of the department: Mgr. Lucie Stefflová
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The final report for the External proficiency testing (EPT) program in the area of quantitative analysis of cell chimerism for the year 2024

Variants:

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

Material:

DNAs were isolated from the buffy coats according to the standard operating procedure (SOP 01, addendum 1).
recipient – X253
donor – X265

The regular round:

1_2024 – X253/X265 at the expected 12 % of the recipient genotype
2_2024 – X253/X265 at the expected 0 % of the recipient genotype
3_2024 – X253/X265 at the expected 30 % of the recipient genotype
4_2024 – X253/X265 at the expected 30 % of the recipient genotype
5_2024 – X253/X265 at the expected 95 % of the recipient genotype
6_2024 – X253/X265 at the expected 100 % of the recipient genotype
7_2024 – X253/X265 at the expected 25 % of the recipient genotype
8_2024 – X253/X265 at the expected 3 % of the recipient genotype
9_2024 – X253/X265 at the expected 0,5 % of the recipient genotype
10_2024 – X253/X265 at the expected 0 % of the recipient genotype

The additional round in 2024 was not organized.

Aims of the regular round:

1. Informativity examination (genotyping of reference alleles) based on examination of the recipient´s and donor´s DNA – two samples – **optional part**.

2. Quantitative examination of chimerism status - 5 samples in the basic variant (10 samples in the extended variant) based on the chosen DNA polymorphisms and/or sex-specific loci including interpretation (recipient/donor ratio) – **compulsory part**.

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Participating laboratories – the regular round:

Domestic participants:
Department of Clinical Biochemistry and Diagnostics, University Hospital, Hradec Králové, Czech Republic
Laboratory of Molecular Genetics, Department of Hematology and Oncology, University Hospital Pilsen, Czech Republic
Laboratory of Molecular biology, Hemato-oncologic clinic, University Hospital Olomouc, Czech Republic
Center for Molecular Biology and Genetics, Internal Hematology and Oncology Clinic, The University of Hospital Brno, Czech Republic
Department of medical genetics, Institute of Clinical and Molecular Pathology, University Hospital Ostrava

Foreign participants:

NZOZ Medigen Diagnostyka Molekularna, Wieliszew, Poland

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

Department of Clinical Immunology, Diagnostic Laboratory of the Department of Immunology, University Children´s Hospital of Cracow, Cracow, Poland

Laboratory of Immunogenetics, Department of Hematology, Transplantation and Internal 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 Laboratory, Gayrettepe Florence Nightingale Hospital, Istanbul, Turkey
SBT laboratory, İstanbul Tıp Fakültesi Temel Bilimler Binası, Tıbbi Biyoloji Anabilim Dalı Doku tipleme laboratuvarı, Istanbul, Turkey

Department for Blood Group Serology &Transfusionsmedicine, Medical University Vienna, General Hospital Vienna, Vienna, Austria

A total of 14 laboratories participated (designation of participants A to N) - 4 in the basic variant, 10 in the extended variant + organizer.

Results:

The optional part, the genotyping of reference alleles, was attended by a total of 7 laboratories.

The results were statistically evaluated using the median values and the standard deviation.

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The standard deviation was determined based on statistical processing of results from previous years of EPT (variance of values, regression) and recalculated to the value of the Z- score (the lower percentile the participant has, the more successful is in comparison with the other laboratories). An overview is given in *Table 1 Comparison of all participants for EPT 2024.*

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 not able to detect the minor genotype – the example: the expected value of minor genotype is 0.2 % and the participant with the sensitivity of 1 % determines the detection of only the majority genotype - this result is considered to be correct. But if the participant detects both genotypes and quantifies them, the result is evaluated by Z score.
- **critical** ($[z] > 3$) – **incorrect result**

To achieve a successful performance of EPT, at least an 80 % success rate is required (that is 8/10 samples in the extended variant, 4/5 samples in the basic variant).

In the regular round, 86 % of the results were in the category Excellent, 11 % in the category Good, 0 % in the category Acceptable, 0 % in the category Critical, and 3 % in the category Under the detection limit of the laboratory. All of the participants achieved a successful performance and reached 100 % successfully analysed samples.

Furthermore, the results of individual participants/methods were evaluated according to percentiles. The percentile graph shows the participant's success in comparison with other laboratories. Due to the offer of two variants (basic and extended) the participants of the basic variant receive one graph; the participants of the extended variant receive two graphs. The first graph compares the success in analysis of five quantification samples (1_2024 – 5_2024) of all participants of this year, the second graph compares the success of laboratories participating in the extended variant and analysed ten quantification samples (1_2024 – 10_2024).

The lower percentile the participant has, the more successful is in comparison with the other laboratories. The results are shown in *Graph 1. EPT 2024 – Basic variant* and in *Graph 2. EPT 2024 – Extended variant.*

The summary of the methods used for the quantitative examination of cell chimerism, their sensitivities, and their interpretations are given in *Table 2.* The summary of used kits is in *Table 3.*

The reproducibility testing:

This year, our EPT was focused on the reproducibility of participant´s methods. Samples 3_2024 and 4_2024 were prepared as 2 aliquots from one original sample. Each participant received a table summarising the results of all participating laboratories and the kits used for the analysis. The results of the testing were satisfactory, most laboratories reported a difference of 1 % or less

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between two identical samples. The maximum difference in the percentage of the recipient genotype in the same sample was 2 %.

Summary of reproducibility

Percentage difference in the result between samples 3_2024 and 4_2024	Number of laboratories	Used kits
0	4	PowerPlex 16HS 2x, AmpFLSTR Identifiler 2x
<1	7	NGStrack 2x, KMRtrack 2x, Devyser NGS, AmpFLSTR Identifiler, in-houseSTR
1	4	VNTR, GenomeLab Human STR Primer Set, AmpFLSTR Identifiler, STR not specified
<2	1	Mentype Chimera
2	1	Investigator ID Plex Plus Kit

Conclusion:

This year's EPT round was very successful. All participants received a certificate with a 100 % success rate.

Processed: Mgr. Lucie Stefflová

In Prague, date 17.9.2024

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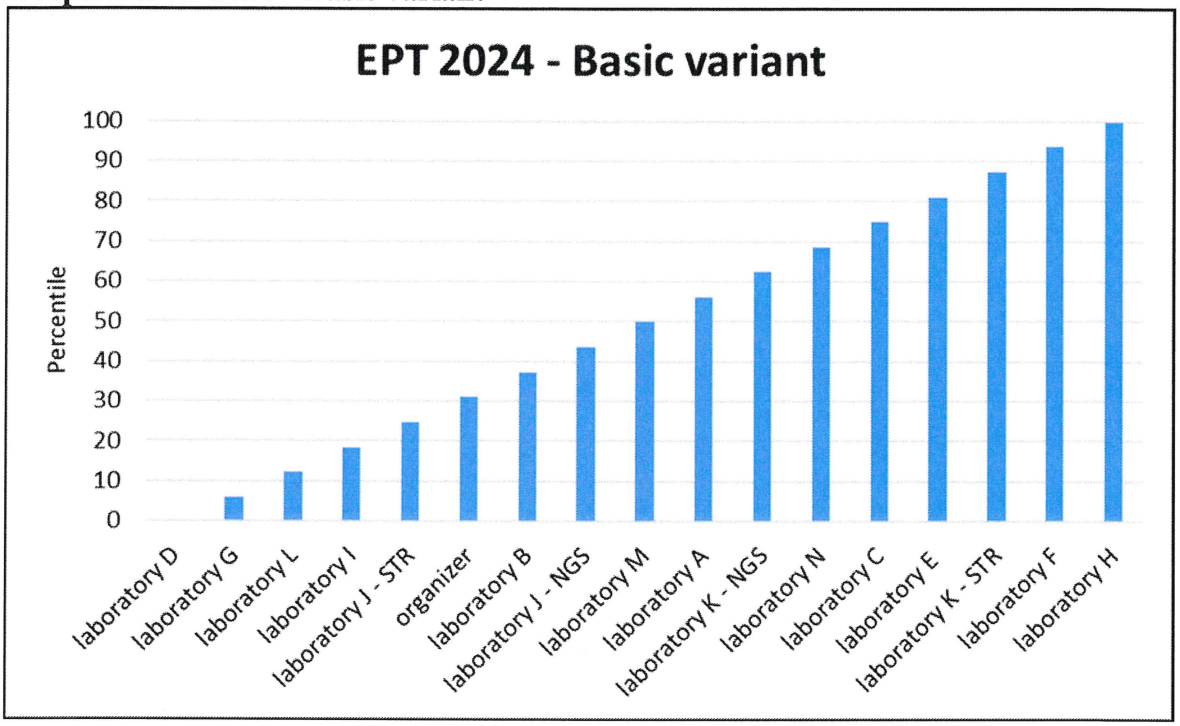
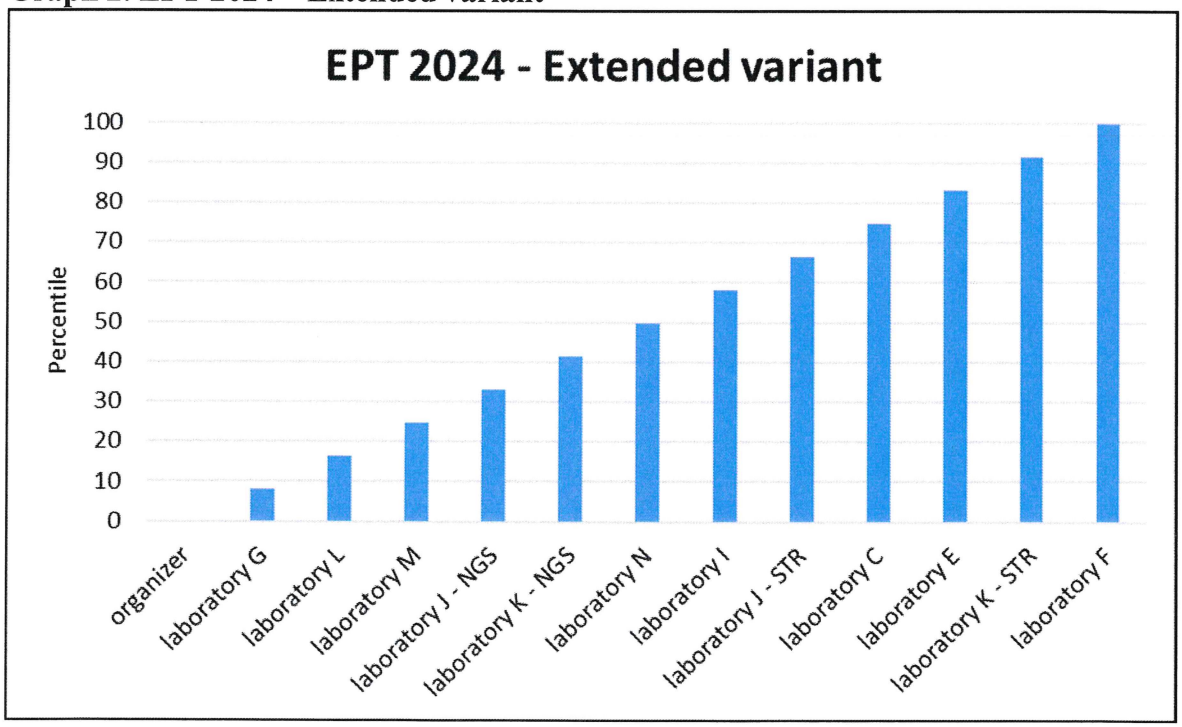
Graph 1. EPT 2024 – Basic variant



Graph 2. EPT 2024 – Extended variant



Comparison of all participants for EPT 2024

expected results*	1_2024 (12%)	2_2024 (0%)	3_2024 (30%)	4_2024 (30%)	5_2024 (95%)	6_2024 (100%)	7_2024 (25%)	8_2024(3%)	9_2024 (0.5%)	10_2024 (0%)
organizer	12,000	0,000	31,000	31,000	95,600	100,000	25,000	3,400	0,583	0,000
laboratory A	13,000	0,000	30,000	30,000	93,000					
laboratory B	14,300	0,000	33,600	33,900	95,800					
laboratory C	16,600	0,000	36,600	35,800	94,800	100,000	29,400	5,000	0,000	0,000
laboratory D	13,000	0,000	32,000	32,000	95,000					
laboratory E	13,200	0,000	41,200	40,600	96,400	100,000	37,600	3,700	0,500	0,000
laboratory F	6,000	0,000	24,000	23,000	98,000	100,000	19,000	4,000	0,000	0,000
laboratory G	13,000	0,000	32,000	31,000	94,000	100,000	25,000	3,000	0,600	0,000
laboratory H	6,200	0,000	23,700	25,100	100,000					
laboratory I	12,000	0,000	31,000	32,000	94,000	97,000	24,000	3,000	0,000	0,000
laboratory J - STR	12,500	0,000	32,000	34,000	93,000	96,000	24,000	4,000	0,000	0,000
laboratory J - NGS	14,500	0,000	34,200	33,600	96,400	98,700	28,900	3,500	0,600	0,000
laboratory K - STR	15,000	1,000	34,000	34,000	95,000	100,000	29,000	5,000	1,000	1,000
laboratory K - NGS	15,100	0,100	34,400	34,300	95,700	99,800	28,300	3,800	0,700	0,100
laboratory L	14,000	0,000	32,000	33,000	94,000	100,000	27,000	4,000	1,000	0,000
laboratory M	13,400	0,000	32,500	32,000	91,700	100,000	26,500	3,600	1,200	0,000
laboratory N	11,980	0,000	32,530	32,890	90,680	100,000	23,140	2,730	0,470	0,000
average	**12,693**	**0,065**	**32,161**	**32,246**	**94,887**	**99,346**	**26,680**	**3,748**	**0,512**	**0,085**
median	**13,00**	**0,00**	**32,00**	**32,89**	**95,00**	**100,00**	**26,50**	**3,70**	**0,58**	**0,00**
standard deviation**	**3,50**	**0,50**	**6,57**	**6,57**	**3,47**	**2,31**	**5,89**	**1,32**	**0,64**	**0,50**

Z score ***	1_2024 (12%)	2_2024 (0%)	3_2024 (30%)	4_2024 (30%)	5_2024 (95%)	6_2024 (100%)	7_2024 (25%)	8_2024(3%)	9_2024 (0.5%)	10_2024 (0%)
organizer	-0,29	0,00	-0,15	-0,29	0,17	0,00	-0,25	-0,23	0,00	0,00
laboratory A	0,00	0,00	-0,30	-0,44	-0,58					
laboratory B	0,37	0,00	0,24	0,15	0,23					
laboratory C	1,03	0,00	0,70	0,44	-0,06	0,00	0,49	0,98	-0,91	0,00
laboratory D	0,00	0,00	0,00	-0,14	0,00					
laboratory E	0,06	0,00	1,40	1,17	0,40	0,00	1,88	0,00	-0,13	0,00
laboratory F	-2,00	0,00	-1,22	-1,50	0,87	0,00	-1,27	0,23	-0,91	0,00
laboratory G	0,00	0,00	0,00	-0,29	-0,29	0,00	-0,25	-0,53	0,03	0,00
laboratory H	-1,94	0,00	-1,26	-1,19	1,44					
laboratory I	-0,29	0,00	-0,15	-0,14	-0,29	-1,30	-0,42	-0,53	-0,91	0,00
laboratory J - STR	-0,14	0,00	0,00	0,17	-0,58	-1,73	-0,42	0,23	-0,91	0,00
laboratory J - NGS	0,43	0,00	0,33	0,11	0,40	-0,56	0,41	-0,15	0,03	0,00
laboratory K - STR	0,57	1,99	0,30	0,17	0,00	0,00	0,42	0,98	0,65	1,99
laboratory K - NGS	0,60	0,20	0,37	0,21	0,20	-0,09	0,31	0,08	0,18	0,20
laboratory L	0,29	0,00	0,00	0,02	-0,29	0,00	0,08	0,23	0,65	0,00
laboratory M	0,11	0,00	0,08	-0,14	-0,95	0,00	0,00	-0,08	0,96	0,00
laboratory N	-0,29	0,00	0,08	0,00	-1,25	0,00	-0,57	-0,73	-0,18	0,00

|z| <= 1 (excellent)
1 < |z| <= 2 (good)
2 < |z| <= 3 (acceptable)
under the detection limit of the laboratory
|z| > 3 (critical)

* Expected values are referred as recipient genotype.

** Standard deviation was determined on the basis of statistical processing of the previous results of EPT (values dispersion, regression).

*** Standard deviation recalculated to Z score (the closer to zero the value is, the better is the result)

Summary of using methods of all participants - quantitative analysis of cell chimerism 2024

	organizer	laboratory A	laboratory B	laboratory C
polymorphism	STR, indel	STR	indel	STR
method	FA, qPCR	FA	NGS	STR-PCR and FA
commercial kit	for FA yes, for qPCR only for polymorphisms HLD markers	yes	yes	no
sensitivity	FA 1%, qPCR 0.035%	1%	1%	1%
interpretation %	recipient genotype	donor genotype	donor genotype	donor genotype

	laboratory D	laboratory E	laboratory F	laboratory G
polymorphism	SNP, indel	indel	VNTR	STR, indel
method	FA, qPCR	qPCR	PCR and gel electrophoresis	FA, ddPCR
commercial kit	yes	yes	no	yes for FA, no for ddPCR
sensitivity	FA 1%, qPCR 0.1%	0.1%	1%	1% STR; 0.05% indel
interpretation %	recipient genotype	both donor and recipient genotype	both donor and recipient genotype	recipient genotype

	laboratory H	laboratory I	laboratory J - STR	laboratory J - NGS
polymorphism	STR, indel	STR	STR	indel
method	FA and qPCR	FA	FA	NGS
commercial kit	yes	yes	yes	yes
sensitivity	0.013-0.2% according to used marker qPCR; 1% STR	5%	5%	0,05%
interpretation %	donor genotype	both donor and recipient genotype	both donor and recipient genotype	both donor and recipient genotype

	laboratory K - STR	laboratory K - NGS	laboratory L	laboratory M
polymorphism	STR	indel	STR, VNTR	STR
method	PCR and gel electrophoresis	NGS	FA	FA
commercial kit	yes	yes	NA	yes
sensitivity	1%	1%	0.5%	1%
interpretation %	both donor and recipient genotype	both donor and recipient genotype	recipient genotype	donor genotype

	laboratory N
polymorphism	SNP and indel
method	qPCR
commercial kit	yes
sensitivity	0,066%
interpretation %	donor genotype

Explanations:

STR = short tandem repeat
SNP = single nucleotide polymorphism
indel = short insertion and deletion
VNTR = variable number of tandem repeat
FA = fragment analysis on genetic analyzer
qPCR = quantitative polymerase chain reaction in real-time
NA = nonavailable
HLD = Human Locus DIP (deletion insertion polymorphisms)
ddPCR = droplet digital PCR

Summary of used kits 2024

STR. ev. VNTR (FA) analýza	number of participants
AmpFLSTR Identifiler (Applied Biosystems)	3
GenomeLab Human STR Primer Set (Beckman-Coulter)	1
Mentype Chimera CE-IVD (Biotype)	2
Investigator ID Plex Plus Kit (Qiagen)	1
PowerPlex 16HS (Promega)	2
home-made	3
not specified	1
indel (qPCR nebo ddPCR) analýza	**number of participants**
Mentype DIPscreen (Biotype)	2
Mentype DIPquant (Biotype)	1
KMRtrack (GenDX)	2
home-made	2
NGS	**number of participants**
NGStrack (GenDx)	2
Devyser chimerism NGS (Devyser)	1