The final report for the External proficiency testing (EPT) program in the area of quantitative analysis of cell chimerism for the year 2023

Variants:

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

Material:

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

recipient – X235

donor – X252

The regular round:

1_2023 – X235/X252 at the expected 22 % of the recipient genotype

2_2023 – X235/X252 at the expected 73 % of the recipient genotype

3_2023 – X235/X252 at the expected 7 % of the recipient genotype

4_2023 – X235/X252 at the expected 0 % of the recipient genotype

5_2023 – X235/X252 at the expected 33 % of the recipient genotype

6_2023 – X235/X252 at the expected 85 % of the recipient genotype

7_2023 – X235/X252 at the expected 14 % of the recipient genotype

8_2023 – X235/X252 at the expected 0.6 % of the recipient genotype

9_2023 – X235/X252 at the expected 10 % of the recipient genotype

10_2023 – X235/X252 at the expected 42 % of the recipient genotype

The additional round in 2023 was not organized.

Aims of the regular round:

1. Informativity examination (genotyping of reference alleles) based on examination of DNA samples – recipient and donor´s DNA – 2 samples – **optional part**.

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

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:

Medirex a.s., GENETIKA department, Bratislava, Slovakia

NZOZ Medigen Diagnostyka Molekularna, Warszawa, 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 15 laboratories participated (designation of participants A to O) - 5 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 8 laboratories.

The results were statistically evaluated using the median values obtained and the standard deviation in the regular round.

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 2023.*

Category rating:

- **Excellent** ([z] ≤ 1)
- **Good** (1 < [z] ≤ 2)
- **Acceptable** (2 < [z] ≤ 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 acceptable 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, **81 % of the results were in the category Excellent, 10 % in the category Good, 4 % in the category Acceptable, 2 % in the category Critical, and 3 % in the category Under the detection limit of the laboratory.**

All of the participants achieved an acceptable performance. One participant achieved 80 % success, one participant achieved 90 % success and the other achieved 100 % success.

Furthermore, the results of individual participants were evaluated according to percentiles. The percentile graph (25 %, 50 %, 75 %, and 100 %) shows the participant's success in comparison with other laboratories. Due to the offer of two variants (basic and extended) the participant of the basic variant receives one graph; the participant of the extended variant receives two graphs. The first graph compares five quantification samples (1_2023 – 5_2023) of all participants of this year, the second graph compares ten quantification samples (1_2023 – 10_2023) only laboratories participating in the extended variant.

The lower percentile the participant has, the more successful is in comparison with the other laboratories. The results are shown in *Graph 1. EPT 2023 – basic variant* and in *Graph 2. EPT 2023 – 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 most common errors and recommendations:

- False negativity, using less sensitive methods for quantification.

- False positivity – mixed chimerism was detected in a sample where only donor DNA or recipient DNA was present.

- Inappropriate selection of polymorphism for quantification (shorter repetition – so-colled stutter peak).

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In Prague, date 30.11.2023

Table 1. Comparison of all participants for EPT 2023

expected results*	1_2023 (22%)	2_2023 (73%)	3_2023 (7%)	4_2023 (0%)	5_2023 (33%)	6_2023 (85%)	7_2023 (14%)	8_2023(0,6%)	9_2023 (10%)	10_2023 (42%)
organizer	20,561	78,147	7,043	0,001	31,093	88,523	14,029	0,618	10,077	39,092
laboratory A	24,000	78,000	8,000	0,000	37,000					
laboratory B	25,000	75,000	8,000	0,000	34,000					
laboratory C	22,000	73,900	6,500	0,000	34,100	85,500	15,200	0,000	9,500	42,600
laboratory D	22,100	68,800	6,900	0,000	31,800					
laboratory E	21,500	80,100	8,500	0,000	32,200	91,200	17,500	0,700	8,300	38,100
laboratory F	8,000	58,000	2,000	0,000	25,000	64,000	12,000	0,000	5,000	35,000
laboratory G	24,000	75,000	8,000	0,000	36,000	88,000	14,000	0,700	11,000	45,000
laboratory H	24,200	86,600	6,900	0,000	44,000					
laboratory I	27,000	75,000	12,000	0,000	38,000	86,000	19,000	0,000	14,000	47,000
laboratory J	29,000	72,000	15,000	0,000	42,000	84,000	22,000	0,000	18,000	45,000
laboratory K	29,000	77,000	13,000	0,000	40,000	85,000	21,000	1,000	13,000	48,000
laboratory L	26,000	76,000	9,000	0,000	36,500	87,000	17,500	1,000	13,000	47,000
laboratory M	27,300	75,600	11,300	4,400	39,300	85,800	19,300	4,200	16,300	46,800
laboratory N	18,530	78,300	5,810	0,000	25,420	87,190	11,040	0,480	9,670	35,750
laboratory O	25,000	74,000	9,000	0,000	36,000					
average	23,324	75,090	8,560	0,275	35,151	84,747	16,597	0,791	11,622	42,667
median	24,10	75,30	8,00	0,00	36,00	86,00	17,50	0,62	11,00	45,00
standard deviation**	5,55	7,08	2,36	0,45	7,10	5,36	4,01	0,63	3,10	7,86

Z score ***	1_2023 (22%)	2_2023 (73%)	3_2023 (7%)	4_2023 (0%)	5_2023 (33%)	6_2023 (85%)	7_2023 (14%)	8_2023(0,6%)	9_2023 (10%)	10_2023 (42%)
organizer	-0,64	0,40	-0,40	0,00	-0,69	0,47	-0,87	0,00	-0,30	-0,75
laboratory A	-0,02	0,38	0,00	0,00	0,14					
laboratory B	0,16	-0,04	0,00	0,00	-0,28					
laboratory C	-0,38	-0,20	-0,63	0,00	-0,27	-0,09	-0,57	-0,99	-0,48	-0,31
laboratory D	-0,36	-0,92	-0,47	0,00	-0,59					
laboratory E	-0,47	0,68	0,21	0,00	-0,54	0,97	0,00	0,13	-0,87	-0,88
laboratory F	-2,90	-2,44	-2,54	0,00	-1,55	-4,11	-1,37	-0,99	-1,93	-1,27
laboratory G	-0,02	-0,04	0,00	0,00	0,00	0,37	-0,87	0,13	0,00	0,00
laboratory H	0,02	1,60	-0,47	0,00	1,13					
laboratory I	0,52	-0,04	1,69	0,00	0,28	0,00	0,37	-0,99	0,97	0,25
laboratory J	0,88	-0,47	2,96	0,00	0,85	-0,37	1,12	-0,99	2,26	0,00
laboratory K	0,88	0,24	2,11	0,00	0,56	-0,19	0,87	0,61	0,64	0,38
laboratory L	0,34	0,10	0,42	0,00	0,07	0,19	0,00	0,61	0,64	0,25
laboratory M	0,58	0,04	1,40	9,73	0,47	-0,04	0,45	5,72	1,71	0,23
laboratory N	-1,00	0,42	-0,93	0,00	-1,49	0,22	-1,61	-0,22	-0,43	-1,18
laboratory O	0,16	-0,18	0,42	0,00	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)

Table 2. Summary of using methods of all participants - quantitative analysis of cell chimerism 2023

	organizer	laboratory A	laboratory B	laboratory C
polymorphism	STR, indel	STR	VNTR	STR
method	FA, qPCR	FA	FA	STR-PCR and FA
commercial kit	for FA yes, for qPCR only for polymorphisms HLD markers	yes	no	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 - screening, qPCR - quantification	qPCR	PCR and gel electrophoresis	FA, ddPCR
commercial kit	yes	yes	no	yes for FA, no for ddPCR
sensitivity	0.05%	0.1%	1%	1% STR; 0.05% indel
interpretation %	recipient genotype	both donor and recipient genotype	both donor and recipient genotype	recipient genotype

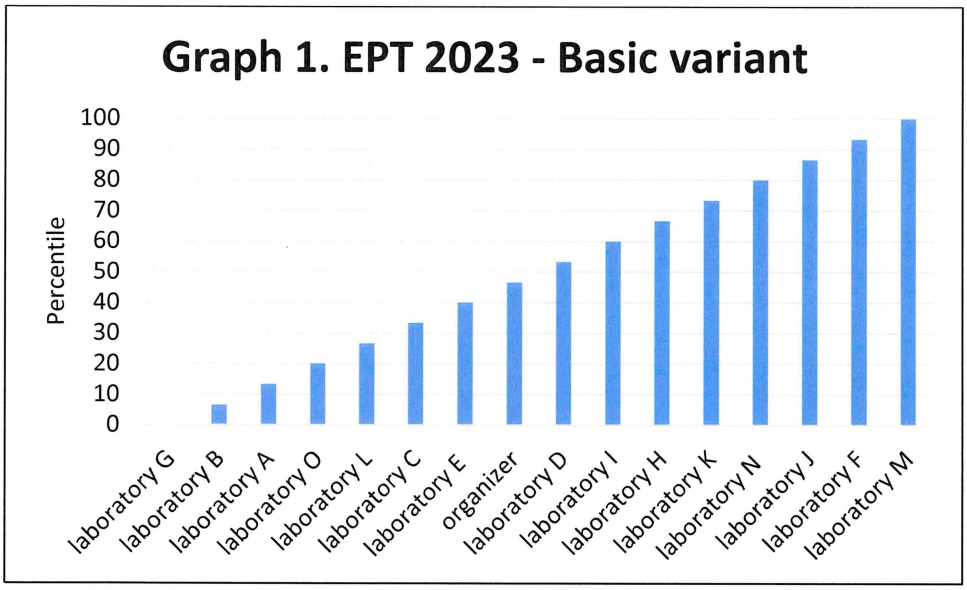
	laboratory H	laboratory I	laboratory J	laboratory K
polymorphism	STR, indel	STR	STR	STR
method	FA and qPCR	FA	FA	FA
commercial kit	yes	yes	yes	yes
sensitivity	0.013-0.2% according to used marker qPCR; 1% STR	5%	5%	1%
interpretation %	donor genotype	both donor and recipient genotype	both donor and recipient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

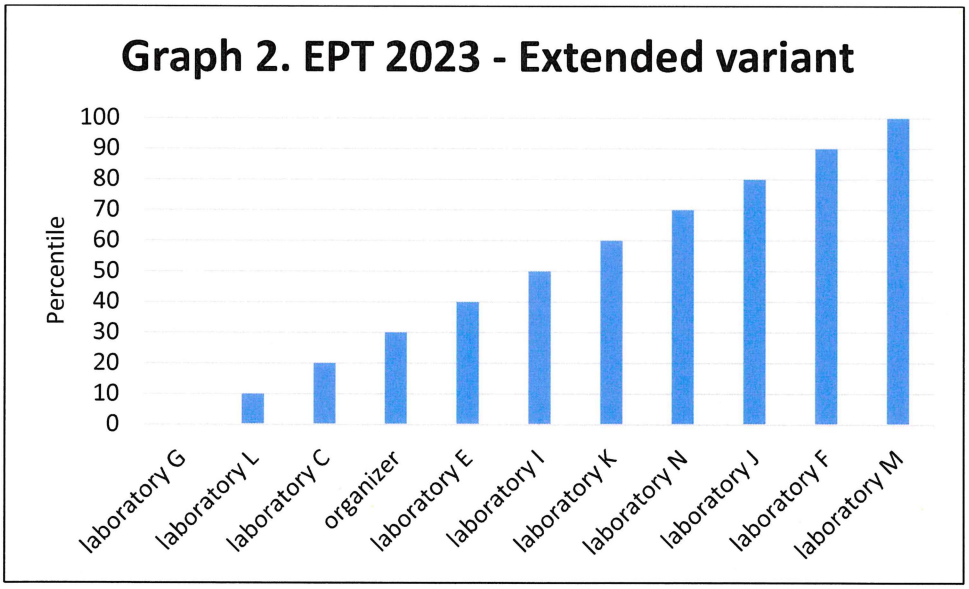
	laboratory L	laboratory M	laboratory N	laboratory O
polymorphism	STR	STR	SNP and indel	STR, indel
method	FA	FA	qPCR	FA and qPCR
commercial kit	NA	yes	yes	yes for FA, no for qPCR
sensitivity	0.5%	1%	0,066%	FA 1%, qPCR 0.1%
interpretation %	recipient genotype	donor genotype	donor genotype	recipient genotype

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

Table 3. Summary of used kits 2023

STR. eventually VNTR (FA) analysis	number of participants
AmpFLSTR™ Identifiler™ PCR Amplification Kit (Applied Biosystems)	4
GenomeLab Human STR Primer Ser (Beckman-Coulter)	1
Mentype Chimera CE-IVD (Biotype)	2
Investigator ID Plex Plus Kit (Qiagen)	1
PowerPlex multiplex kits - ESI17FAST, CS7 (Promega)	1
PowerPlex multiplex kits - PP16HS (Promega)	2
PowerPlex monoplex kits (Promega)	1
home-made	3
not specified	1
indel (qPCR or ddPCR) analysis	**number of participants**
Mentype DIPscreen (Biotype)	3
Mentype DIPquant (Biotype)	3
HLA-KMR Assay (GenDX)	2
home-made	3


Graph 1. EPT 2023 - Basic variant
Percentile
100
90
80
70
60
50
40
30
20
10
0
laboratory G
laboratory B
laboratory A
laboratory O
laboratory L
laboratory C
laboratory E
organizer
laboratory D
laboratory I
laboratory H
laboratory K
laboratory N
laboratory J
laboratory F
laboratory M


Graph 2. EPT 2023 - Extended variant
Percentile
100
90
80
70
60
50
40
30
20
10
0
laboratory G
laboratory L
laboratory C
organizer
laboratory E
laboratory I
laboratory K
laboratory N
laboratory J
laboratory F
laboratory M