The final report for the External proficiency testing (EPT) program in the area of quantitative analysis of cell chimerism for the year 2022

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 – X234

donor – X233

The regular round:

1_2022 – X234/X233 at the expected 98 % of the recipient genotype

2_2022 – X234/X233 at the expected 5 % of the recipient genotype

3_2022 – X234/X233 at the expected 0 % of the recipient genotype

4_2022 – X234/X233 at the expected 20 % of the recipient genotype

5_2022 – X234/X233 at the expected 25 % of the recipient genotype

6_2022 – X234/X233 at the expected 3 % of the recipient genotype

7_2022 – X234/X233 at the expected 0.4 % of the recipient genotype

8_2022 – X234/X233 at the expected 12 % of the recipient genotype

9_2022 – X234/X233 at the expected 37 % of the recipient genotype

10_2022 – X234/X233 at the expected 80 % of the recipient genotype

The additional round:

11_2022 – X234/X233 at the expected 30 % of the recipient genotype

12_2022 – X234/X233 at the expected 8 % of the recipient genotype

13_2022 – X234/X233 at the expected 0 % of the recipient genotype

14_2022 – X234/X233 at the expected 40 % of the recipient genotype

15_2022 – X234/X233 at the expected 65 % of the recipient genotype

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**.

Aims of the additional round:

1. Informativity examination (genotyping of reference alleles) based on examination of DNA samples - recipient and donor´s DNA – 2 samples – **optional part. DNA samples are the same as sent in the regular round (EPT 2022). They are not sent again.**

2. Quantitative examination of chimerism status - 5 samples 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

Foreign participants:

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

Laboratory of clinical and molecular genetics, Department of Paediatrics, National Institute of Pediatric Diseases, 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.

The additional round:

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

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 obtained and the standard deviation in the regular round. Due to the participation of only one participant in the additional round, the statistical evaluation was performed using the expected value and standard deviation.

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* – regular round, in *Table 2* – the additional round.

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 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, **86 % of the results were in the category Excellent, 5 % in the category Good, 1 % in the category Acceptable, 4 % in the category Critical, and 4 % in the category Under the detection limit of the laboratory.**

One participant did not meet the conditions for successful participation (70 % success), two achieved 90 % success and the other twelve achieved 100 % success. An additional round was offered to all participants, only 1 participant showed interest.

In the additional round, **70 % of the results were in the category Excellent, 10 % in the category Good, 0 % in the category Acceptable, 20 % in the category Critical, and 0 % in the category Under the detection limit of the laboratory.**

The participant in the additional round achieved a 60 % success rate. He did not meet the conditions for successful participation.

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_2022 – 5_2022) of all participants of this year, the second graph compares ten quantification samples (1_2022 – 10_2022) only laboratories participating in the extended variant. The third graph compares 5 quantification samples (11_2022 to 15_2022) of the organizer and one participant in the additional round.

The lower percentile the participant has, the more successful is in comparison with the other laboratories. The results are shown in *Graph 1* (a basic variant of the regular round), *Graph 2* (an extended variant of the regular round), and *Graph 3* (additional round).

The summary of the methods used for the quantitative examination of cell chimerism, their sensitivities, and their interpretations are given in *Table 3.* The summary of used kits is in *Table 4.*

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 5.10.2022

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Comparison of all participant for EPT 2022

expected results*	1_2022 (98%)	2_2022 (5%)	3_2022 (0%)	4_2022 (20%)	5_2022 (25%)	6_2022 (3%)	7_2022 (0.4%)	8_2022 (12%)	9_2022 (37%)	10_2022 (80%)
organizer	97,854	4,558	0,000	18,791	22,713	2,624	0,348	10,652	34,039	83,038
laboratory A	98,000	6,000	0,000	21,000	27,000					
laboratory B	96,000	6,000	0,000	17,000	22,000					
laboratory C	97,300	6,600	0,000	21,800	27,400	4,400	0,000	13,500	38,900	82,200
laboratory D	100,000	8,600	0,000	35,700	34,400					
laboratory E	100,000	6,700	0,000	27,400	29,400	3,500	0,400	15,500	29,900	73,500
laboratory F	100,000	5,000	0,000	17,000	24,000	3,000	0,000	11,000	35,000	79,000
laboratory G	96,000	6,000	0,000	23,000	29,000	4,000	0,400	14,000	41,000	80,000
laboratory H	98,300	7,700	0,000	22,300	28,900					
laboratory I	97,000	8,000	6,000	23,000	29,000	8,000	5,000	15,000	41,000	82,000
laboratory J	100,000	11,000	0,000	25,000	30,000	10,000	0,000	16,000	42,000	80,000
laboratory K	98,000	6,000	0,000	26,000	31,000	4,000	0,000	17,000	43,000	80,000
laboratory L	97,500	5,000	0,000	20,000	23,000	3,000	0,500	11,900	36,000	79,000
laboratory M	95,700	9,300	0,000	23,600	29,000	7,800	0,000	16,100	39,900	81,000
laboratory N	98,190	4,060	0,000	18,300	22,080	2,660	0,330	12,020	32,360	82,120
laboratory O	97,000	5,000	0,000	21,000	26,000					
průměr	**97,928**	**6,595**	**0,375**	**22,556**	**27,181**	**4,817**	**0,634**	**13,879**	**37,554**	**80,169**
median	**97,93**	**6,00**	**0,00**	**22,05**	**28,15**	**4,00**	**0,33**	**14,00**	**38,90**	**80,00**
standard deviation**	**3,38**	**1,78**	**0,28**	**5,44**	**6,37**	**1,20**	**0,40**	**3,65**	**8,00**	**6,99**

Z score ***	1_2022 (98%)	2_2022 (5%)	3_2022 (0%)	4_2022 (20%)	5_2022 (25%)	6_2022 (3%)	7_2022 (0.4%)	8_2022 (12%)	9_2022 (37%)	10_2022 (80%)
organizer	-0,02	-0,81	0,00	-0,60	-0,85	-1,15	0,04	-0,92	-0,61	0,43
laboratory A	0,02	0,00	0,00	-0,19	-0,18					
laboratory B	-0,57	0,00	0,00	-0,93	-0,97					
laboratory C	-0,19	0,34	0,00	-0,05	-0,12	0,33	-0,82	-0,14	0,00	0,31
laboratory D	0,61	1,46	0,00	2,51	0,98					
laboratory E	0,61	0,39	0,00	0,98	0,20	-0,42	0,17	0,41	-1,12	-0,93
laboratory F	0,61	-0,56	0,00	-0,93	-0,65	-0,83	-0,82	-0,82	-0,49	-0,14
laboratory G	-0,57	0,00	0,00	0,17	0,13	0,00	0,17	0,00	0,26	0,00
laboratory H	0,11	0,95	0,00	0,05	0,12					
laboratory I	-0,27	1,12	21,81	0,17	0,13	3,34	11,64	0,27	0,26	0,29
laboratory J	0,61	2,80	0,00	0,54	0,29	5,01	-0,82	0,55	0,39	0,00
laboratory K	0,02	0,00	0,00	0,73	0,45	0,00	-0,82	0,82	0,51	0,00
laboratory L	-0,13	-0,56	0,00	-0,38	-0,81	-0,83	0,42	-0,57	-0,36	-0,14
laboratory M	-0,66	1,85	0,00	0,28	0,13	3,17	-0,82	0,57	0,12	0,14
laboratory N	0,08	-1,09	0,00	-0,69	-0,95	-1,12	0,00	-0,54	-0,82	0,30
laboratory O	-0,27	-0,56	0,00	-0,19	-0,34					

|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 1

Comparison of all participant for EPT 2022 - the additional round

expected value*	11_2022 (30%)	12_2022 (8%)	13_2022 (0%)	14_2022 (40%)	15_2022 (65%)
organizer	28,064	7,737	0,000	37,046	68,915
laboratory I	39,000	19,000	3,000	48,000	68,000

mean	33,532	13,369	1,500	42,523	68,458
median	**33,53**	**13,37**	**1,50**	**42,52**	**68,46**
expected value*	**30,00**	**8,00**	**0,00**	**40,00**	**65,00**
standard deviation**	7,15	2,62	0,28	8,28	8,56

Z score ***	11_2022 (30%)	12_2022 (8%)	13_2022 (0%)	14_2022 (40%)	15_2022 (65%)
organizer	-0,27	-0,10	0,00	-0,36	0,46
laboratory I	1,26	4,20	10,91	0,97	0,35

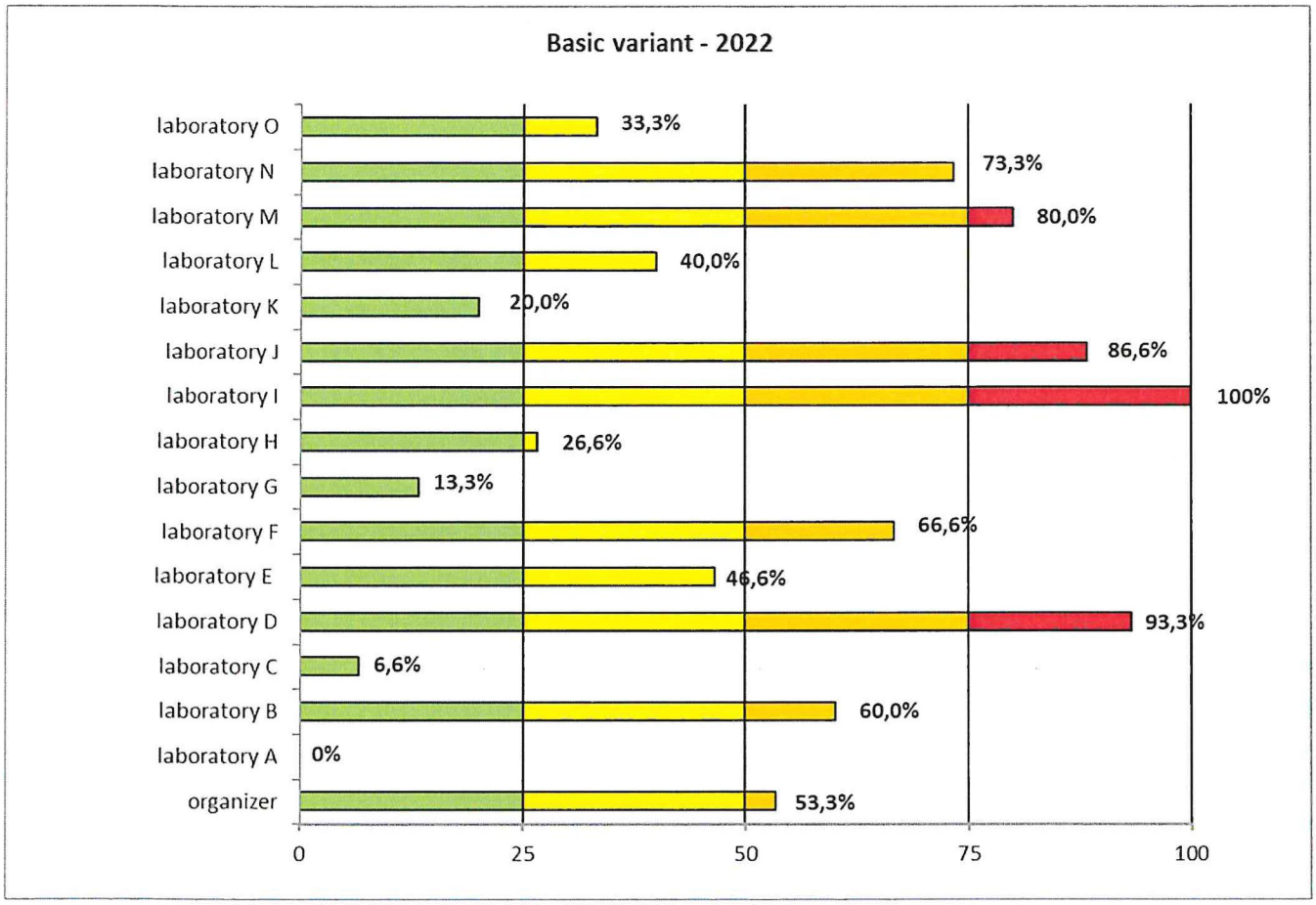
|z| <= 1 (excellent)
1 < |z| <= 2 (good)
2 < |z| <= 3 (acceptable)
under the detection limit of the laboratory
|z| > 3 (critical)

* Expected values are referred as recipient genotype.

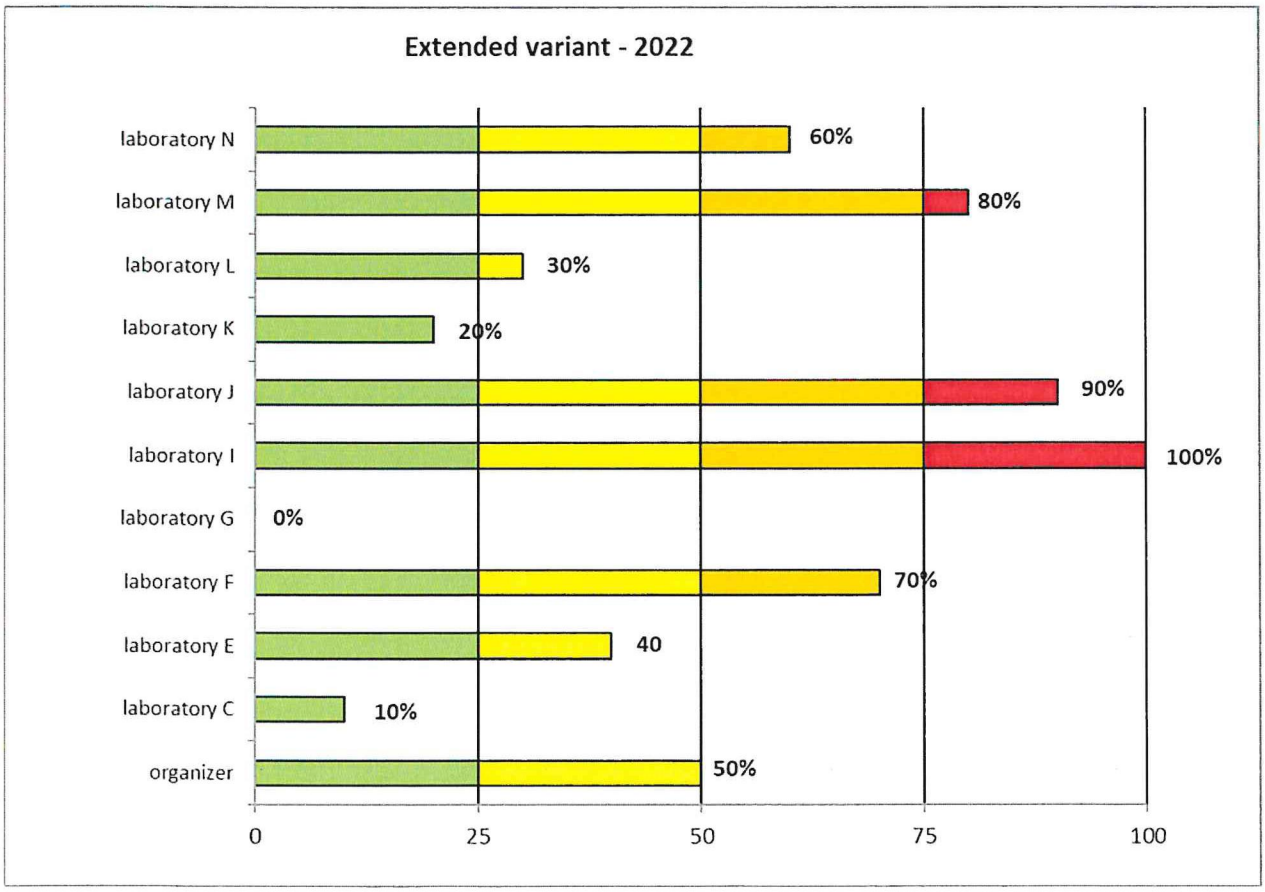
** Standard deviation was determined on the basis of statistical processing of the previous results of EPT (values dispersion, regression).

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

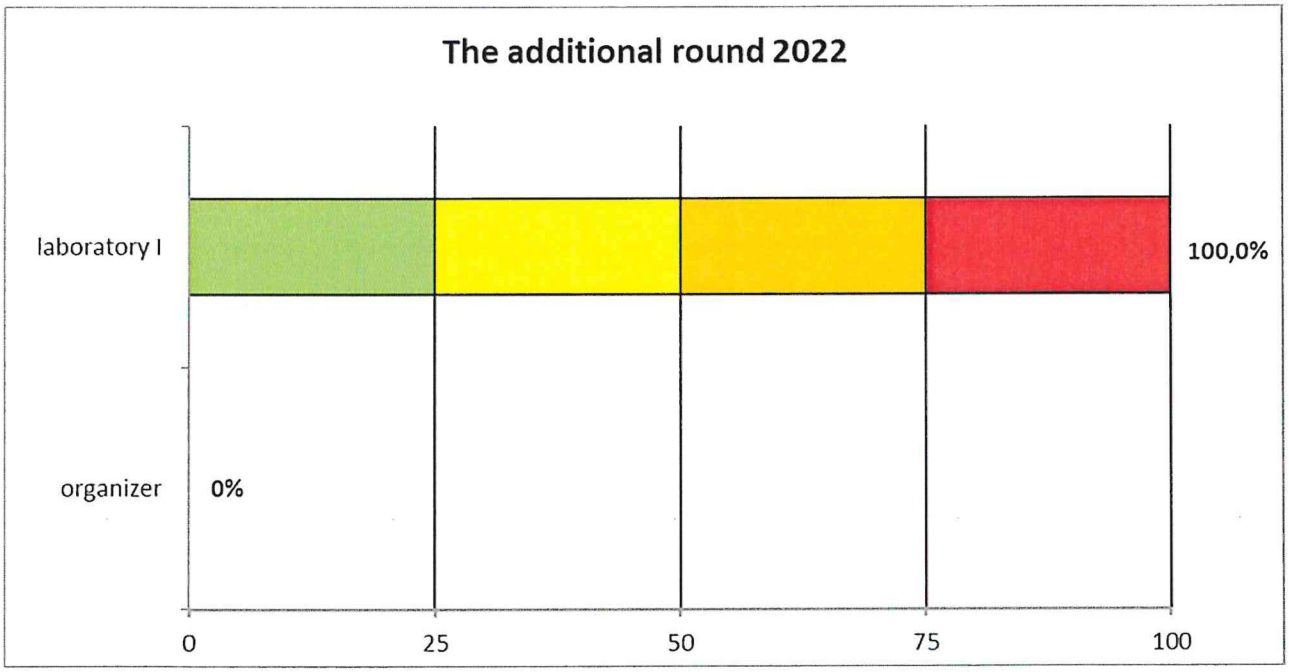
Table 2



Graph 1



Graph 2


The additional round 2022
laboratory I
100,0%
organizer
0%
0
25
50
75
100

Graph 3

Summary of using method of all participants - quantitative analysis of cell chimerism 2022

	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 %	% of recipient genotype	% of donor genotype	% of donor genotype	% of 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 %	% of donor and recipient genotype	% of donor and recipient genotype	% of donor and recipient genotype	% of 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 for FA, no for qPCR	yes	yes	yes
sensitivity	0.1%	5%	5%	1%
interpretation %	% of recipient genotype	% of donor and recipient genotype	% of donor and recipient genotype	% of donor and recipient genotype

	laboratory L	laboratory M	laboratory N	laboratory O
polymorphism	VNTR, 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 %	% of recipient genotype	% of donor genotype	% of donor genotype	% of 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 2022

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)	1
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
PowerPlex ESX17	1
not specified	1
indel (qPCR or ddPCR) analysis	**number of participants**
Mentype DIPscreen (Biotype)	2
Mentype DIPquant (Biotype)	2
HLA-KMR Assay (GenDX)	2
home-made	3

Table 4