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The Final Report of the External Proficiency Testing (EPT) Program in Quantitative Analysis of Cell Chimerism for the Year 2026

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 – X275
Donor – X274

Regular Round:

1_2026 – X275/X274, expected recipient genotype: 6%
2_2026 – X275/X274, expected recipient genotype: 6%
3_2026 – X275/X274, expected recipient genotype: 98%
4_2026 – X275/X274, expected recipient genotype: 100%
5_2026 – X275/X274, expected recipient genotype: 0.9%
6_2026 – X275/X274, expected recipient genotype: 50%
7_2026 – X275/X274, expected recipient genotype: 52%
8_2026 – X275/X274, expected recipient genotype: 14%
9_2026 – X275/X274, expected recipient genotype: 0%
10_2026 – X275/X274, expected recipient genotype: 9%

EPT Assignment:

Quantitative examination of cell chimerism:

Basic variant - 5 samples
Extended variant - 10 samples

(Recipient and donor DNA samples were submitted along with the quantification 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

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

National HLA Laboratory, National Institute of Blood Transfusion "Prof. Dr. C.T. Nicolau", Bucharest, Romania

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In 2026, a total of 15 laboratories participated (designated A - N): four in the basic variant, ten in the extended variant, and the organizer. One laboratory analyzed the samples separately using both STR analysis and NGS.

Results:

The results were statistically evaluated, with the robust mean established as the consensus reference value. The robust standard deviation was determined from historical data as the median absolute deviation (MAD), depending on the percentage of the recipient genotype in the analyzed sample. The results were further assessed using the robust Z-score (the closer the value is to zero, the more accurate the result). An overview of the robust Z-scores for individual participants is provided in the table „***Statistical Evaluation of EPT Chimerism 2026***", included in the appendices to this report.

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 2026, 80% of the results fell into the Excellent category, 10.7% into the Good category, 4.3% into the Acceptable category, 1.4% into the Critical category, and 3.6% 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 80%, while the remaining participants achieved 100%.

Reproducibility Testing:

This year's round once again provided participants the opportunity to test the reproducibility of their methods. Samples 1_2026 and 2_2026 were prepared as two aliquots from one original sample. The results of all participants are summarized in the table no.1. The testing yielded highly consistent results: most laboratories reported a difference of 1% or less between the two identical samples. The maximum reported difference in the percentage of the recipient genotype in the identical samples was less than 2%.

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Table 1: Evaluation of the reproducibility of methods used by participants in the EPT Chimerism 2026.

Percentage difference in the result between samples 1_2026 and 2_2026	Number of laboratories	Used kits
0	4	in-house STR, in-house qPCR; AmpFLSTR Identifiler; in-house STR; PowerPlex 16HS;
≤ 0.2	2	KMRtrack, NGStrack
≤ 0.5	4	NGStrack; KMRtrack; Mentype DIPquant; in-house STR, in-house VNTR
≤ 1	5	in-house STR; AmpFLSTR Identifiler Plus, Mentype Chimera; AlloSeq HCT; not specified STR; Qiagen InvestigatorID Plex Plus Kit
< 2	1	AmpFLSTR Identifiler Plus

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, 2026***. A list of commercial kits used in the participating laboratories is presented in the table ***Overview of Utilized Kits in 2026***. 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

Prague, 30 June 2026

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Statistical Evaluation of EPT Chimerism 2026

expected results*	1_2026 (6%)	2_2026 (6%)	3_2026 (98%)	4_2026 (100%)	5_2026 (0,9%)	6_2026 (50%)	7_2026 (52%)	8_2026 (14%)	9_2026 (0%)	10_2026 (9%)
organizer	6,000	6,000	98,000	100,000	0,761	48,000	50,000	12,000	0,000	8,000
laboratory A	7,000	7,000	98,500	100,000	2,000					
laboratory B	6,280	6,570	98,010	99,990	0,950					
laboratory C	5,440	6,070	97,360	100,000	0,000	49,150	51,410	14,450	0,000	8,210
laboratory D	7,000	6,000	98,000	100,000	1,000					
laboratory E	5,500	5,170	100,000	100,000	0,760	50,930	62,620	14,340	0,000	7,130
laboratory F	6,620	5,880	98,030	100,000	1,000	50,880	52,620	14,410	0,000	9,410
laboratory G	6,000	6,000	98,000	100,000	0,880	49,000	50,000	14,000	0,000	9,000
laboratory H	7,510	7,900	97,820	99,070	1,070					
laboratory I	7,000	8,000	100,000	100,000	0,000	50,000	54,000	14,000	0,000	12,000
laboratory J	10,000	9,000	100,000	100,000	0,000	49,000	54,000	16,000	0,000	13,000
laboratory K - STR	8,000	8,000	98,000	100,000	1,000	50,000	51,000	15,000	0,000	11,000
laboratory K - NGS	6,200	6,400	97,700	99,900	1,100	49,700	52,200	14,700	0,000	9,400
laboratory L	6,500	6,000	98,000	100,000	1,000	51,000	53,000	16,000	0,000	10,000
laboratory M	7,950	9,840	96,630	100,000	3,550	50,990	53,220	16,960	0,000	9,830
laboratory N	5,080	5,260	97,630	99,050	0,520	50,280	51,300	13,320	0,000	7,080

robust mean	6.56 %	6.24 %	98.00 %	100.00 %	1.00 %	50.00 %	52.41 %	14.43 %	0.00 %	9.41 %
robust SD**	1,227	1,179	0,913	0,598	0,354	4,526	4,526	2,287	0,186	1,634
excellent	5.33 - 7.79	5.06 - 7.41	97.09 - 98.91	99.40 - 100.00	0.65 - 1.35	45.47 - 54.53	47.88 - 56.94	12.14 - 16.72	0.00 - 0.19	7.77 - 11.04
good	4.11 - 9.01	3.88 - 8.59	96.17 - 99.83	98.80 - 100.00	0.29 - 1.71	40.95 - 59.05	43.36 - 61.46	9.86 - 19.00	0.00 - 0.37	6.14 - 12.67
accepted	2.88 - 10.24	2.70 - 9.77	95.26 - 100.00	98.21 - 100.00	0.00 - 2.06	36.42 - 63.58	38.83 - 65.99	7.57 - 21.29	0.00 - 0.56	4.50 - 14.31
uncertainty	0,383	0,368	0,305	0,187	0,123	1,633	1,633	0,825	0,067	0,590
n***	16	16	14	16	13	12	12	12	12	12

robust Z-score										
	1_2026 (6%)	2_2026 (6%)	3_2026 (98%)	4_2026 (100%)	5_2026 (0,9%)	6_2026 (50%)	7_2026 (52%)	8_2026 (14%)	9_2026 (0%)	10_2026 (9%)
organizer	-0,456	-0,199	0,000	0,000	-0,675	-0,442	-0,532	-1,062	0,000	-0,860
laboratory A	0,359	0,649	0,547	0,000	2,823					
laboratory B	-0,228	0,284	0,011	-0,017	-0,141					
laboratory C	-0,913	-0,140	-0,701	0,000	-2,823	-0,188	-0,221	0,009	0,000	-0,731
laboratory D	0,359	-0,199	0,000	0,000	0,000					
laboratory E	-0,864	-0,904	2,190	0,000	-0,678	0,205	2,256	-0,039	0,000	-1,392
laboratory F	0,049	-0,301	0,033	0,000	0,000	0,194	0,046	-0,009	0,000	0,003
laboratory G	-0,456	-0,199	0,000	0,000	-0,339	-0,221	-0,532	-0,188	0,000	-0,248
laboratory H	0,774	1,413	-0,197	-1,556	0,198					
laboratory I	0,359	1,497	2,190	0,000	-2,823	0,000	0,351	-0,188	0,000	1,588
laboratory J	2,804	2,346	2,190	0,000	-2,823	-0,221	0,351	0,686	0,000	2,200
laboratory K - STR	1,174	1,497	0,000	0,000	0,000	0,000	-0,312	0,249	0,000	0,976
laboratory K - NGS	-0,293	0,140	-0,328	-0,167	0,282	-0,066	-0,046	0,118	0,000	-0,003
laboratory L	-0,049	-0,199	0,000	0,000	0,000	0,221	0,130	0,686	0,000	0,364
laboratory M	1,133	3,053	-1,500	0,000	7,199	0,219	0,179	1,106	0,000	0,260
laboratory N	-1,206	-0,827	-0,405	-1,590	-1,355	0,062	-0,245	-0,485	0,000	-1,423

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

* Expected values correspond to the recipient´s genotype.

** The robust standard deviation was estimated from previous EPT results as the median absolute deviation (MAD).

*** Number of results used for the calculation of the robust mean.

Summary of Methods Used by All Participants – Quantitative Analysis of Cell Chimerism, 2026

	organizer	laboratory A	laboratory B	laboratory C
polymorphism	STR, indel	STR	indel	STR
method	FA, qPCR	FA	NGS	FA
commercial kit	genotypization yes, quantification no (STR and qPCR)	yes	yes	no
sensitivity	FA 1%, qPCR 0.035%	1%	0,5%	1%

	laboratory D	laboratory E	laboratory F	laboratory G
polymorphism	STR, indel	indel	SNP	STR, indel
method	FA, qPCR	qPCR	NGS	FA, ddPCR
commercial kit	STR yes, qPCR no	yes	yes	STR genotypization yes, quantification no (STR and qPCR)
sensitivity	FA 1%, qPCR 0.1%	0.1%	0.22%	FA 1%; ddPCR 0.05%

	laboratory H	laboratory I	laboratory J	laboratory K - STR
polymorphism	STR, indel	STR	STR	STR
method	FA, qPCR	FA	FA	FA
commercial kit	yes	not specified	yes	yes
sensitivity	FA 1%; qPCR 0.013-0.2% based on the marker	5%	5%	1%

	laboratory K - NGS	laboratory L	laboratory M	laboratory N
polymorphism	indel	STR, VNTR	STR	indel
method	NGS	FA	FA	qPCR
commercial kit	yes	no	yes	yes
sensitivity	0.5%	0.5%	1-5%	0.066%

Explanations:
STR = short tandem repeat
NGS= next generation sequencing
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
ddPCR = droplet digital PCR

Overview of Utilized Kits in 2026

STR, VNTR (FA)	number of participants
in-house	4
AmpFLSTR Identifiler (Applied Biosystems)	2
AmpFLSTR Identifiler Plus (Applied Biosystems)	1
PowerPlex 16HS (Promega)	1
Mentype Chimera CE-IVD (Biotype)	1
Mentype DIPscreen CE-IVD (Biotype)	1
Investigator ID Plex Plus Kit (Qiagen)	1
not specified	1
indel (qPCR)	**number of participants**
KMRtrack (GenDX)	2
in-house	2
Mentype DIPquant (Biotype)	1
droplet digital PCR (ddPCR)	**number of participants**
in-house	1
NGS	**number of participants**
NGStrack (GenDx)	2
AlloSeq HCT (CareDx)	1