BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

EXTERNAL QUALITY ASSESSMENT*

DEFINITIVE GLOBAL ANNUAL REPORT
FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS
CD34+ STEM CELL ENUMERATION
2024

* RD 03/12/1999

Sciensano/Flow cytometry/92/E

Biological health risks
Quality of laboratories
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The experts were invited to send their comments via e-mail.

Authorization of the report: by Lobna Bouacida, coordinator

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1. LYMPHOCYTE SUBSET ANALYSIS

1.1. Surveys

A triannual external quality assessment scheme for lymphocyte immunophenotyping is operational in Belgium since 2000. Each survey, participating laboratories are sent 2 fresh K_2EDTA anticoagulated whole blood samples by overnight mail. The laboratories are surveyed for methodology and are asked to report white blood cell count (WBC), percentage of lymphocytes, percentages and absolute numbers of T (CD3+), B (CD19+) and NK cells, and of the CD4+ and CD8+ T cell subsets as well as the percentages of κ and λ chain expressing B cells and the κ/λ ratio.

The samples are sent by Taxipost 24h and the laboratories are informed by e-mail of the send-out of the control material (day 0).

In 2024, surveys were conducted in February (FC/20511, FC/20512), May (FC/20687, FC/20688) and November (FC/21146, FC/21147).

Fifty-two clinical laboratories took part in these surveys, but not all participants completed every survey.

1.2. Methodology of the clinical laboratories Survey 2024/3 (n=49)

Six laboratories (12%) used a single platform approach for determining the absolute lymphocyte subset counts. Of these laboratories, 3 used Flow-Count beads (Beckman-Coulter) and 3 Trucount technology (BD Biosciences).

Following tables provide an overview of the haematology analysers and flow cytometers used:

Haematology analyser	Number of participants
Sysmex XN 1000/ XN 2000/ XN 3000/ XN 9000	39
Beckman Coulter UniCel DxH 800 / DxH 900	5
Siemens Advia 2120	1
Abbott Cell-Dyn Ruby	1
Not mentioned	3

Flow cytometer	Number of participants
BD Biosciences FACSLyric	20
Beckman Coulter Navios	10
BD Biosciences FACSCanto II	9
Beckman Coulter DxFLEX	5
Beckman Coulter AQUIOS CL	2
Beckman Coulter Cytomics FC 500	1
BD Biosciences FACSVia	1
Sysmex XF-1600	1

Monitoring of flow cytometer performance

Performance characteristics such as precision and fluorescence sensitivity that can change rapidly due to fluidic problems and affect the alignment of the sample in the optical path, should be checked each day the instrument is used. This is achieved using stable bead mixtures during the daily start-up routine for each instrument[1].

All participants mentioned monitoring the performance of their flow cytometer. One laboratory gave no further details, all others reported the use of commercial bead material (85% daily, 8% per batch and 7% weekly).

The following table summarises the bead material used:

Bead material	**Number of laboratories**
BD Biosciences, cytometer Setup and Tracking beads (CST beads)	29
Beckman-Coulter Flow-Check Fluorospheres	7
Beckman-Coulter Flow-Check Pro Fluorospheres	5
BD Biosciences 7-color setup beads	1
Beckman-Coulter Flow-Set Fluorospheres	1
Not mentioned	6

80% of the participants (n=39) also make use of commercial control material.

The following table summarises the control material used:

Control material	**Number of laboratories**
Streck CD-Chex Plus	10
Beckman-Coulter IMMUNO-TROL Cells	10
BD Biosciences Multi-Check Control	9
BD Biosciences Multi-Check CD4 Low Control	2
Streck CD-Chex Plus CD4 Low, Normal	2
Beckman-Coulter ClearLLab Control Cells Abnormal	2
Beckman-Coulter DxFLEX Daily QC Fluorospheres	2
Streck CD-Chex Plus BC	1
R&D Systems StatusFlow	1

1. Tanqri et al. Validation of Cell-based Fluorescence Assays: Practice Guidelines from the ICSH and ICCS – Part III – Analytical Issues. *Cytometry Part B (Clinical Cytometry) 84B:291–308 (2013)*

CD3+, CD4+, CD8+, CD19+, and NK cells

All the laboratories mentioned applying the whole blood lysis technique, of which 55% used a lyse no wash procedure.

The following table summarises the lysing reagents used (n=48, responding laboratories).

Lysing reagent	Number of laboratories
BD Biosciences FACS Lysing Solution	25
Beckman-Coulter VersaLyse	11
Ammonium chloride (NH_4Cl)	5
Beckman-Coulter Optilyse C	3
BD Biosciences Pharm Lyse	3
Beckman-Coulter Immunoprep reagent system	1

Most laboratories used 6 and 8-colour combinations (n=48, responding laboratories).

	Number of participants				
	$CD3^+$	**$CD4^+$**	**$CD8^+$**	**$CD19^+$**	**NK**
6 colours	23	23	23	23	23
7 colours	10	10	10	10	10
8 colours	12	12	12	12	12
10 colours	3	3	3	3	3

A consensus set of reagents suitable for general use in the diagnosis and monitoring of hematopoietic neoplasms has been repeatedly defined[1,2,3,4,5]. All laboratories used the recommended monoclonal antibody panels for performing CD3, CD4 and CD8 determinations (two colour systems: CD3/CD4 and CD3/CD8; three colour systems: CD3/CD4/CD45 and CD3/CD8/CD45; four colour systems: CD3/CD4/CD8/CD45).

To identify NK cells, 31% of the participants used CD56 alone and 69% used the combination of CD16 and CD56.

All laboratories mentioned their gating technique (n=49), they all used CD45 as gating agent.

Following table displays the sample quality control assessment procedures used by the participating laboratories:

Sample quality control assessment	Number
Lymphosum	19
100% CD45 positive cells[6,7] + lymphosum + CD3 consistency check	12
100% CD45 positive cells[6,7] + lymphosum	10
Lymphosum + CD3 consistency check	6
100% CD45 positive cells[6,7]	2

Lymphosum: sum of CD3+% plus CD19+% plus CD3-CD16+ and/or CD56+% should equal the purity of lymphocytes in the gate ± 5%, with a maximum variability of ≤ 10%.

CD3 consistency check: replicate results within a panel (e.g. CD3+%) for the same sample should be within 5% of each other for FSC/SSC gating or within 3% for CD45/SSC gating.

1. Van Bockstaele DR, Verhasselt B, Philippe J, De Waele M, Offner F, Noens L, et al. Belgian consensus recommendations for flow cytometric immunophenotyping. *Acta Clin Belg. 1999 Apr;54(2):88-98.*
2. Braylan RC, Orfao A, Borowitz MJ, Davis BH. Optimal number of reagents required to evaluate hematolymphoid neoplasias: results of an international consensus meeting. *Cytometry. 2001 Feb 15;46(1):23-7.*
3. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, et al. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry B Clin Cytom. 2007;72 Suppl 1:S14-22.*
4. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood. 2008 Apr 15;111(8):3941-67.*
5. Van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia. 2012 Sep;26(9):1908-75.*
6. Stelzer GT, Shults KE, Loken MR. CD45 gating for routine flow cytometric analysis of human bone marrow specimens. *Ann N Y Acad Sci. 1993;677:265-80.*
7. Nicholson JK, Hubbard M, Jones BM. Use of CD45 fluorescence and side-scatter characteristics for gating lymphocytes when using the whole blood lysis procedure and flow cytometry. *Cytometry. 1996;26:16-21.*

κ and λ % B lymphocytes and κ/λ ratio (43 participants)

All laboratories performed 2 (35%) or more (65%) washing steps. Following table shows the number of washing steps performed by the laboratories.

	2 washing steps	3 washing steps	4 washing steps	Total
Washing before incubation with anti-κ/anti-λ reagents, followed by RBC lysing after ab incubations	11	17		28
Washing/RBC lysing before incubation with anti-κ/anti-λ reagents	4	8	2	14
Incubation with B-cell marker (CD19) before washing and incubation with anti-κ/anti-λ reagents		1		1
Total	15	26	2	43

The majority of the participants (74%) employed polyclonal anti-kappa/anti-lambda reagents.

All laboratories combined anti-kappa and anti-lambda antibodies with CD19 in a single tube.

The participants used different gating strategies to identify lymphocytes: 69% used CD19/SSC, 26% used CD45/SSC followed by CD45/CD19 or CD3/CD19 within the lymphocyte population, and 5% used CD19/SSC and CD20/SSC.

All laboratories that reported their sample quality control assessment indicated that they utilized the sum of the kappa and lambda chain expressing B cells for the technical validation of their analyses.

1.3. Results

Sample receipt

Survey 2024/1: All the laboratories received the samples on day 1 or 2.

Survey 2024/2: All the laboratories received the samples on day 1 or 2.

Survey 2024/3: 98% of the laboratories received the samples on day 1 or 2.

Sample analysis

Survey 2024/1: Most of the laboratories conducted the analyses promptly, with 80% performing them on day 1 and 18% on day 2.

Survey 2024/2: Most of the laboratories conducted the analyses promptly, with 69% performing them on day 1 and 29% on day 2.

Survey 2024/3: Most of the laboratories conducted the analyses promptly, with 76% performing them on day 1 and 22% on day 2.

The evaluation statistics were based exclusively on the results of the clinical laboratories. The evaluation statistics of the WBC count, the percentage of lymphocytes by haematology analyser, and the absolute counts for the different lymphocyte subsets were based exclusively on the results of the clinical laboratories that performed the analyses on day 1 or 2.

The following tables show the medians and coefficients of variation obtained for the different parameters on the samples sent in 2024:

WBC 10^9/L

	Median	CV,%	N
FC/20511	7.37	1.6	48
FC/20512	8.60	2.7	48
FC/20687	7.50	3.2	50
FC/20688	5.78	1.9	50
FC/21146	16.23	1.9	47
FC/21147	7.75	3.5	47

Lymphocytes %
Haematology analyser

	Median	CV,%	N
FC/20511	25.6	2.6	46
FC/20512	16.0	5.6	46
FC/20687	20.6	4.0	49
FC/20688	25.7	3.5	49
FC/21146	30.8	3.4	45
FC/21147	23.2	3.5	45

Lymphocytes %
Flow cytometer

	Median	CV,%	N
FC/20511	25.0	5.6	42
FC/20512	15.4	6.7	42
FC/20687	20.0	7.4	44
FC/20688	24.1	6.8	44
FC/21146	29.9	14.8	43
FC/21147	21.9	10.1	43

CD3 %

	Median	CV,%	N
FC/20511	78.1	2.5	49
FC/20512	73.3	4.1	49
FC/20687	67.6	3.1	52
FC/20688	80.8	2.1	52
FC/21146	70.9	2.7	49
FC/21147	76.6	2.8	49

CD3 10^9/L

	Median	CV,%	N
FC/20511	1.480	6.2	49
FC/20512	1.008	9.9	49
FC/20687	1.033	4.5	51
FC/20688	1.190	6.7	51
FC/21146	3.538	6.6	48
FC/21147	1.370	6.5	48

CD4 %

	Median	CV,%	N
FC/20511	39.3	6.4	49
FC/20512	47.6	4.4	49
FC/20687	45.2	5.6	52
FC/20688	48.6	4.1	52
FC/21146	40.0	5.9	49
FC/21147	39.6	7.7	49

CD4 10^9/L

	Median	CV,%	N
FC/20511	0.740	9.4	49
FC/20512	0.664	12.1	49
FC/20687	0.700	6.0	51
FC/20688	0.711	7.3	51
FC/21146	2.016	8.4	48
FC/21147	0.696	11.0	48

CD8 %

	Median	CV,%	N
FC/20511	36.9	7.8	49
FC/20512	22.4	4.3	49
FC/20687	20.4	5.8	52
FC/20688	25.0	5.0	52
FC/21146	29.2	5.6	49
FC/21147	35.3	6.5	49

CD8 10^9/L

	Median	CV,%	N
FC/20511	0.700	8.6	49
FC/20512	0.310	7.9	49
FC/20687	0.312	9.3	51
FC/20688	0.364	8.2	51
FC/21146	1.446	9.5	48
FC/21147	0.623	10.6	48

CD19 %

	Median	CV,%	N
FC/20511	16.2	11.9	49
FC/20512	10.2	14.5	49
FC/20687	11.6	7.0	52
FC/20688	9.6	10.0	52
FC/21146	14.4	12.8	49
FC/21147	17.9	7.4	49

CD19 10^9/L

	Median	CV,%	N
FC/20511	0.303	13.9	49
FC/20512	0.145	19.4	49
FC/20687	0.177	11.9	51
FC/20688	0.140	13.2	51
FC/21146	0.705	14.0	48
FC/21147	0.312	14.0	48

NK %

	Median	CV,%	N
FC/20511	5.0	22.2	49
FC/20512	15.6	10.9	49
FC/20687	19.9	13.8	52
FC/20688	8.9	11.6	52
FC/21146	13.5	13.2	49
FC/21147	4.9	16.6	49

NK 10^9/L

	Median	CV,%	N
FC/20511	0.094	30.7	49
FC/20512	0.213	16.0	49
FC/20687	0.300	13.1	51
FC/20688	0.130	14.8	51
FC/21146	0.676	13.7	48
FC/21147	0.086	15.5	48

κ % B lymphocytes

	Median	CV,%	N
FC/20511	62.6	11.2	39
FC/20512	61.9	3.2	40
FC/20687	58.0	3.3	44
FC/20688	58.8	5.2	44
FC/21146	61.9	4.1	41
FC/21147	61.0	10.1	41

λ % B lymphocytes

	Median	CV,%	N
FC/20511	36.0	22.8	39
FC/20512	37.5	6.1	40
FC/20687	41.7	3.5	44
FC/20688	40.6	5.3	44
FC/21146	37.4	9.3	41
FC/21147	38.5	19.4	41

κ/λ ratio

	Median	CV,%	N
FC/20511	1.78	35.8	39
FC/20512	1.65	8.7	40
FC/20687	1.38	5.9	44
FC/20688	1.44	11.3	44
FC/21146	1.66	13.4	41
FC/21147	1.59	38.6	41

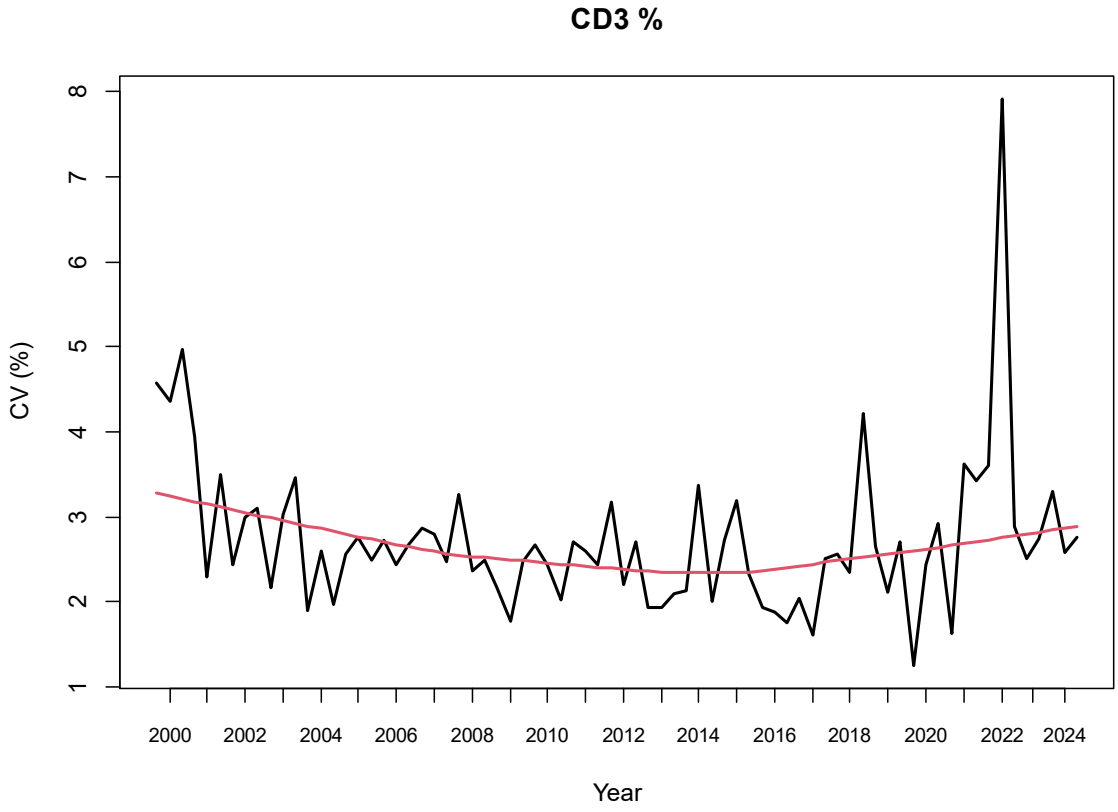
κ+λ % B lymphocytes

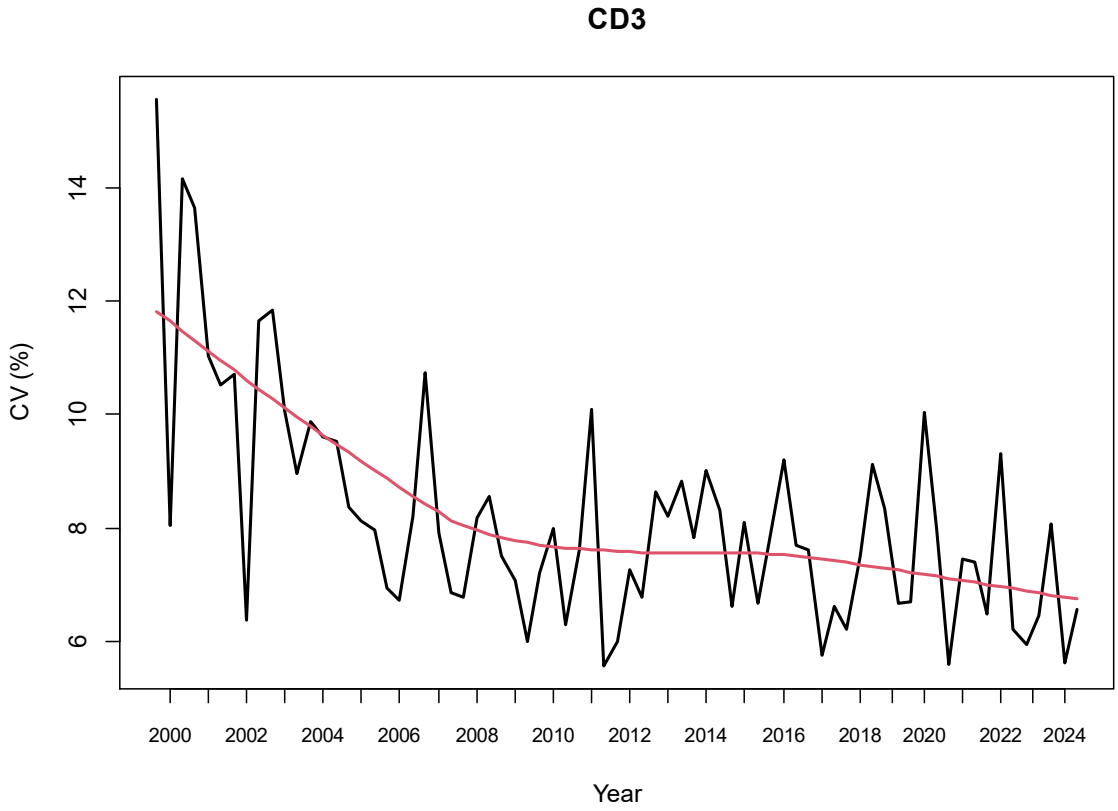
	Median	CV,%	N
FC/20511	99.9	0.8	39
FC/20512	99.9	0.5	40
FC/20687	99.9	0.6	44
FC/20688	99.9	0.4	44
FC/21146	99.7	1.0	41
FC/21147	99.8	0.4	41

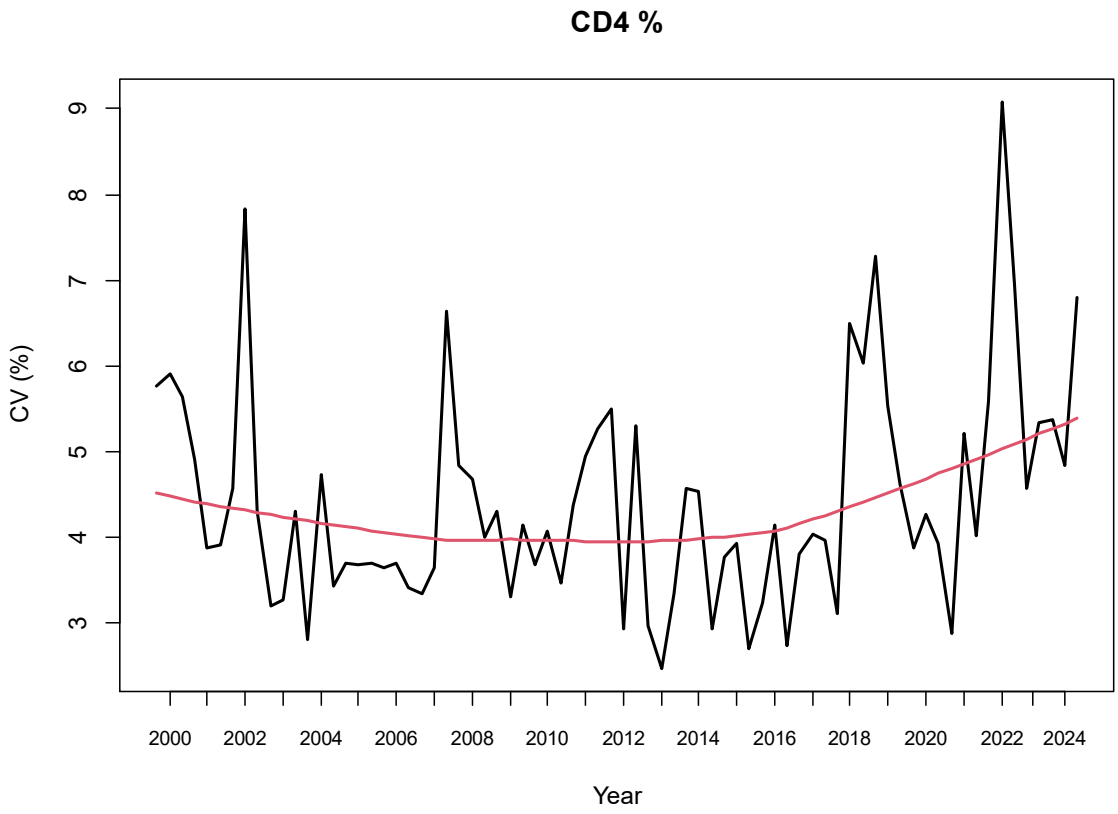
Lymphosum %

	Median	CV,%	N
FC/20511	99.5	0.7	49
FC/20512	99.0	1.3	49
FC/20687	98.8	1.2	52
FC/20688	99.1	0.7	52
FC/21146	99.1	1.3	49
FC/21147	99.4	0.6	49

The following graphs show for the different parameters the evolution of the interlaboratory variability over the years. The black lines show the mean CV per survey. The red lines are a smoothed representation of the black lines and depict the evolution of the mean CV over time.






CD4 %
CV (%)
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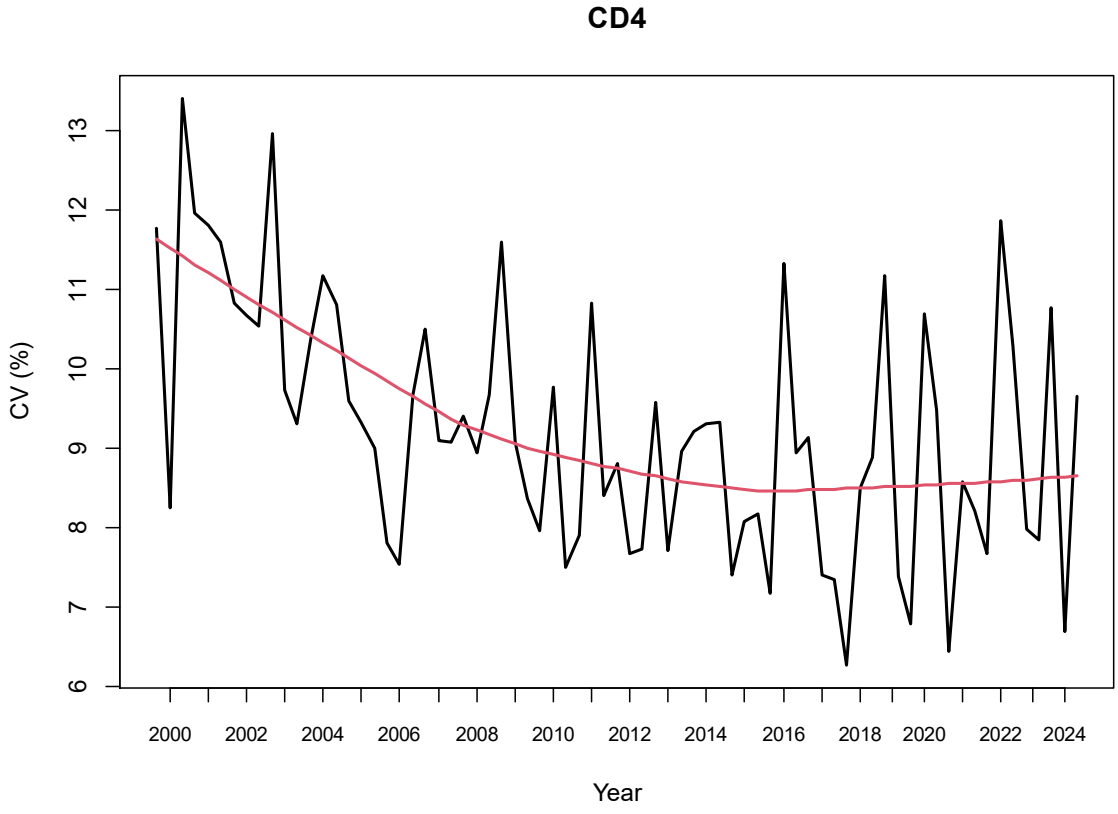
Year


CD4
CV (%)
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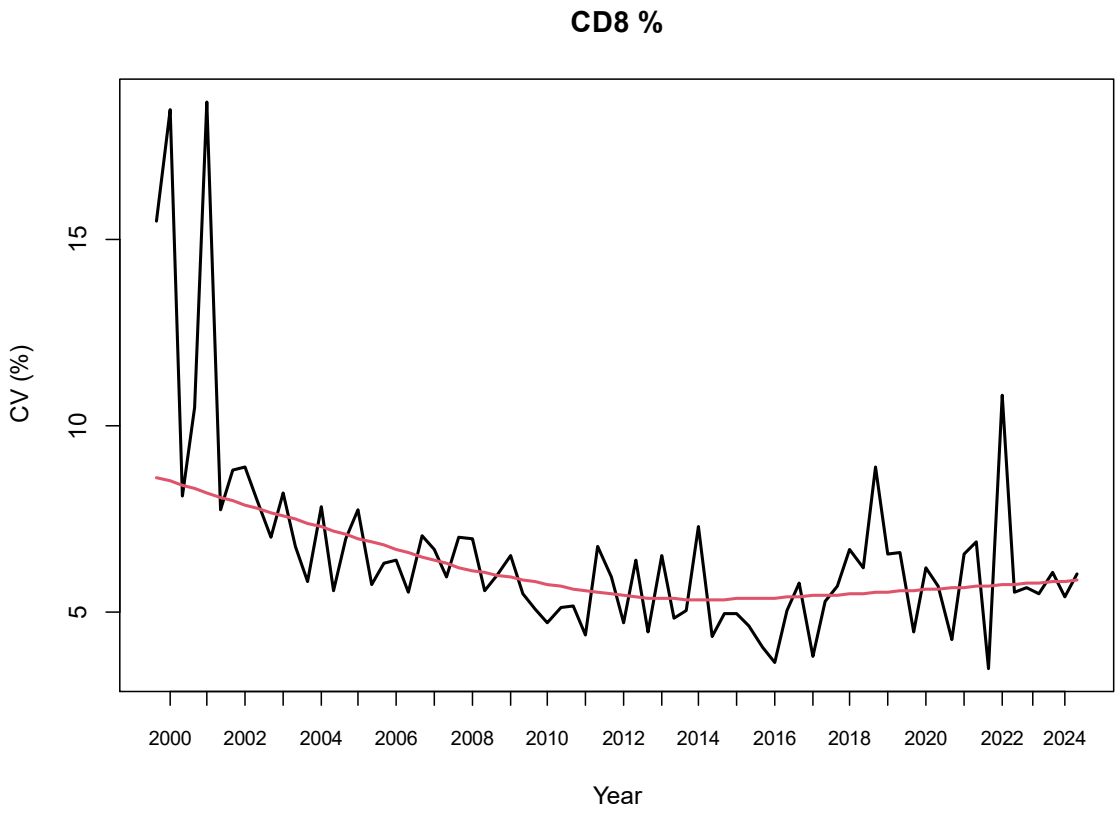
CD8 %
CV (%)
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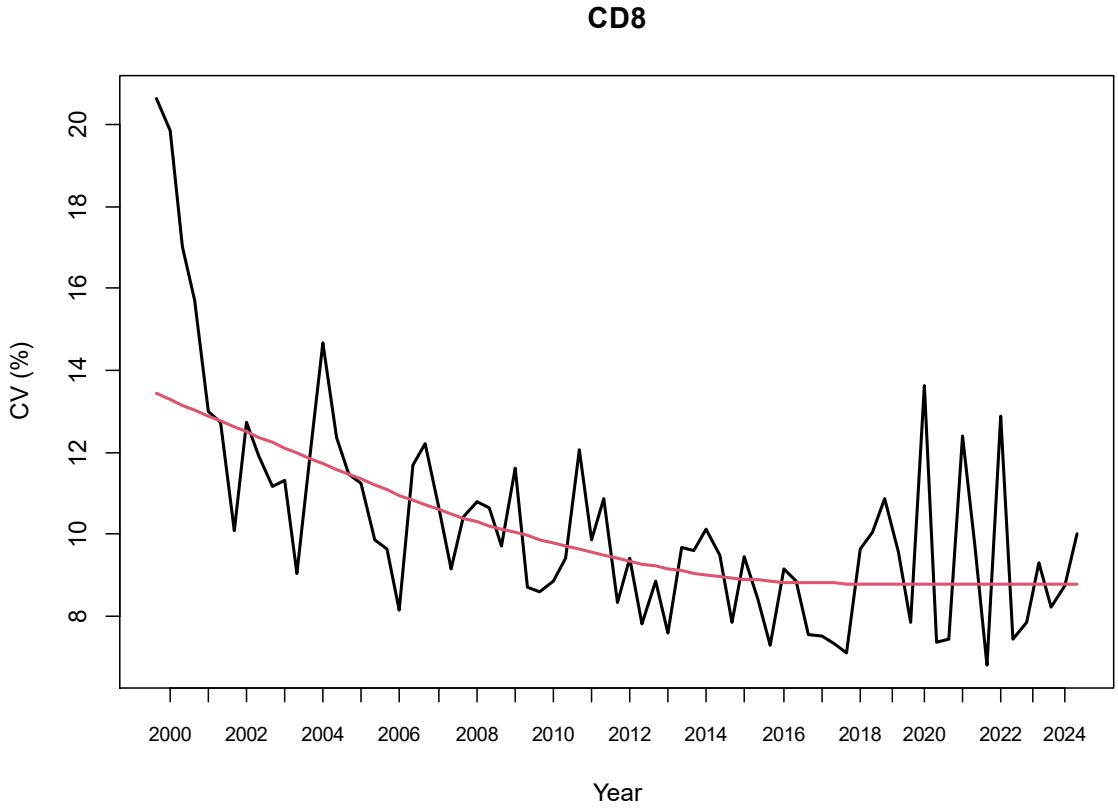
Year


CD8
CV (%)
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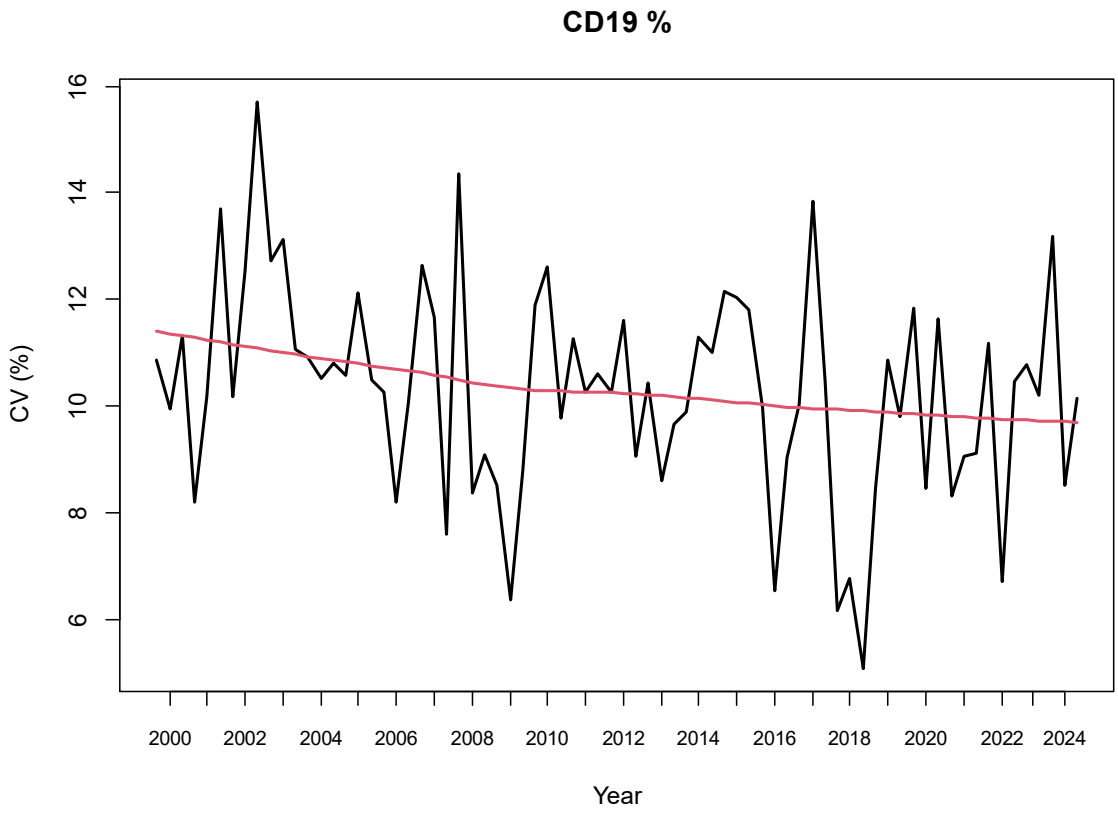
2024
Year


CD19 %
CV (%)
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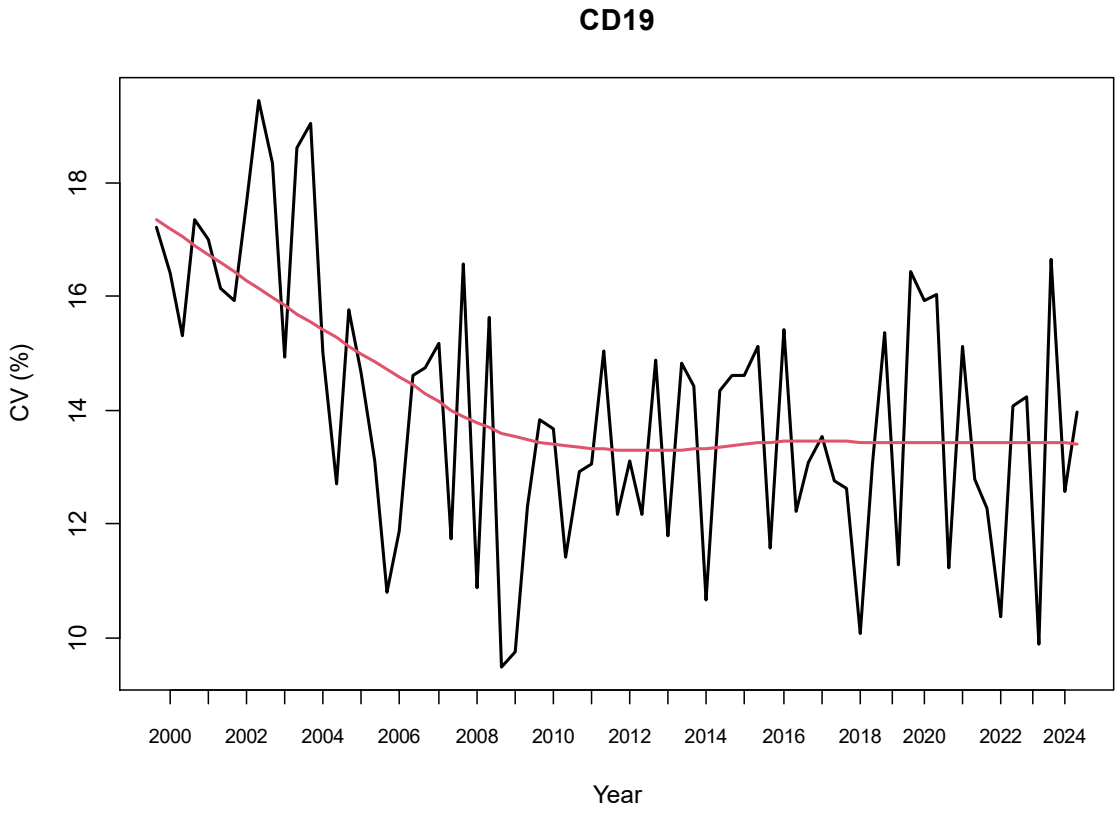
2024
Year


CD19
CV (%)
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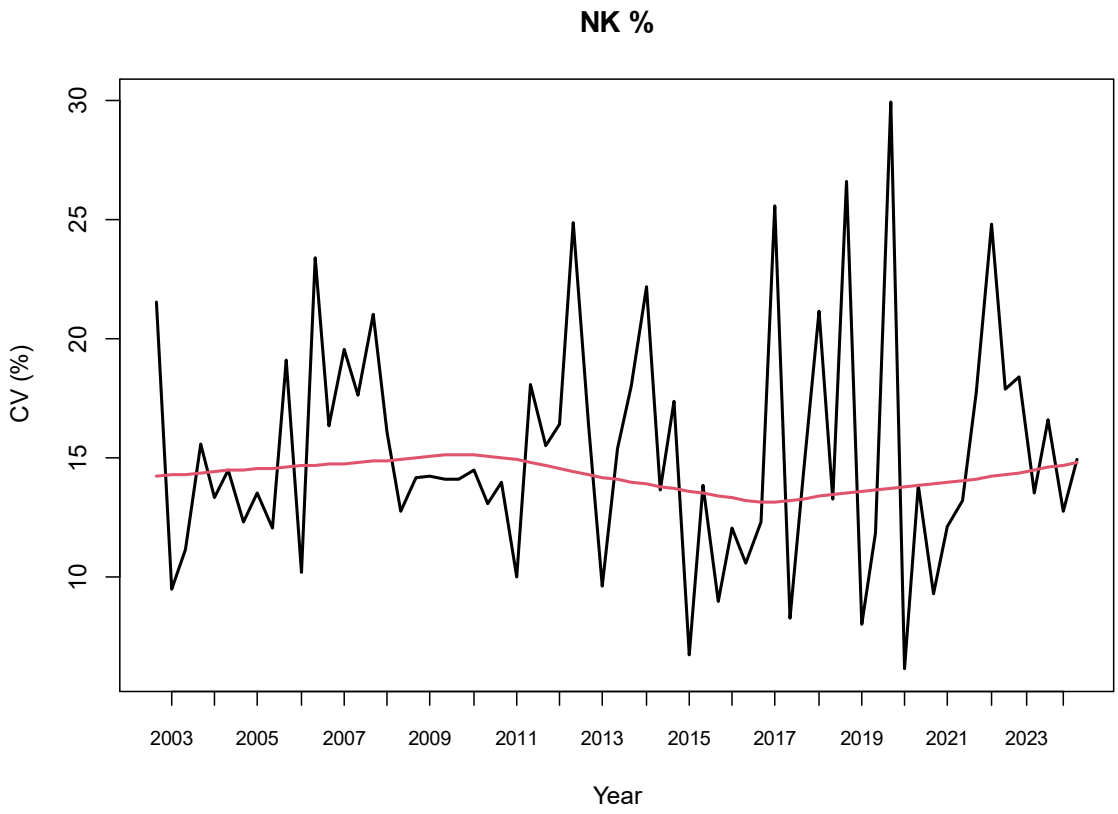
Year


NK %
CV (%)
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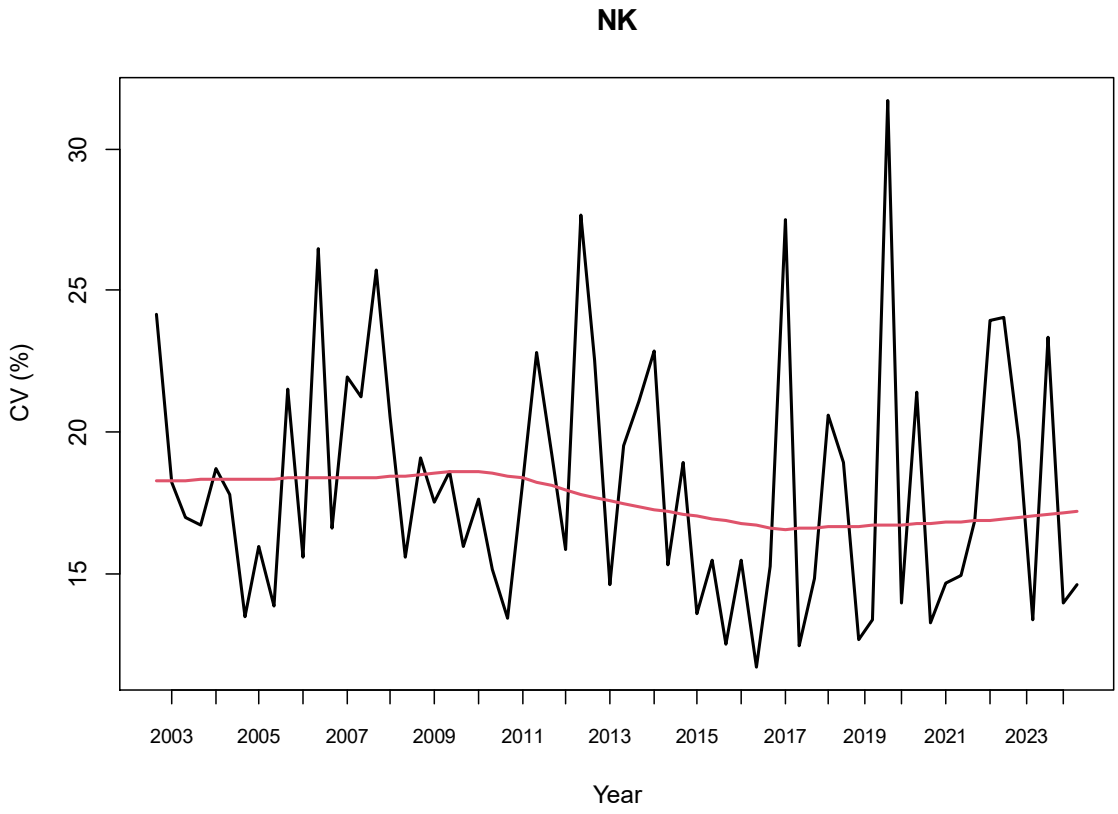
2023
Year


NK
CV (%)
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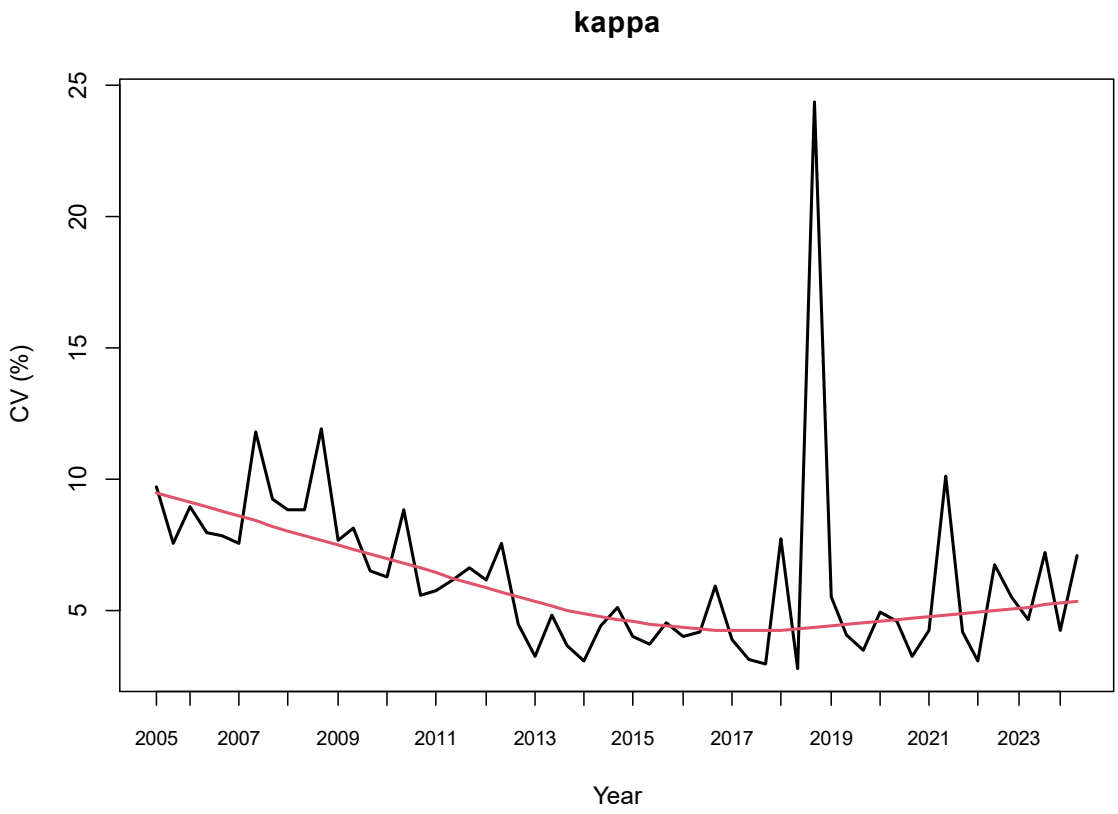
Year


kappa
CV (%)
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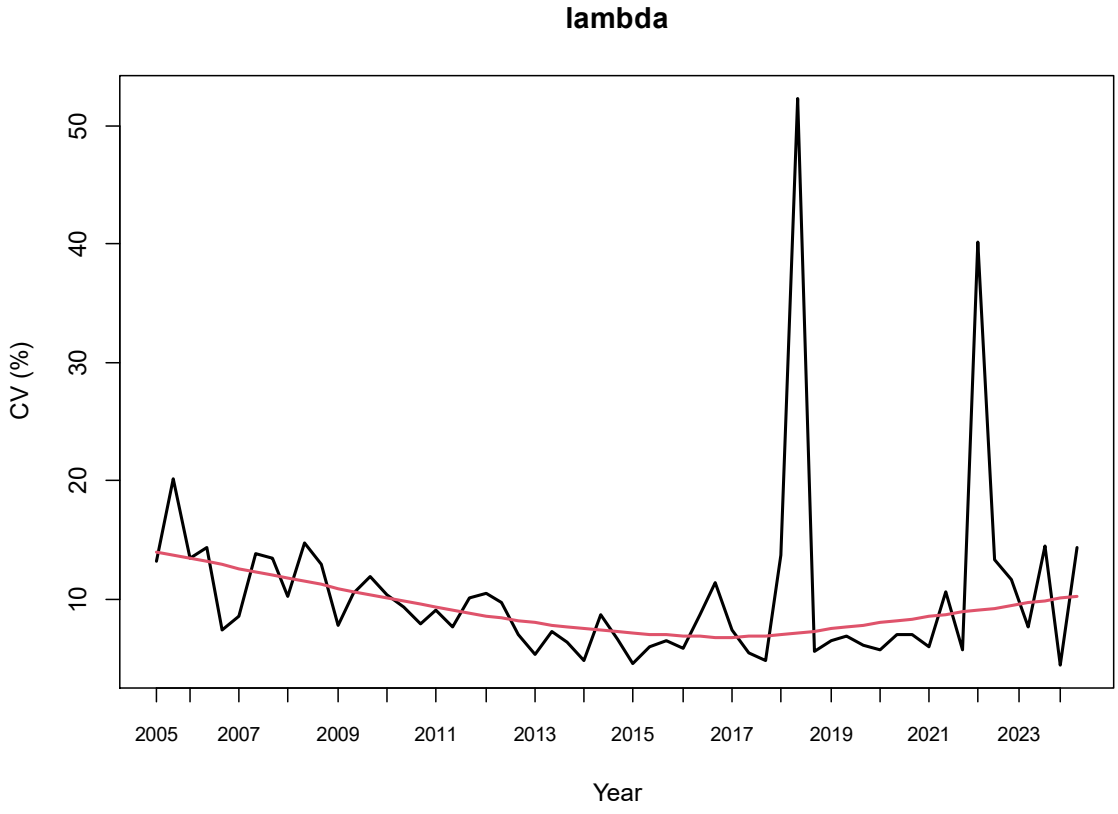
2019
2021
2023
Year


lambda
CV (%)
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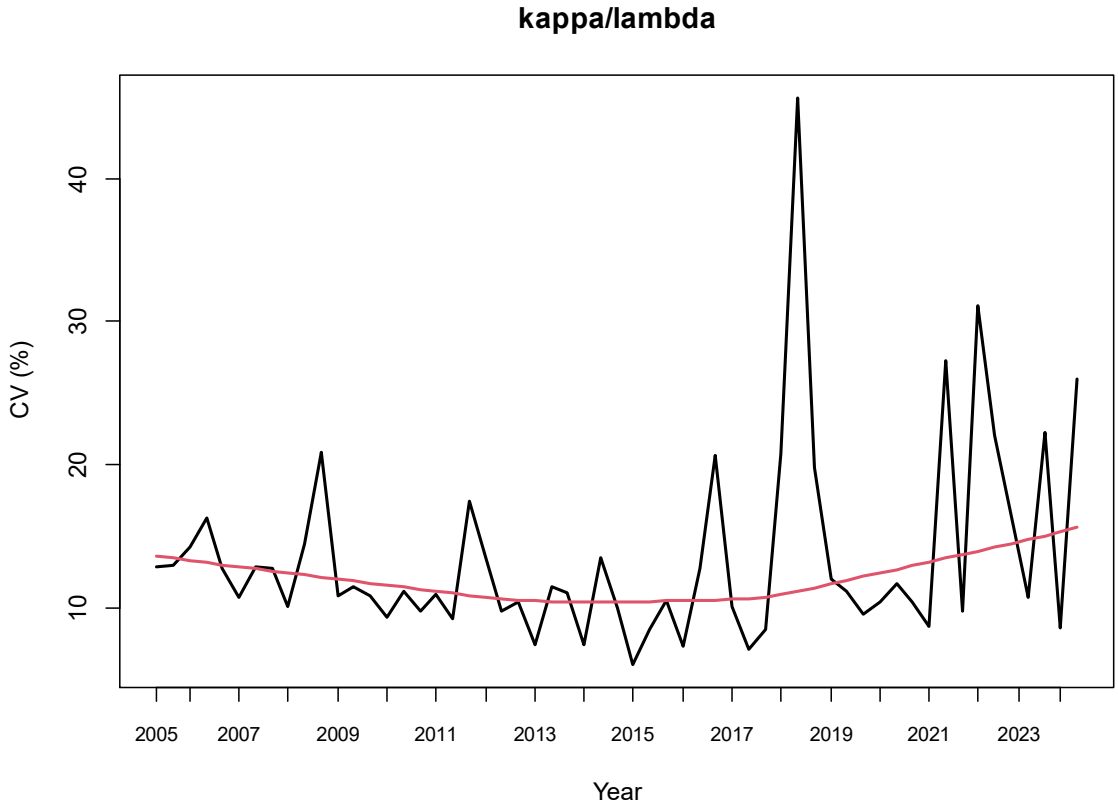
Year


kappa/lambda
CV (%)
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2005
2007
2009
2011
2013
2015
2017
2019
2021
2023
Year

1.4. P_Z evaluation

The performance of the laboratories was scored by means of the P_Z evaluation.

Methodology

Each reported result is evaluated by means of a z-score:

$$z = \left(\frac{x - M}{SD} \right)$$

x: result
M: median
SD: standard deviation

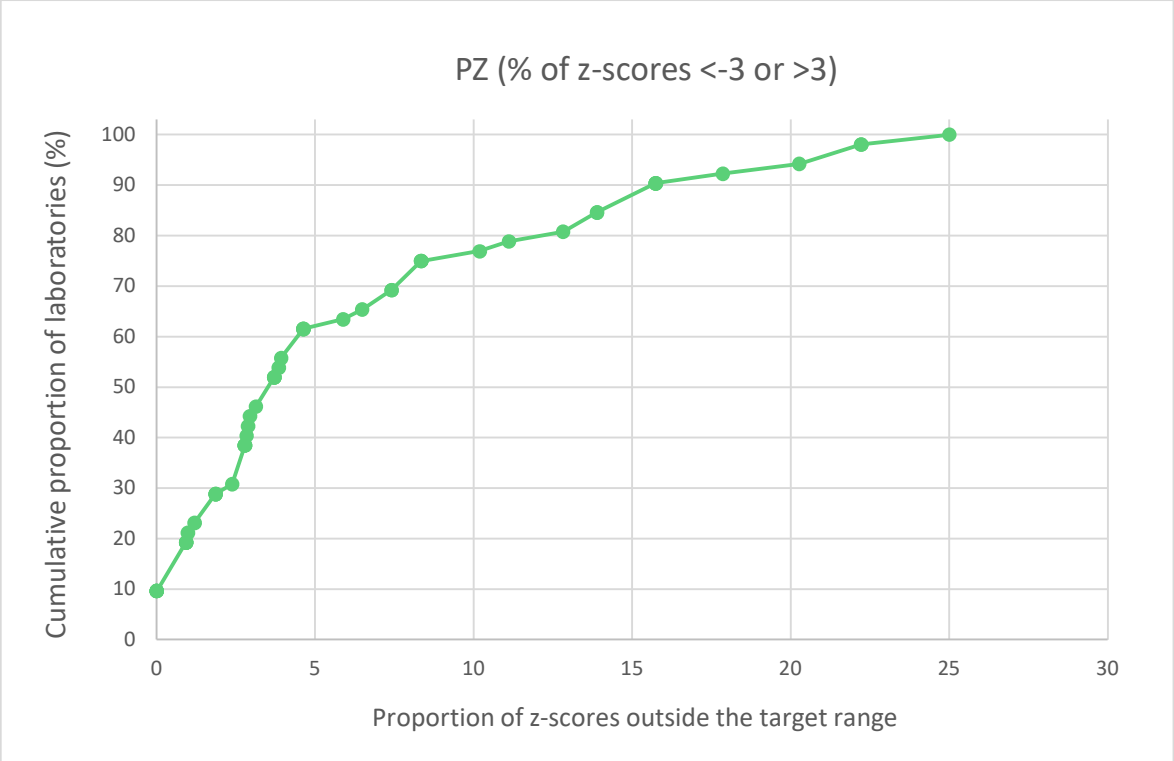
Z-scores reflect the performance of a laboratory with respect to its peer group. Z-scores <-3 or >3 (results falling beyond 3 SD from the median) are considered unacceptable.
The performance of the laboratories is evaluated by means of the percentage of unacceptable z-scores (P_Z, % of results falling beyond 3 SD from the median) obtained in the course of 1 year.

$$P_Z = \left(\frac{N_Z}{N} \right) \times 100 \quad (\%)$$

N_Z: number of results falling beyond 3 SD from the median
N: number of reported results

Each participant is provided with an individual annual report summarising for each sample and parameter the result and z-score and mentioning the global P_Z score. A result falling beyond 3 SD from the median (z-score <-3 or >3) is depicted in bold.

Participants can compare their performance with that of other laboratories by means of the graph below. The P_Z value is situated on the X-axis, the corresponding value on the Y-axis reflects the percentage of laboratories having an equal or better performance.



Participants who obtained ≥ 10% of results with a z-score <-3 or >3 (P_Z value ≥ 10%) are considered as having unsatisfactory performance[1].

If they are interested, participants who reported an outlying result for one or more parameters can contact the members of the expert committee to examine their data in order to find a possible explanation for the erroneous result.

The next table shows the characteristics of the distribution of the P_Z values since 2012: number of evaluated participants (N), average (m) ± standard deviation (SD), percentiles, minimum and maximum:

Year	N	m ± SD	P_{25}	P_{50}	P_{75}	P_{90}	P_{95}	P_{99}	Min-max
2012	48	5.9 ± 7.7	0.8	2.6	10.0	14.4	17.6	32.7	0 - 40.3
2013	46	5.9 ± 6.9	0.8	4.0	9.0	13.9	17.3	29.1	0 - 32.5
2014	47	5.9 ± 7.8	0	3.1	6.9	18.9	22.0	27.8	0 - 28.9
2015	46	5.4 ± 7.1	0.6	3.4	7.4	14.3	17.2	29.9	0 - 32.7
2016	48	6.2 ± 6.7	0.6	3.7	8.8	16.3	20.1	23.5	0 – 25.0
2017	50	5.8 ± 8,8	0.6	2.6	8.3	11.8	23.6	37.7	0 – 49.0
2018	49	6.8 ± 7.5	1.4	4.2	11.1	15.4	19.7	32.2	0 – 34.5
2019	52	6.7 ± 6.7	2.0	5.9	9.0	12.7	17.7	29.6	0 – 37.0
2020	53	8.4 ± 8.4	2.0	5.6	11.1	19.9	25.3	31.5	0 – 32.6
2021	52	6.9 ± 7.1	0.9	4.6	11.3	15.7	20.4	26.5	0 – 29.0
2022	52	6.5 ± 6.3	1.7	4.6	9.0	15.6	20.2	22.3	0 – 22.4
2023	53	7.0 ± 8.2	1.4	4.2	11.1	16.7	22.4	33.1	0 – 35.9
2024	52	6.5 ± 6.7	1.8	3.7	8.8	15.7	21.1	23.6	0 – 25.0

The maximum number of evaluated results per laboratory was 108.

Analysis of the data reveals that, on average, laboratories reported 6.5% of results exceeding 3 SD, while 25% of laboratories reported less than 1.8% of results beyond 3 SD in 2024.

The next table summarises for the different parameters the number of evaluated results and the percentage of results beyond 3 SD:

	2023		2024	
Parameter	**Number of evaluated results**	**% results >3 SD**	**Number of evaluated results**	**% results >3 SD**
Leukocytes 10^9/L	203	3.9	290	6.2
Lymphocytes % HA	195	9.2	280	6.7
Lymphocytes % FC	185	5.9	258	8.5
CD3 %	207	8.2	300	4.6
CD3 10^9/L	203	7.8	296	8.1
CD4 %	207	7.2	300	6.0
CD4 10^9/L	203	8.3	296	7.1
CD8 %	207	4.8	300	4.6
CD8 10^9/L	203	9.3	296	6.4
CD19 %	207	8.2	300	5.6
CD19 10^9/L	203	8.3	296	7.4
NK cells %	207	4.3	300	5.0
NK cells 10^9/L	203	7.8	296	7.7
κ % B lymphocytes	173	6.3	249	3.6
λ % B lymphocytes	173	6.3	249	4.0
κ/λ ratio	173	8.0	249	6.0
κ+λ % B lymphocytes	173	8.6	249	11.2
Lymphosum	207	6.2	300	5.3

1. Wood B et al. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria. *Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):315-23.*

The following 3 tables show the percentage of results beyond 3 SD according to the methodology used (double vs single platform, lyse no wash vs lyse wash, use of polyclonal vs monoclonal antibodies for the determination of the κ and λ chain expressing B cells):

Parameter	Number of evaluated results		% results >3 SD	
	Double platform	Single platform	Double platform	Single platform
CD3 10^9/L	268	28	6%	25%
CD4 10^9/L	268	28	6%	18%
CD8 10^9/L	268	28	5%	18%
CD19 10^9/L	268	28	7%	14%
NK cells 10^9/L	268	28	7%	14%

Parameter	Number of evaluated results		% results >3 SD	
	Lyse and wash	Lyse no wash	Lyse and wash	Lyse no wash
CD3 %	142	158	6%	4%
CD3 10^9/L	138	158	8%	8%
CD4 %	142	158	10%	3%
CD4 10^9/L	138	158	9%	6%
CD8 %	142	158	8%	2%
CD8 10^9/L	138	158	8%	5%
CD19 %	142	158	8%	4%
CD19 10^9/L	138	158	9%	6%
NK cells %	142	158	6%	4%
NK cells 10^9/L	138	158	7%	8%
Lymphosum	142	158	5%	6%

Parameter	Number of evaluated results		% results >3 SD	
	Monoclonal anti-κ/anti-λ reagent	Polyclonal anti-κ/anti-λ reagent	Monoclonal anti-κ/anti-λ reagent	Polyclonal anti-κ/anti-λ reagent
κ % B lymphocytes	57	192	2%	4%
λ % B lymphocytes	57	192	4%	4%
κ/λ ratio	57	192	2%	7%
κ+λ % B lymphocytes	57	192	16%	10%

The following table shows the percentage of results beyond 3 SD according to the monitoring of the flow cytometer performance.

Parameter	Commercial control material usage			
	Number of evaluated results		% results >3 SD	
	YES	NO	YES	NO
CD3 %	222	78	5%	4%
CD3 10^9/L	218	78	9%	5%
CD4 %	222	78	4%	13%
CD4 10^9/L	218	78	7%	8%
CD8 %	222	78	5%	3%
CD8 10^9/L	218	78	6%	6%
CD19 %	222	78	5%	6%
CD19 10^9/L	218	78	9%	4%
NK cells %	222	78	5%	4%
NK cells 10^9/L	218	78	10%	1%
Lymphosum	222	78	6%	4%

2. CD34+ STEM CELL ENUMERATION

2.1. Surveys

A triannual external quality assessment (EQA) scheme for the enumeration of CD34+ hematopoietic stem cells is operational in Belgium since 2011.

In 2024, two surveys were conducted, in May (FC/20466, FC/20467) and November (FC/20615, FC/20616).

Upon dispatch via Taxipost 24h service, notification of the control material's shipment was promptly communicated to the participating laboratories via electronic mail on the initial day of the survey.

The four samples provided were stabilized, affording the laboratories the flexibility to conduct analyses at any juncture during the survey timeframe.

The participating laboratories were instructed to undertake the quantification of CD34+ stem cells utilizing flow cytometry, to record the date of receipt, the date of acquisition, and to furnish comprehensive details encompassing the flow cytometer model employed, the methodology of sample preparation, the source of the antibodies utilized, the gating strategy, and the data analysis software used.

Twenty clinical laboratories participated in these surveys.

2.2. Methodology of the clinical laboratories Survey 2024/3 (n=20)

Single or dual-platform

75% of the laboratories used a single-platform approach.

- Among these, 11 laboratories utilized Trucount technology (BD Biosciences).
- 3 laboratories employed Flow-Count or Stem-count beads (Beckman-Coulter).
- One participant used a volumetric single platform approach with the MACSQuant analyzer (Miltenyi Biotec).

The next table gives an overview of the **flow cytometers** used:

Flow cytometer	Number of laboratories
BD Biosciences FACSLyric	9
Beckman-Coulter Navios	4
BD Biosciences FACSCanto II	4
Beckman Coulter AQUIOS CL	2
Miltenyi Biotec MACSQuant analyzer	1

Sample preparation

Among the participants, twelve used a sample volume of 100 µL, five used 50 µL, one used 43 µL, another used 30 µL, and one more used 25 µL. All participants employed a lyse-no-wash method.

Below is a summary of the lysing reagents used:

Lysing reagent	Number of laboratories
BD Biosciences Ammonium chloride lysing solution	7
Ammonium chloride (NH_4Cl)	5
BD Biosciences Pharm Lyse	3
Beckman-Coulter VersaLyse Lysing Solution	1
Beckman-Coulter Ammonium chloride	1
BD Biosciences FACS Lysing Solution	1
Qiagen EL-buffer	1
Beckman-Coulter AQUIOS STEM Lysing Solution	1

Monoclonal antibodies

In all laboratories except one (PC5.5/PE-Cy5.5), a phycoerythrin (PE)-conjugated CD34 monoclonal antibody was used.

Except for three participants (Horizon V500, Krome Orange, VioBlue), all others utilized a fluorescein isothiocyanate (FITC)-conjugated CD45 monoclonal antibody.

Gating strategy

Out of the participants, 14 followed the ISHAGE (International Society of Hematotherapy and Graft Engineering) gating protocol, 4 utilized the BD Biosciences Stem Cell Enumeration kit, one participant employed the BD Biosciences ProCount Kit, and another used the Stem-Kit from Coulter/Immunotech.

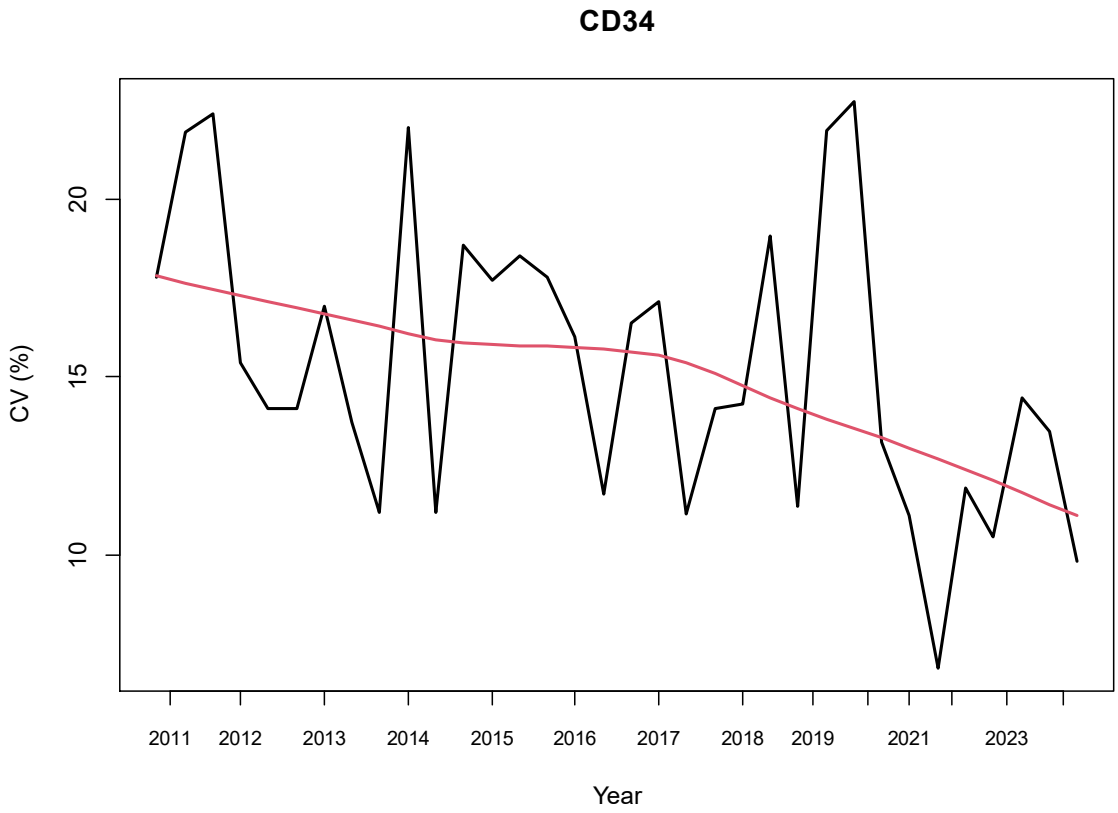
2.3. Results

Since the samples were stabilized, the laboratories were able to carry out the analysis throughout the full duration of survey. Statistics for the evaluation are therefore based on all results from the clinical laboratories regardless of the date of analysis.

The following table shows the median % viable CD34+ cells within total WBC and the median absolute CD34+ cell counts and coefficients of variation obtained for the samples sent in 2024:

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/µL	CV %	N
FC/20466	0.160	12.7	20	9.9	13.5	20
FC/20467	0.640	8.1	20	39.7	11.5	20
FC/20615	0.130	9.4	20	8.3	9.8	20
FC/20616	0.489	5.1	20	30.1	4.4	20

The following graph shows the evolution of the interlaboratory variability over the years. The black line shows the mean CV per survey. The red line is a smoothed representation of the black line and depicts the evolution of the mean CV over time.



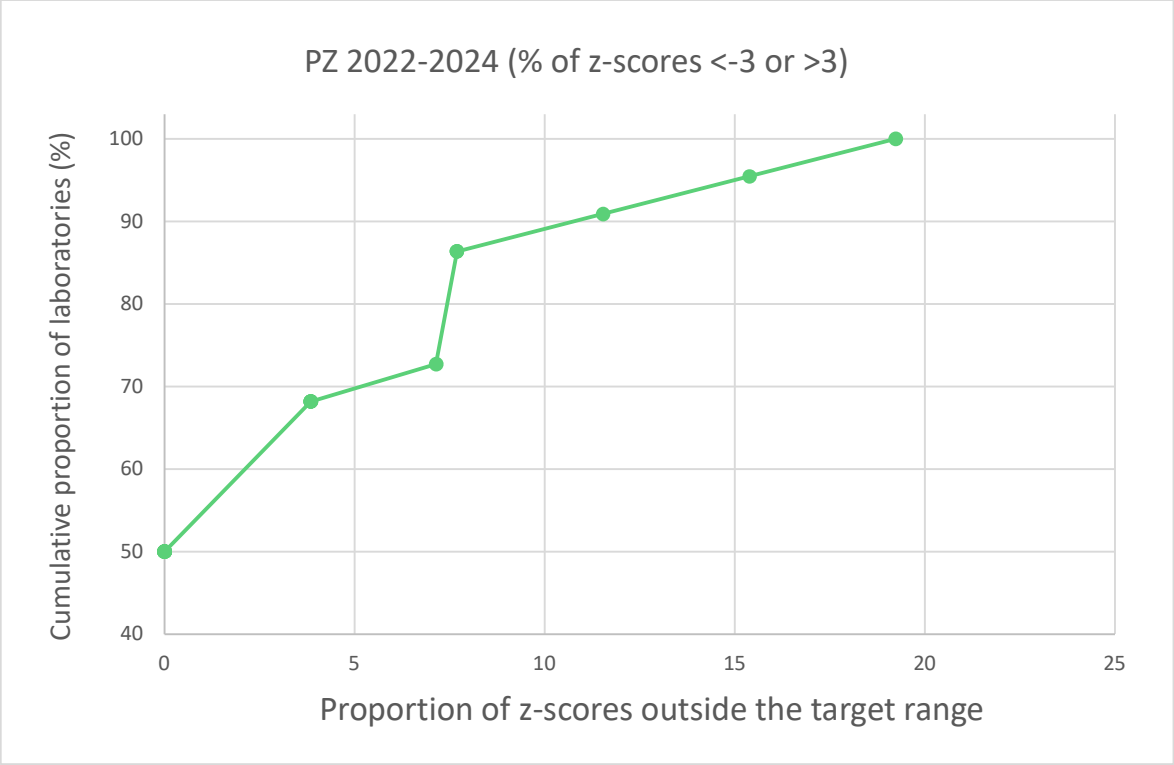
2.4. PZ evaluation

The performance of the laboratories was examined by means of the P_Z evaluation.

Given the very limited number of results available per year (2022: n=6, 2023: n=12, 2024: n=8), the P_Z evaluation was based on the results obtained over 3 years.

Each participant is provided with an individual annual report summarising for each sample and parameter the result and z-score and mentioning the global P_Z score. A result falling beyond 3 SD from the median (z-score <-3 or >3) is depicted in bold.

Participants can compare their performance with that of other laboratories by means of the graph below. The Pz value is situated on the X-axis, the corresponding value on the Y-axis reflects the percentage of laboratories having an equal or better performance.



Participants who obtained ≥ 10% of results with a z-score <-3 or >3 (PZ value ≥ 10%) are considered as having unsatisfactory performance.

Participants who reported an outlying result for one or more parameters are encouraged to engage with the expert committee. This collaborative review of their data aims to elucidate potential factors contributing to the aberrant results.

The next table shows the characteristics of the distribution of the P_Z values during the period 2022-2024: number of evaluated participants (N), average (m) ± standard deviation (SD), percentiles, minimum and maximum:

Period	N	m ± SD	P_{25}	P_{50}	P_{75}	P_{90}	P_{95}	P_{99}	Min-max
2022-2024	22	4.1 ± 5.5	0	1.9	7.6	11.1	15.2	18.4	0 – 19.2

During the period 2022-2024, the maximum of evaluated results per laboratory was 26.
Analysis of the data reveals that, on average, laboratories documented 4.1% of results that exceeded the threshold of 3 SD.
Furthermore, a significant 50% (eleven laboratories) reported no results beyond 3 SD during this period.

END