BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
COMMITTEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

DEFINITIVE GLOBAL ANNUAL REPORT

FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS

CD34+ STEM CELL ENUMERATION

2023

Sciensano/Flow cytometry/88-E

Biological health risks
Quality of laboratories
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EEQ Cytométrie en flux : Analyse des sous-populations lymphocytaires et Numération des cellules souches CD34+ | sciensano.be

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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 2023, surveys were conducted in February (FC/19753, FC/19754), May (FC/19939, FC/19940) and November (FC/20326, FC/20327).

51 Belgian clinical laboratories participated in these surveys.

1.2. Methodology of the Belgian clinical laboratories Survey 2023/3 (n=51)

Five laboratories (10%) used a single platform approach for determining the absolute lymphocyte subset counts. Of these laboratories, 3 used Flow-Count beads (Beckman-Coulter) and 2 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	38
Beckman Coulter UniCel DxH 800 / DxH 900	6
Siemens Advia 2120	1
Sysmex XE 2100/XE 5000	1
Abbott Cell-Dyn Ruby	1
Not mentioned	4

Flow cytometer	Number of participants
BD Biosciences FACSLyric	21
Beckman Coulter Navios	13
BD Biosciences FACSCanto II	9
Beckman Coulter DxFLEX	4
Beckman Coulter Cytomics FC 500	1
Beckman Coulter AQUIOS CL	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 (78% daily, 10% per batch, 10% weekly, and 2% twice a week).

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	10
Beckman-Coulter Flow-Check Pro Fluorospheres	6
BD Biosciences 7-color setup beads	2
Beckman-Coulter Flow-Set Fluorospheres	1
Beckman-Coulter CytoFLEX Daily QC Fluorospheres	1
Not mentioned	1

78% of the participants (n=40) also make use of commercial control material.

The following table summarises the control material used:

Control material	**Number of laboratories**
Streck CD-Chex Plus	11
Beckman-Coulter IMMUNO-TROL Cells	11
BD Biosciences Multi-Check Control	10
BD Biosciences Multi-Check CD4 Low Control	2
Streck CD-Chex Plus CD4 Low, Normal	2
Streck CD-Chex Plus BC	1
Beckman-Coulter ClearLLab Control Cells Normal	1
Beckman-Coulter ClearLLab Control Cells Abnormal	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 47% 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	26
Beckman-Coulter VersaLyse	9
Ammonium chloride (NH_4Cl)	4
Beckman-Coulter Optilyse C	4
BD Biosciences Pharm Lyse	3
Beckman-Coulter Immunoprep reagent system	1
Sysmex CyLyse FX	1

Most laboratories used 6 and 8-colour combinations (n=50, responding laboratories).

	Number of participants				
	$CD3^+$	**$CD4^+$**	**$CD8^+$**	**$CD19^+$**	**NK**
6 colours	20	20	20	20	20
7 colours	5	5	5	5	5
8 colours	15	15	15	15	15
10 colours	10	10	10	10	9

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, 29% of the participants used CD56 alone and 71% used the combination of CD16 and CD56.

All laboratories mentioned their gating technique (n=51), 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	20
100% CD45 positive cells[6,7] + lymphosum + CD3 consistency check	13
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 (45 participants)

All laboratories performed 2 (36%) or more (64%) 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	12	18		30
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	16	27	2	45

The majority of the participants (73%) 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: 77% used CD19/SSC, 18% 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 2023/1: A postal strike prevented the laboratories from receiving the samples within 48 hours of collection.

Survey 2023/2: The samples arrived on day 1 or 2 for 98% of the Belgian laboratories.

Survey 2023/3: All the Belgian laboratories received the samples on day 1 or 2.

Sample analysis

Survey 2023/1: Of the Belgian laboratories that participated in the survey, 69% performed the analyses on day 3 and 31% on day 4. The committee of experts decided not to evaluate the laboratories for any parameter.

Survey 2023/2: The analyses were performed on day 1 by 64% of the Belgian laboratories and on day 2 by 34%.

Survey 2023/3: All the Belgian laboratories performed the analyses on day 1 or 2: 71% on day 1 and 29% on day 2.

The evaluation statistics were based exclusively on the results of the Belgian 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 Belgian 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 2023:

WBC 10^9/L

	Median	CV,%	N
FC/19939	5.17	2.8	52
FC/19940	6.66	3.0	52
FC/20326	5.10	2.2	49
FC/20327	9.43	2.1	50

Lymphocytes %
Haematology analyser

	Median	CV.%	N
FC/19939	27.2	2.7	51
FC/19940	24.0	2.3	51
FC/20326	28.8	3.3	46
FC/20327	32.6	3.6	47

Lymphocytes %
Flow cytometer

	Median	CV.%	N
FC/19939	26.6	11.7	47
FC/19940	22.9	8.4	47
FC/20326	27.8	7.7	45
FC/20327	31.9	6.3	46

CD3 %

	Median	CV.%	N
FC/19939	68.5	2.3	53
FC/19940	77.7	2.8	53
FC/20326	79.4	2.2	50
FC/20327	62.4	3.3	51

CD3 10^9/L

	Median	CV.%	N
FC/19939	0.970	6.4	52
FC/19940	1.244	5.5	52
FC/20326	1.158	6.5	49
FC/20327	1.902	6.5	50

CD4 %

	Median	CV.%	N
FC/19939	44.0	3.2	53
FC/19940	41.1	5.9	53
FC/20326	56.2	2.5	50
FC/20327	33.5	8.2	51

CD4 10^9/L

	Median	CV.%	N
FC/19939	0.620	7.9	52
FC/19940	0.650	8.1	52
FC/20326	0.811	7.2	49
FC/20327	1.020	8.5	50

CD8 %

	Median	CV.%	N
FC/19939	22.0	3.7	53
FC/19940	34.9	7.6	53
FC/20326	21.2	5.6	50
FC/20327	27.3	5.4	51

CD8 10^9/L

	Median	CV.%	N
FC/19939	0.310	5.7	52
FC/19940	0.556	10.0	52
FC/20326	0.312	10.4	49
FC/20327	0.825	8.2	50

CD19 %

	Median	CV.%	N
FC/19939	13.6	9.3	53
FC/19940	18.0	12.3	53
FC/20326	7.4	11.0	50
FC/20327	13.0	9.4	51

CD19 10^9/L

	Median	CV.%	N
FC/19939	0.192	13.1	52
FC/19940	0.284	15.4	52
FC/20326	0.108	8.9	49
FC/20327	0.394	10.9	50

NK %

	Median	CV.%	N
FC/19939	17.2	9.0	53
FC/19940	3.2	27.8	53
FC/20326	12.2	12.2	50
FC/20327	22.8	14.8	51

NK 10^9/L

	Median	CV.%	N
FC/19939	0.240	11.1	52
FC/19940	0.055	28.3	52
FC/20326	0.177	13.0	49
FC/20327	0.691	13.8	50

κ % B lymphocytes

	Median	CV.%	N
FC/19939	58.2	2.9	45
FC/19940	62.2	8.1	45
FC/20326	54.0	3.8	41
FC/20327	63.0	5.4	42

λ % B lymphocytes

	Median	CV.%	N
FC/19939	41.3	5.7	45
FC/19940	36.5	17.4	45
FC/20326	45.0	3.6	41
FC/20327	36.0	11.5	42

κ/λ ratio

	Median	CV.%	N
FC/19939	1.42	7.8	45
FC/19940	1.70	24.8	45
FC/20326	1.20	6.8	41
FC/20327	1.72	14.6	42

κ+λ % B lymphocytes

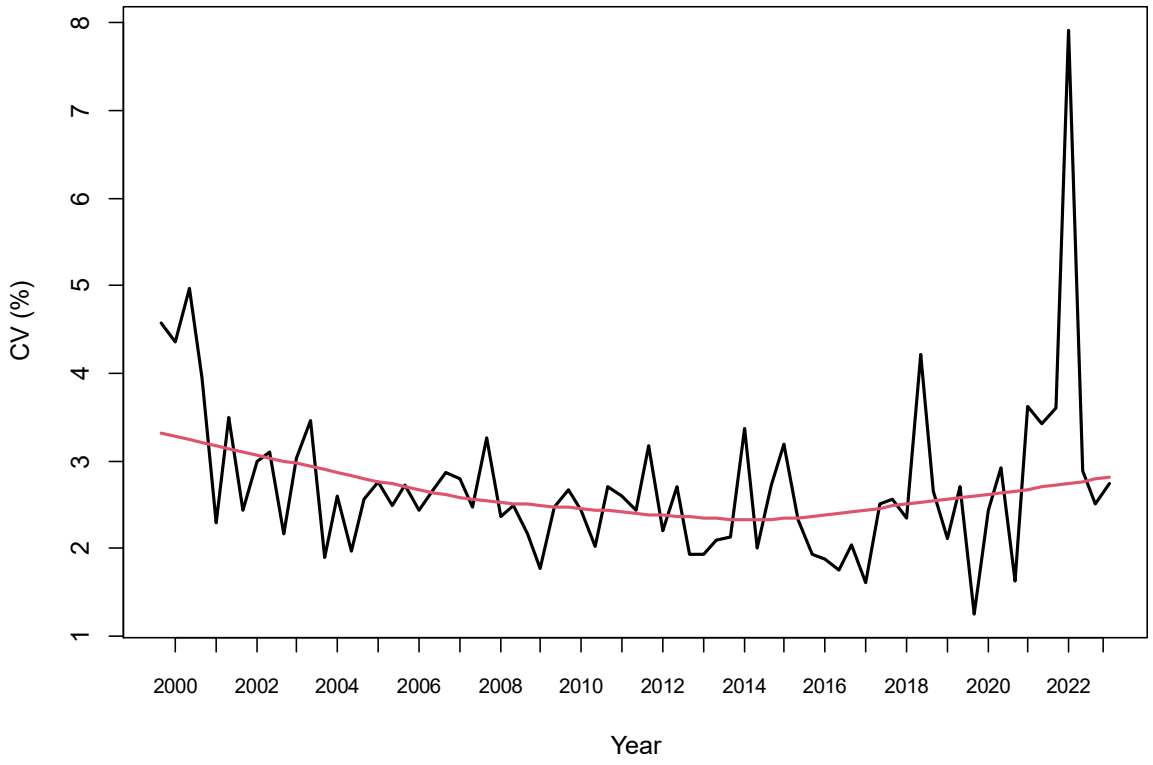
	Median	CV.%	N
FC/19939	99.8	0.5	45
FC/19940	99.8	1.2	45
FC/20326	99.8	0.7	41
FC/20327	99.9	0.8	42

Lymphosum %

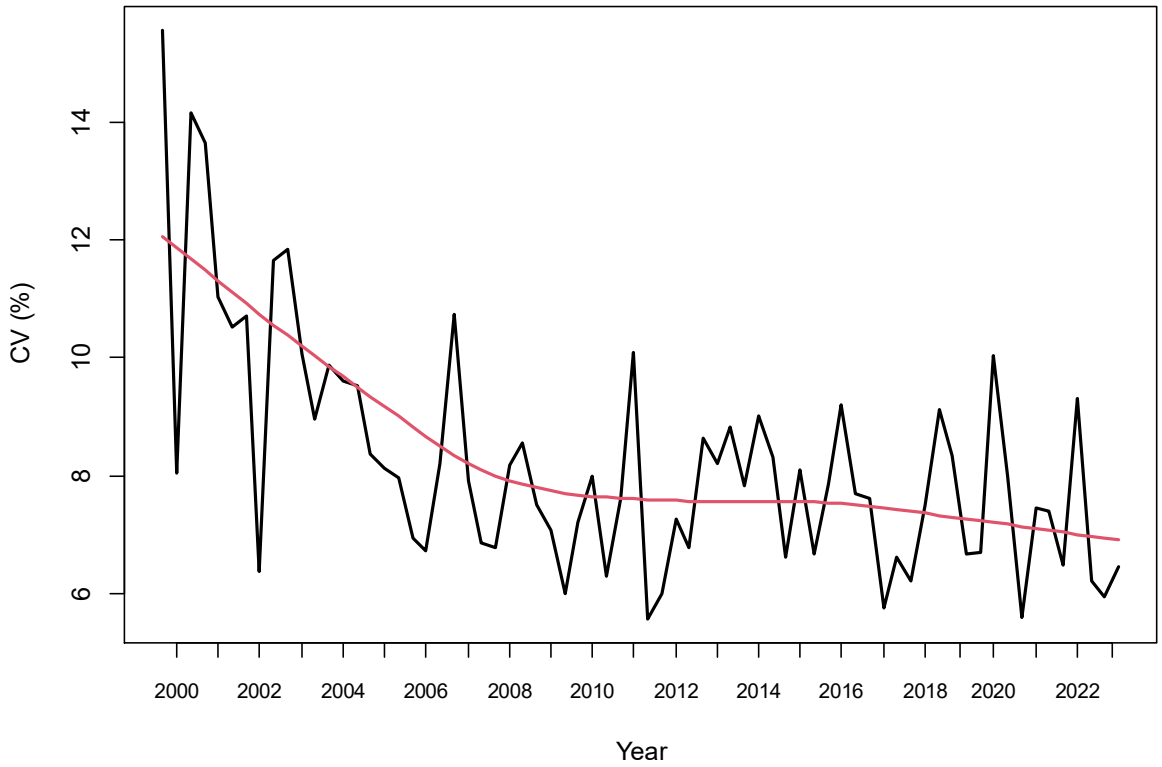
	Median	CV.%	N
FC/19939	99.0	1.1	53
FC/19940	99.3	1.0	53
FC/20326	99.4	0.8	50
FC/20327	99.1	1.6	51

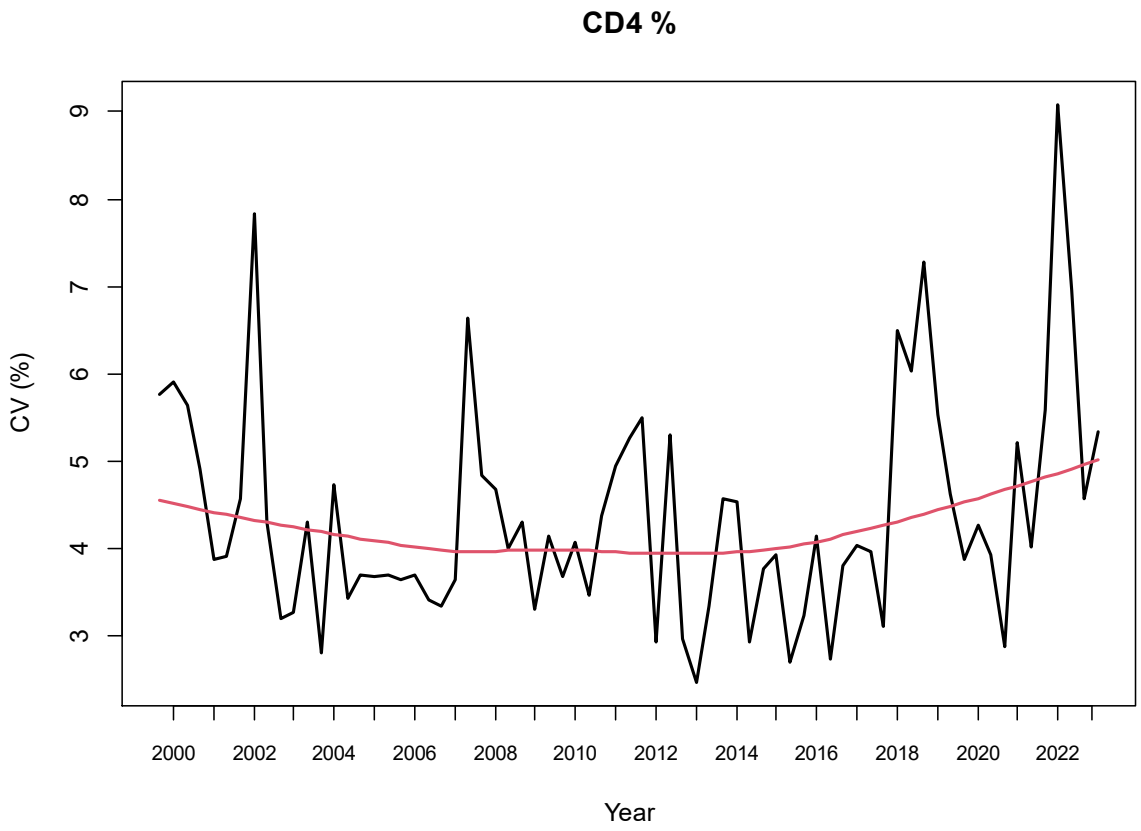
The following graphs show for the different parameters the evolution of the interlaboratory variability over the years. The black lines show the mean CV per survey. The red lines are a smoothed representation of the black lines and depict the evolution of the mean CV over time.

CD3 %



CD3




CD4 %
CV (%)
3
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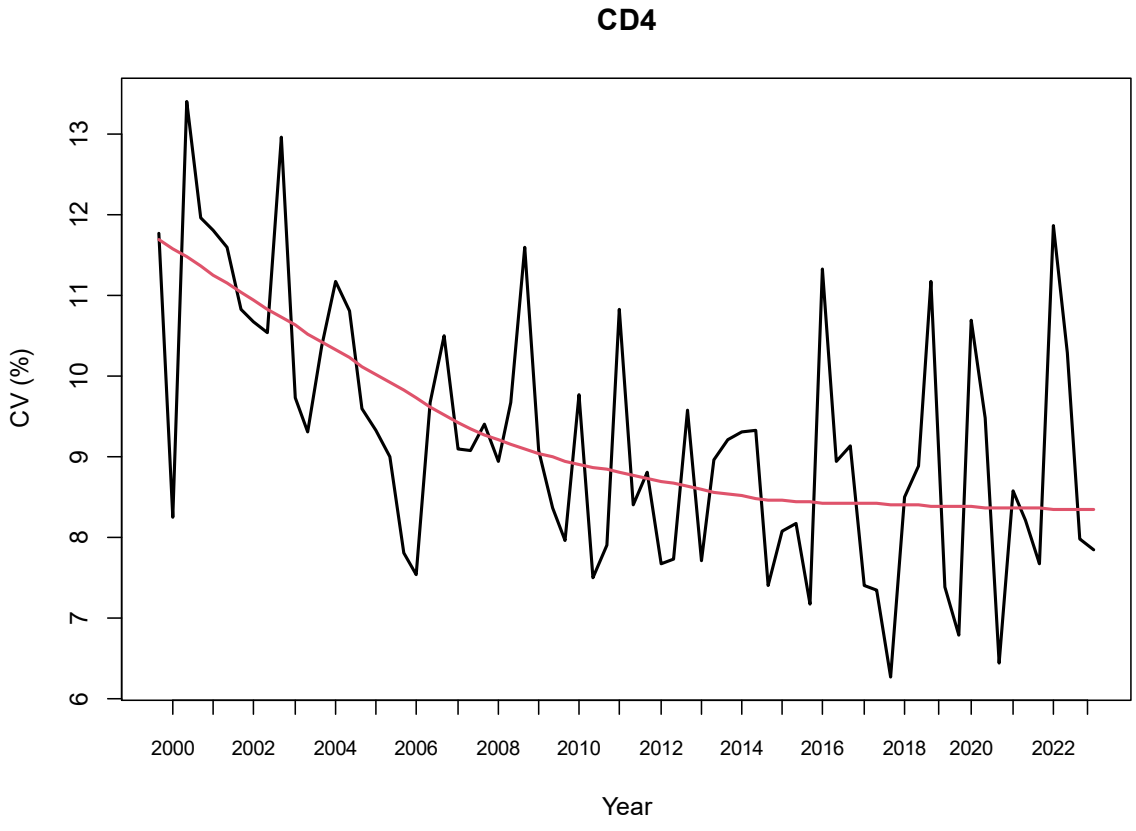
2020
2022
Year


CD4
CV (%)
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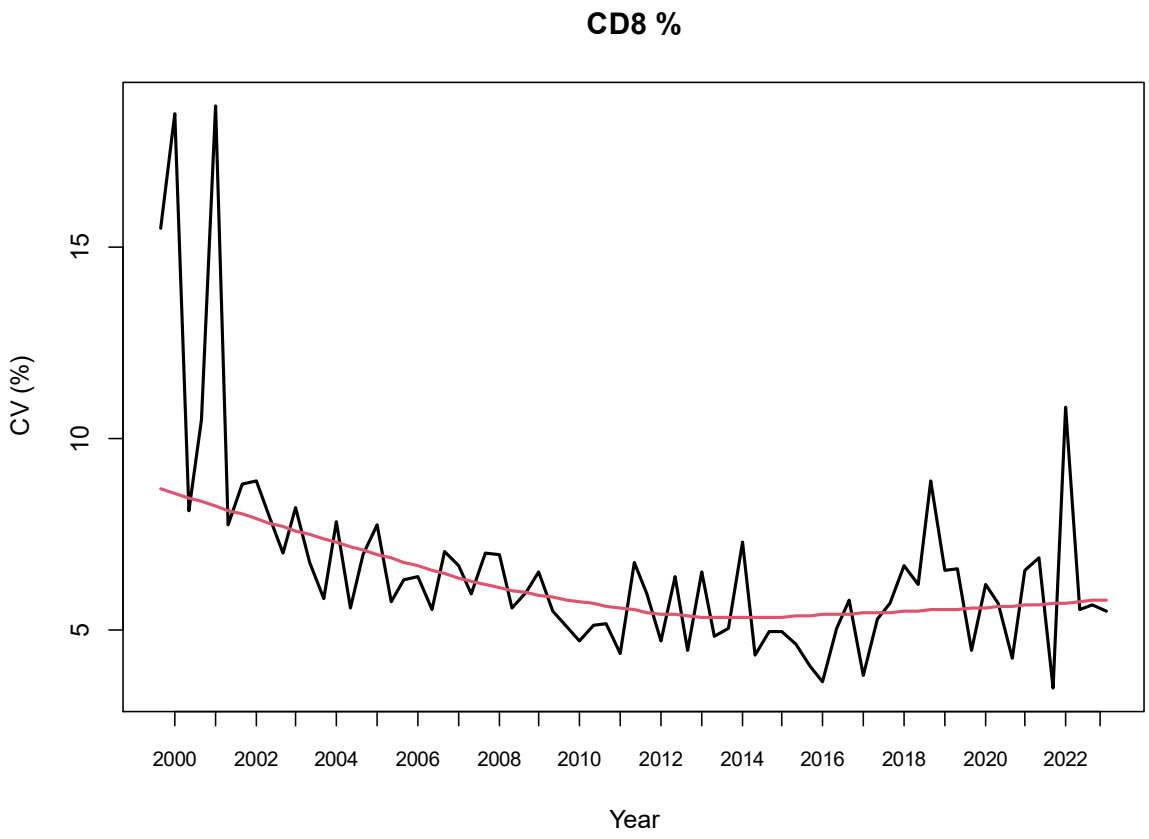
2020
2022
Year


CD8 %
CV (%)
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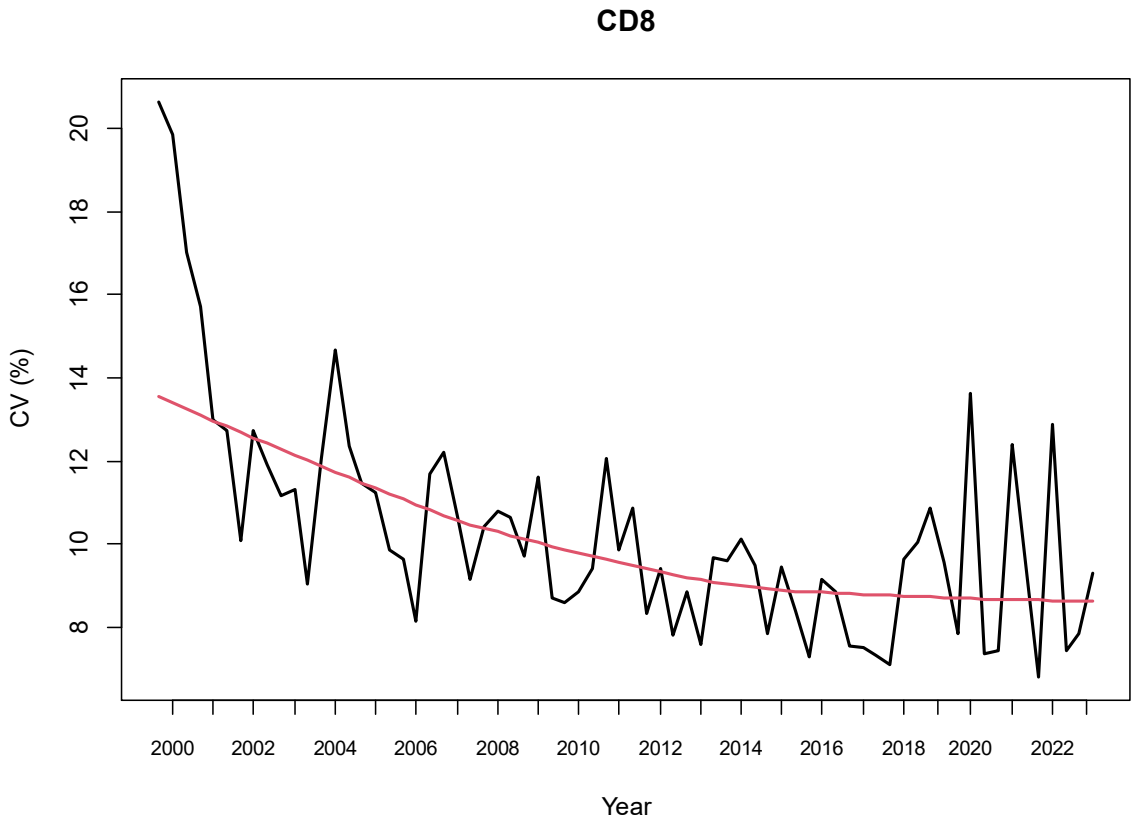
Year


CD8
CV (%)
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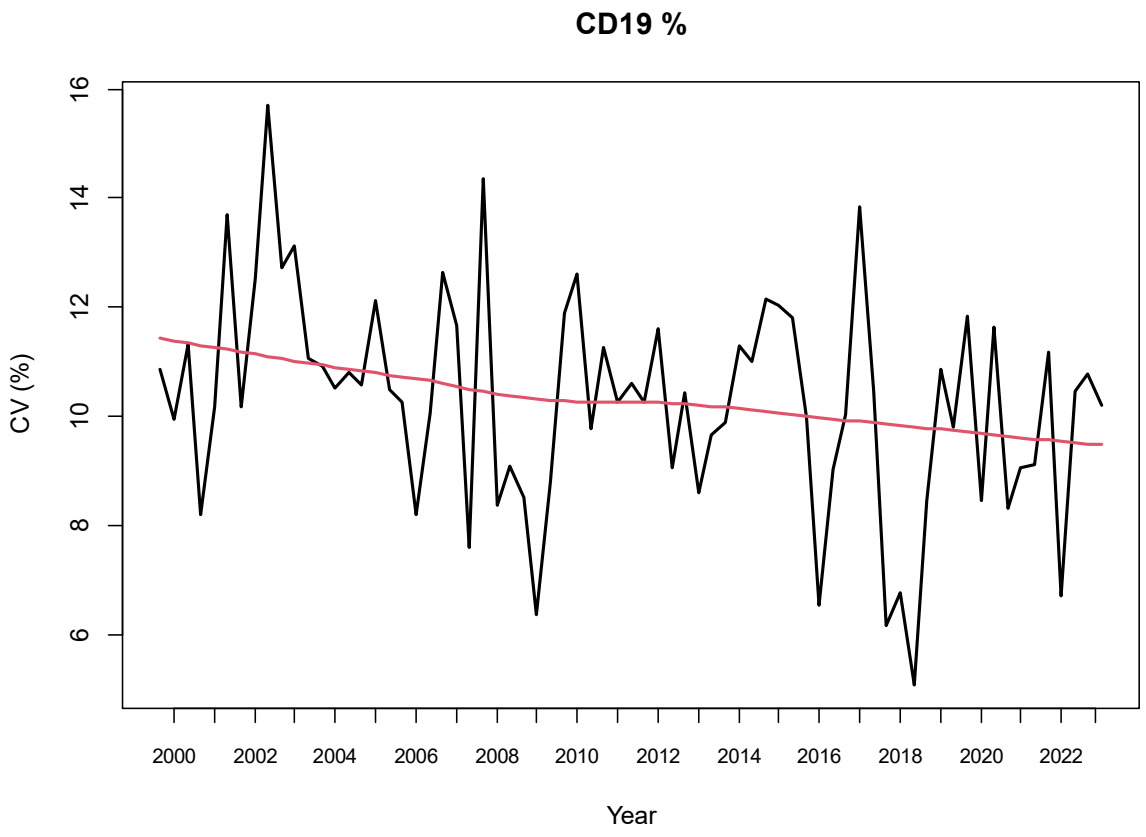
2022
Year


CD19 %
CV (%)
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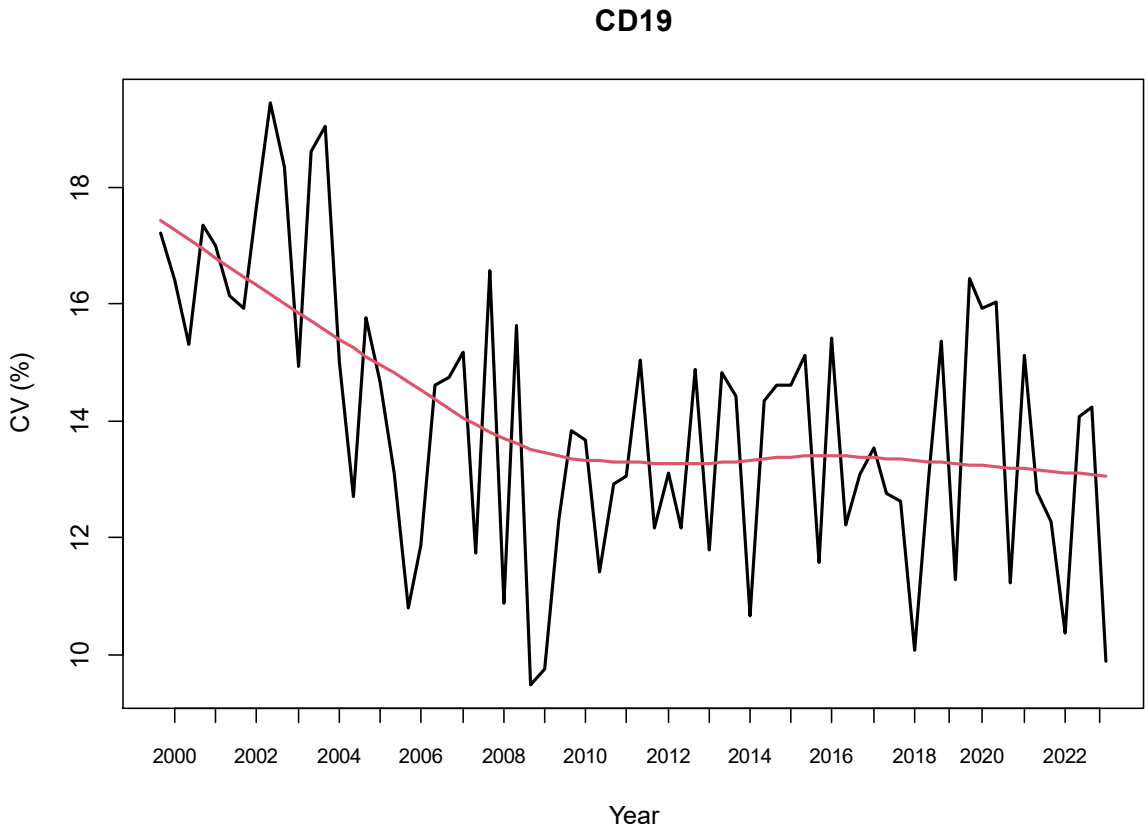
2020
2022
Year


CD19
CV (%)
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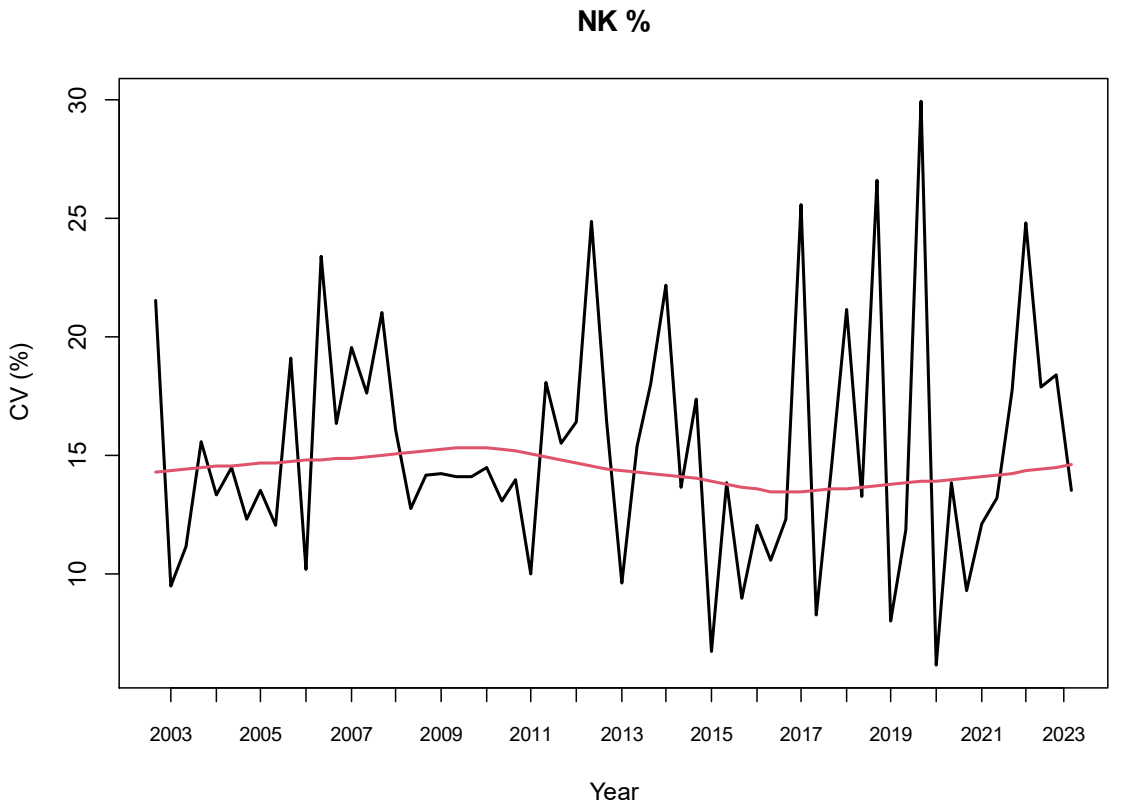
2020
2022
Year


NK %
CV (%)
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25
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2003
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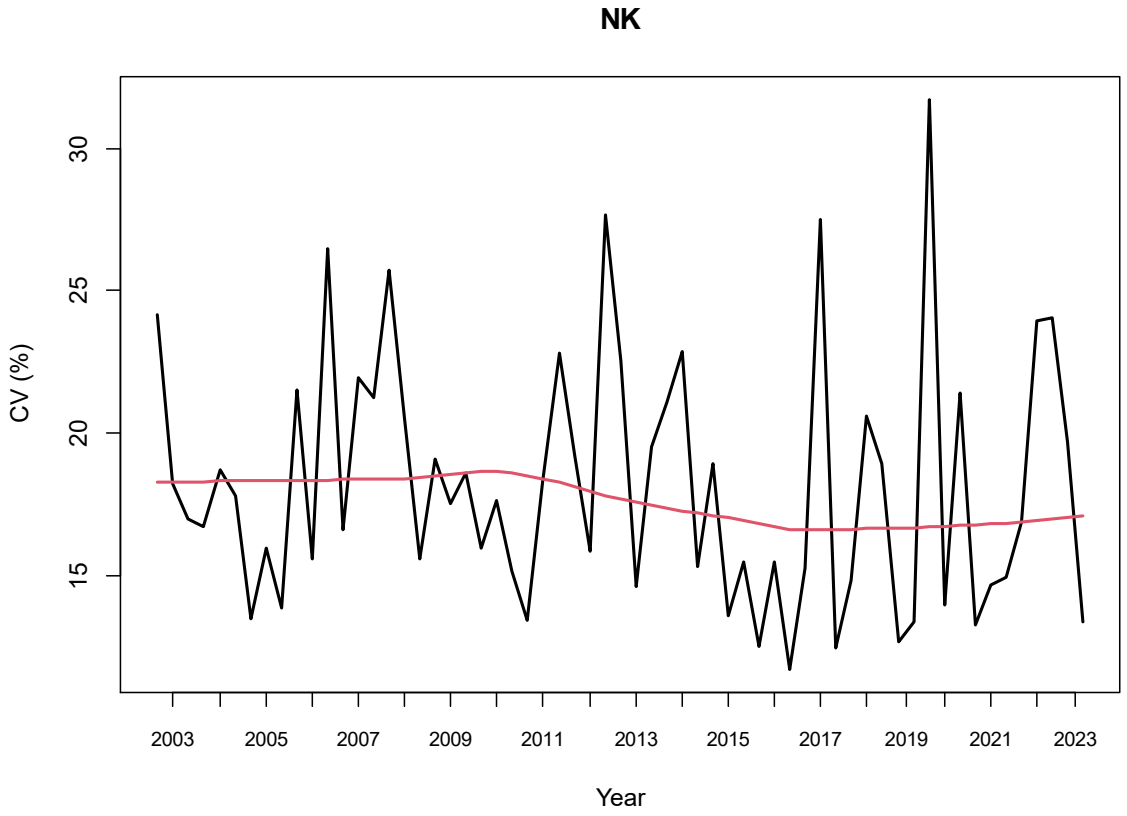
2023
Year


NK
CV (%)
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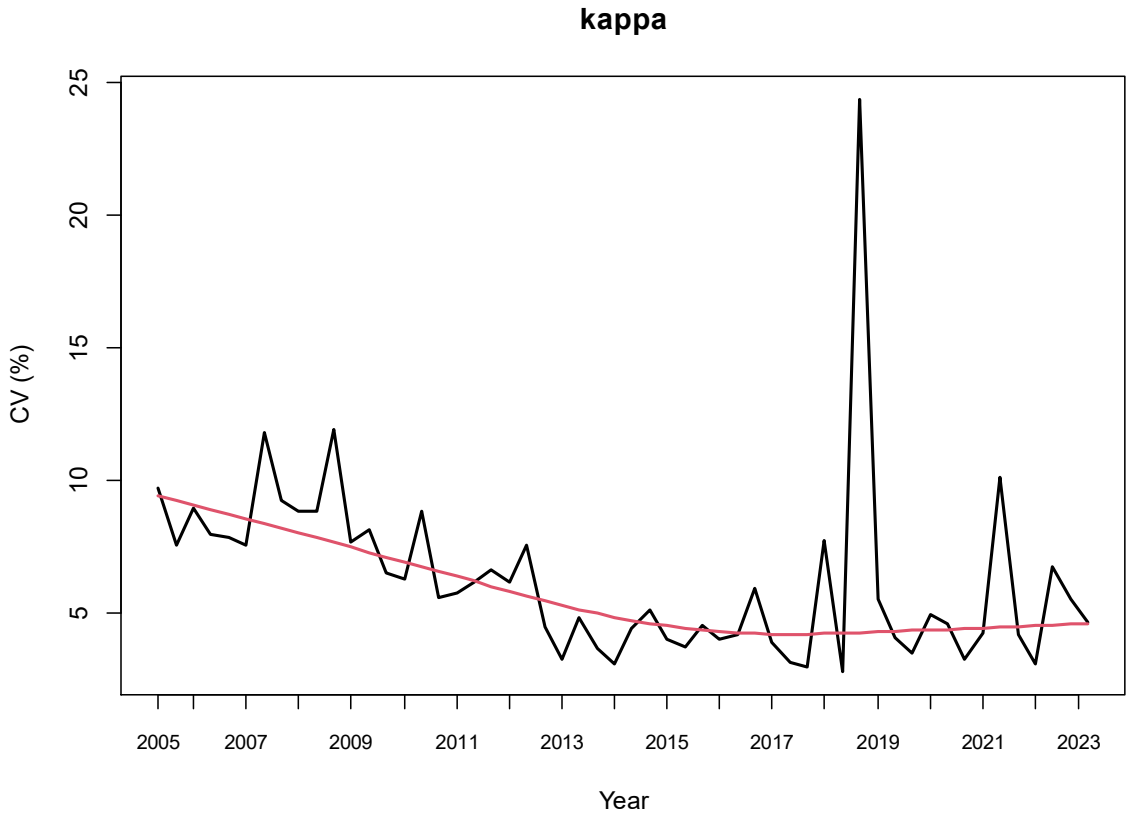
2019
2021
2023
Year


kappa
CV (%)
5
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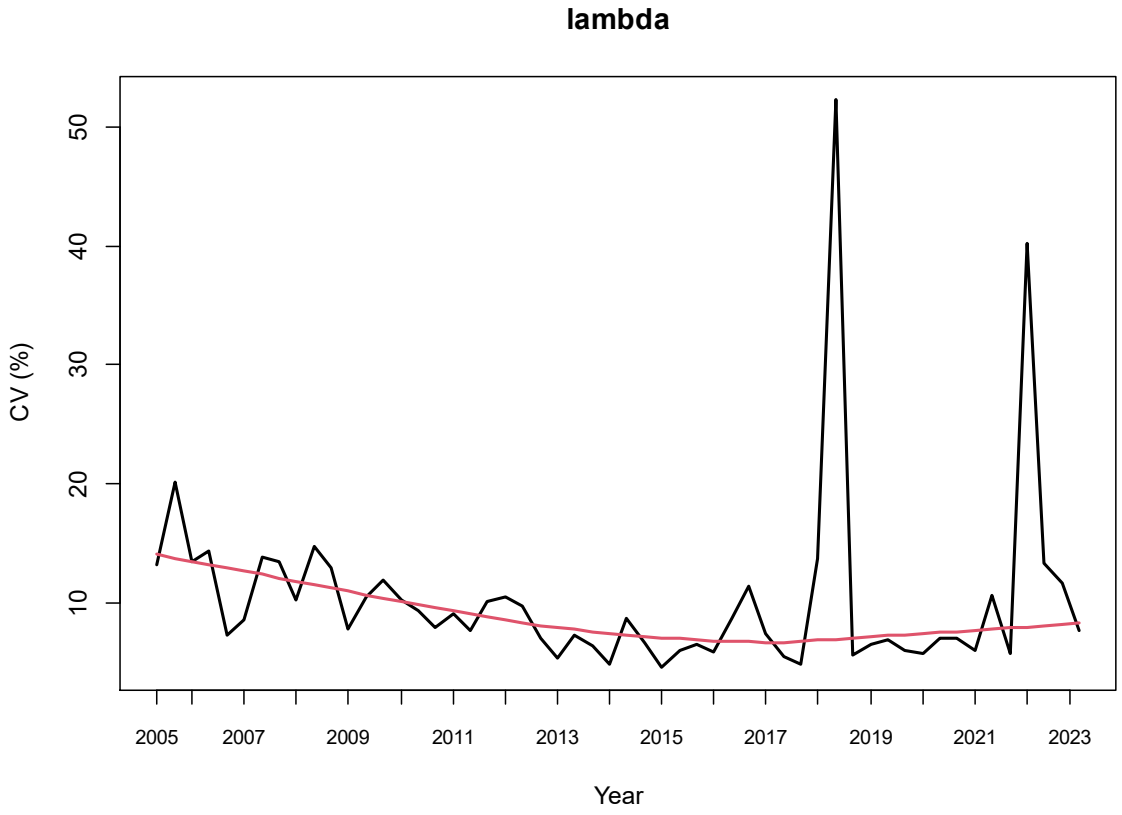
2017
2019
2021
2023
Year


lambda
CV (%)
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50
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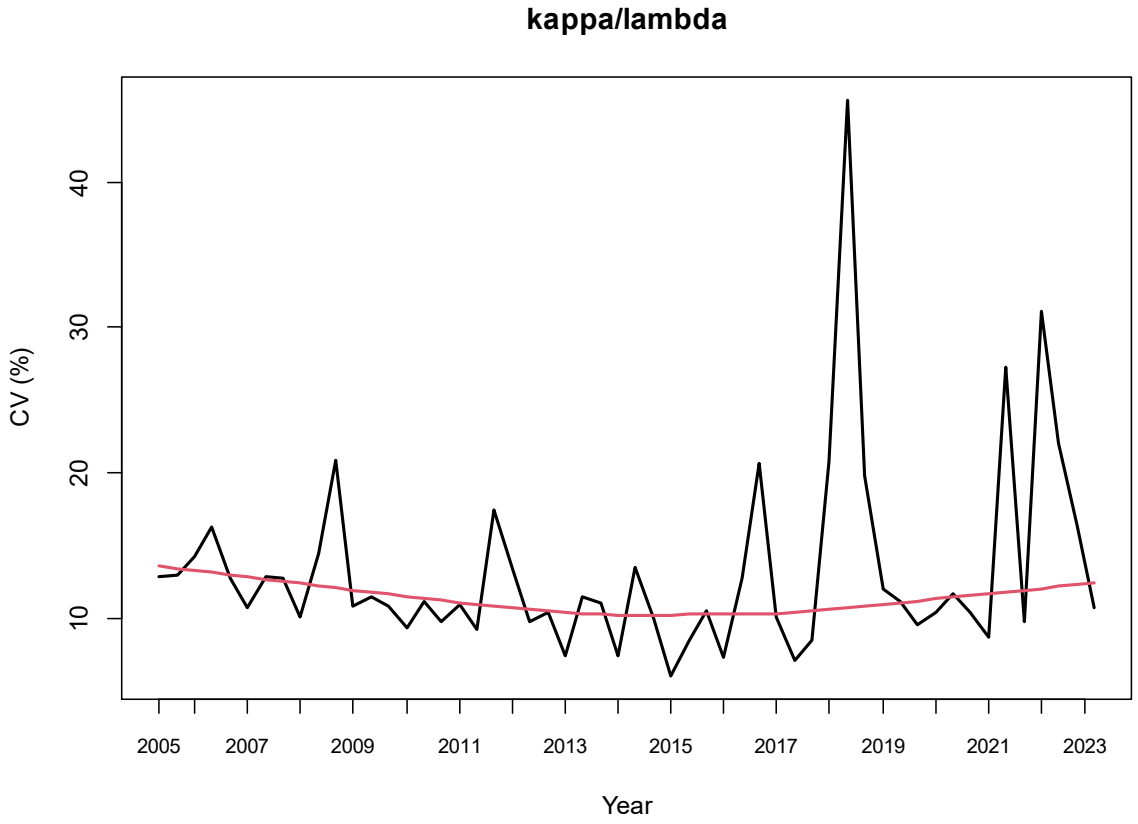
2017
2019
2021
2023
Year


kappa/lambda
CV (%)
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30
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2007
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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

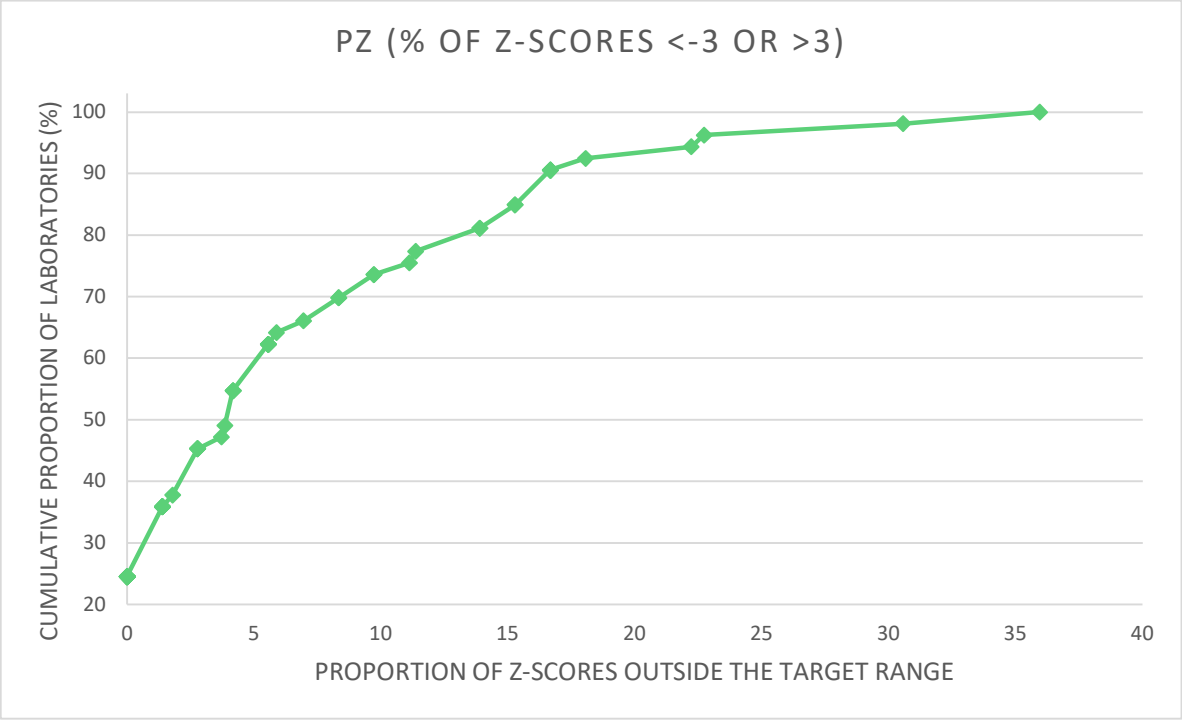
Z-scores reflect the performance of a laboratory with respect to its peer group. Z-scores <-3 or >3 (results falling beyond 3 SD from the median) are considered unacceptable.
The performance of the laboratories is evaluated by means of the percentage of unacceptable z-scores (P_Z, % of results falling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

The maximum of evaluated results per laboratory was 72.
This table shows a.o. that Belgian laboratories reported an average of 7.0% results beyond 3 SD and that 25% of laboratories got less than 1.4% of results beyond 3 SD in 2023.
The next table summarises for the different parameters the number of evaluated results and the percentage of results beyond 3 SD:

	2022		2023	
Parameter	**Number of evaluated results**	**% results >3 SD**	**Number of evaluated results**	**% results >3 SD**
Leukocytes 10^9/L	290	5.9	203	3.9
Lymphocytes % HA	283	5.7	195	9.2
Lymphocytes % FC	266	6.4	185	5.9
CD3 %	298	2.7	207	8.2
CD3 10^9/L	292	9.6	203	7.8
CD4 %	298	4.4	207	7.2
CD4 10^9/L	292	7.5	203	8.3
CD8 %	298	5.0	207	4.8
CD8 10^9/L	292	8.9	203	9.3
CD19 %	296	3.0	207	8.2
CD19 10^9/L	284	4.2	203	8.3
NK cells %	298	4.7	207	4.3
NK cells 10^9/L	292	7.2	203	7.8
κ % B lymphocytes	252	7.5	173	6.3
λ % B lymphocytes	252	7.9	173	6.3
κ/λ ratio	249	7.6	173	8.0
κ+λ % B lymphocytes	252	7.9	173	8.6
Lymphosum	298	6.4	207	6.2

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	191	12	6%	33%
CD4 10^9/L	191	12	7%	25%
CD8 10^9/L	191	12	8%	33%
CD19 10^9/L	191	12	7%	33%
NK cells 10^9/L	191	12	6%	33%

Parameter	Number of evaluated results		% results >3 SD	
	Lyse and wash	Lyse no wash	Lyse and wash	Lyse no wash
CD3 %	109	98	12%	4%
CD3 10^9/L	105	98	10%	6%
CD4 %	109	98	14%	0%
CD4 10^9/L	105	98	10%	6%
CD8 %	109	98	6%	4%
CD8 10^9/L	105	98	11%	7%
CD19 %	109	98	10%	6%
CD19 10^9/L	105	98	11%	5%
NK cells %	109	98	7%	1%
NK cells 10^9/L	105	98	11%	4%
Lymphosum	109	98	6%	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	42	131	5%	7%
λ % B lymphocytes	42	131	5%	7%
κ/λ ratio	42	131	7%	8%
κ+λ % B lymphocytes	42	131	19%	5%

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 %	149	58	9%	7%
CD3 10^9/L	145	58	9%	5%
CD4 %	149	58	9%	3%
CD4 10^9/L	145	58	9%	7%
CD8 %	149	58	6%	2%
CD8 10^9/L	145	58	10%	9%
CD19 %	149	58	9%	7%
CD19 10^9/L	145	58	8%	10%
NK cells %	149	58	4%	5%
NK cells 10^9/L	145	58	6%	12%
Lymphosum	149	58	7%	3%

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 2023, three surveys were conducted, in February (FC/19711, FC/19712), in May (FC/19925, FC/19926) and November (FC/20141, FC/20142).

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 six 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.

In total, twenty-three Belgian clinical laboratories participated in these surveys. However, not all participants took part in all surveys.

2.2. Methodology of the Belgian clinical laboratories Survey 2023/3 (n=20)

Fourteen laboratories (70%) used a single platform approach for determining the absolute CD34+ cell count. Of these laboratories, 10 used Trucount technology (BD Biosciences), 3 Flow-Count or Stem-count beads (Beckman-Coulter) and one participant used a volumetric single platform approach (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

Eleven participants used a sample volume of 100 µL, six a sample volume of 50 µL, one a volume of 43 µL, one a volume of 30 µL and one a volume of 25 µL. All participants used a lyse no wash method.

The following table summarises the lysing reagents used:

Lysing reagent	Number of laboratories
BD Biosciences Ammonium chloride lysing solution	6
Ammonium chloride (NH_4Cl)	6
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

All but 2 laboratories (PC5.5/PE-Cy5.5, APC) used a phycoerythrin (PE)-conjugated CD34 monoclonal antibody. All but 4 participants (Horizon V500 (n=2), Krome Orange, VioBlue) used a fluorescein isothiocyanate (FITC)-conjugated CD45 monoclonal antibody.

Gating strategy

15 participants applied the ISHAGE (International Society of Hematotherapy and Graft Engineering) gating protocol, 4 used the BD Biosciences Stem Cell Enumeration kit and one participant used the BD Biosciences ProCount Kit.

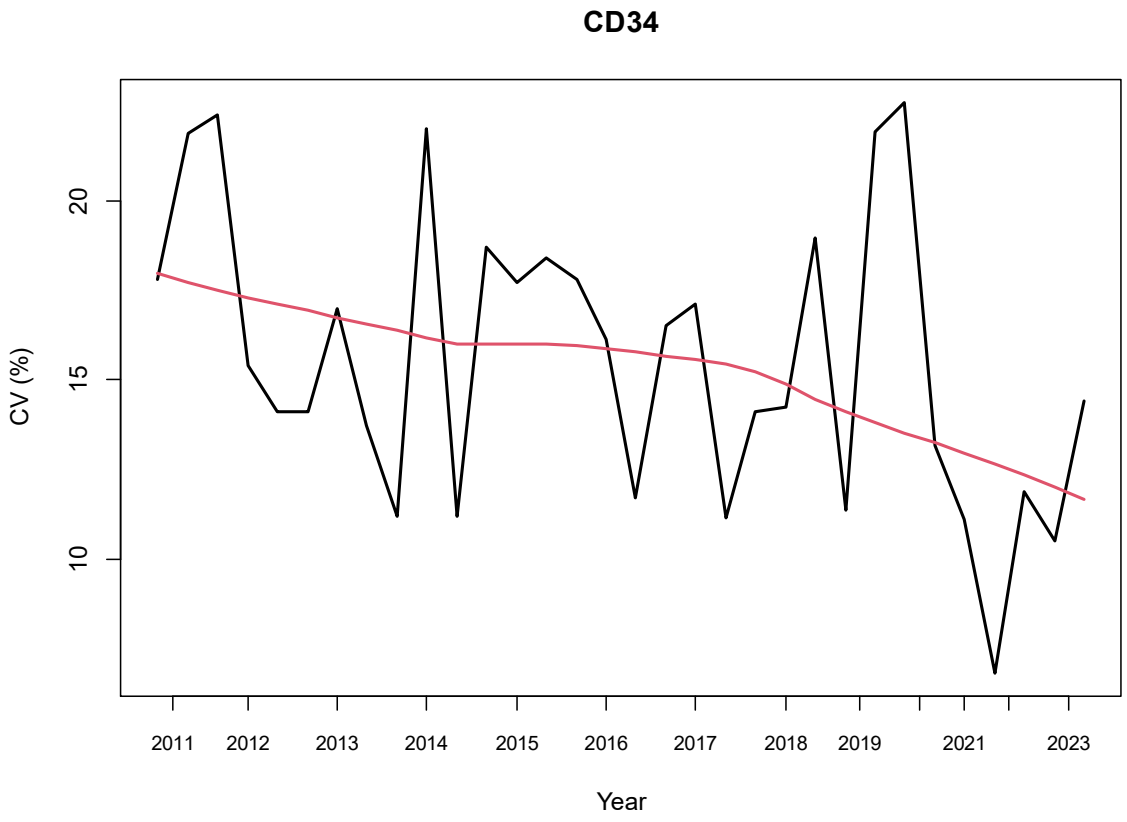
2.3. Results

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 Belgian 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 2023:

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/µL	CV %	N
FC19711	0.534	8.3	22	32.1	9.2	22
FC19712	0.173	15.0	22	10.3	7.2	22
FC19925	0.200	7.4	22	11.3	10.5	22
FC19926	0.594	6.4	22	34.3	9.5	22
FC20141	0.169	8.1	20	9.5	14.4	20
FC20142	0.595	4.4	20	34.1	12.2	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.



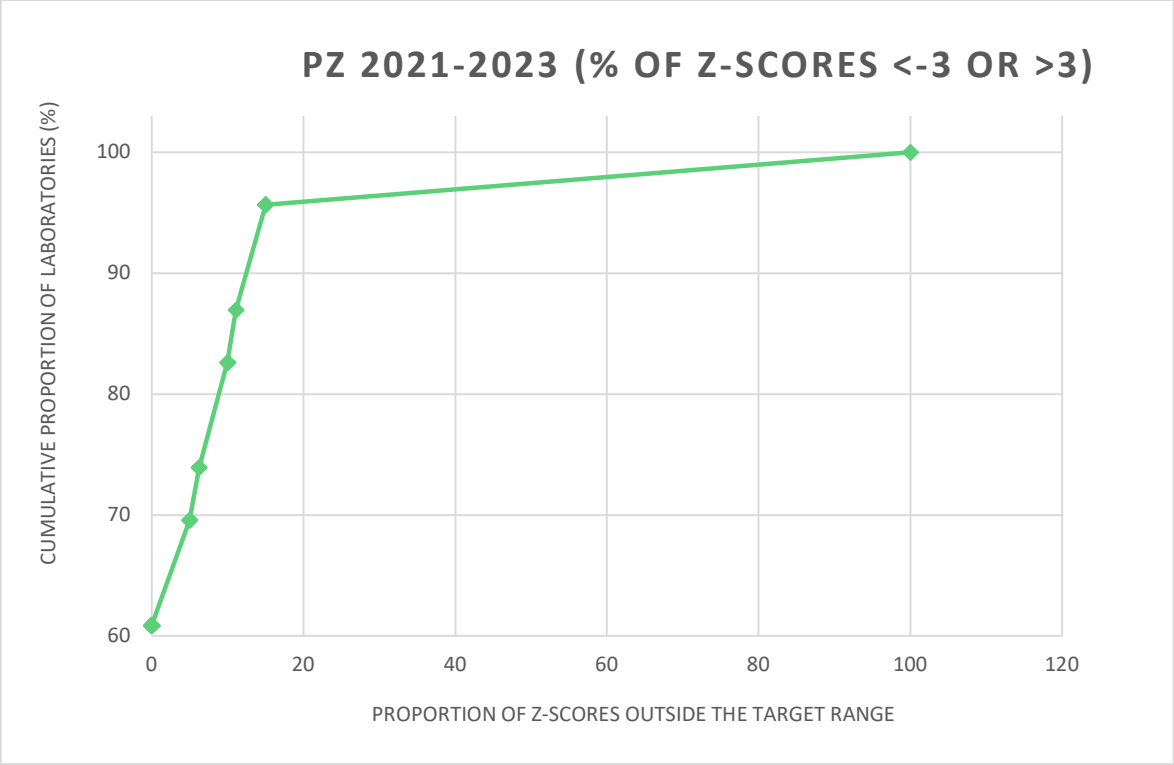
2.4. PZ evaluation

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

Given the very limited number of results available per year (2021: n=2, 2022: n=6, 2023: n=12), 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 2021-2023: 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
2021-2023	23	7.7 ± 20.7	0	0	8.1	14.2	15.0	81.3	0 – 100.0

During the period 2021-2023, the maximum of evaluated results per laboratory was 20.
Analysis of the data reveals that, on average, Belgian laboratories documented 7.7% of results that exceeded the threshold of 3 SD.
Furthermore, a significant 61% (fourteen laboratories) reported no results beyond 3 SD during this period.

END