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

2022

Sciensano/Flow cytometry/84-E

Biological health risks
Quality of laboratories
J. Wytsmanstreet, 14
1050 Brussels | Belgium

www.sciensano.be

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Dr. Bouacida L.	Scheme coordinator	PHONE:	02/642.53.83		
		e-mail:	lobna.bouacida@sciensano.be		
Dr. Vernelen K.	Alternate coordinator	PHONE:	02/642.55.29		
		e-mail:	kris.vernelen@sciensano.be		
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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 2022, surveys were conducted in February (FC/18847, FC/18848), May (FC/19104, FC/19105) and November (FC/19570 and FC/19571).

51 Belgian clinical laboratories participated in these surveys.

1.2. Methodology of the Belgian clinical laboratories Survey 2022/3 (n=51)

Six laboratories (12%) used a single platform approach for determining the absolute lymphocyte subset counts. Of these laboratories, 4 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	39
Beckman Coulter UniCel DxH 800 / DxH 900	5
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	16
BD Biosciences FACSCanto II	15
Beckman Coulter Navios	14
Beckman Coulter DxFLEX	2
Beckman Coulter Cytomics FC 500	2
BD Biosciences FACSCanto	1
BD Biosciences FACSVia	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. Three laboratories gave no further details, all others reported the use of commercial bead material (77% daily, 13% weekly, 8% per batch 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)	28
Beckman-Coulter Flow-Check Fluorospheres	10
Beckman-Coulter Flow-Check Pro Fluorospheres	6
BD Biosciences 7-color setup beads	3
Beckman-Coulter Flow-Set Fluorospheres	1

75% of the participants (n=38) 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
BD Biosciences Multi-Check Control	10
Beckman-Coulter IMMUNO-TROL Cells	9
BD Biosciences Multi-Check CD4 Low Control	2
Streck CD-Chex Plus BC	2
Beckman-Coulter Other	2
R&D Systems StatusFlow	1
Streck CD-Chex Plus CD4 Low, Normal	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=49, responding laboratories).

Lysing reagent	Number of laboratories
BD Biosciences FACS Lysing Solution	25
Beckman-Coulter VersaLyse	10
Ammonium chloride (NH_4Cl)	5
Beckman-Coulter Optilyse C	4
BD Biosciences Pharm Lyse	4
Beckman-Coulter Immunoprep reagent system	1

Most laboratories used 6-colour combination (n=50, responding laboratories).

	Number of participants				
	$CD3^+$	**$CD4^+$**	**$CD8^+$**	**$CD19^+$**	**NK**
4 colours	3	3	3	3	3
5 colours	5	5	5	5	4
6 colours	19	19	19	19	19
7 colours	5	5	5	5	3
8 colours	12	12	12	12	12
10 colours	6	6	6	6	6

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]. 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, 30% of the participants used CD56 alone and 70% 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	21
100% CD45 positive cells[5,6] + lymphosum + CD3 consistency check	11
100% CD45 positive cells[5,6] + lymphosum	11
Lymphosum + CD3 consistency check	6
100% CD45 positive cells[5,6]	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 et al. Belgian consensus recommendations for flow cytometric immunophenotyping. *Acta Clin Belg. 1999 Apr;54(2):88-98.*
2. Braylan RC. et al. 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 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. Van Dongen JJ 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*.
5. CD45 Gating for routine flow cytometric analysis of human bone marrow specimens. Stelzer GT, Shults KE, Loken MR. *Annals of the New York Academy of Sciences 1993; 677: 265–280.*
6. Use of CD45 fluorescence and side-scatter characteristics for gating lymphocytes when using the whole blood lysis procedure and flow cytometry. Nicholson JK, Hubbard M, Jones BM. *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	13	17		
Washing/RBC lysing before incubation with anti-κ/anti-λ reagents	2	8	1	
Incubation with B-cell marker (CD19) before washing and incubation with anti-κ/anti-λ reagents		2		
Total	15	27	1	**43**

73% of the participants used polyclonal anti-κ/anti-λ reagents.
All laboratories used anti-κ and anti-λ antibodies in combination with CD19 in one tube.
84% of the participants used CD19/SSC gating and 16% used CD45/SSC gating to identify lymphocytes, then CD45/CD19 or CD3/CD19 within lymphocytes.
All laboratories that specified their sample quality control assessment mentioned that they use the sum of the κ and λ chain expressing B cells for the technical validation of their analyses.

1.3. Results

Receipt of samples

Survey 2022/1: 100% of the Belgian laboratories received the samples on day 1 or 2.

Survey 2022/2: 98% of the Belgian laboratories received the samples on day 1 or 2. One laboratory received the samples on day 3.

Survey 2022/3: 94% of the Belgian laboratories received the samples on day 1 or 2. Three laboratories received the samples beyond 48 hours.

Sample analysis

Survey 2022/1: 71% of the Belgian laboratories performed the analyses on day 1, 27% (n=14) on day 2 and 2% (n=1) on day 3.

Survey 2022/2: 78% of the Belgian laboratories performed the analyses on day 1, 14% on day 2 and 8% on day 3.

Survey 2022/3: 67% of the Belgian laboratories performed the analyses on day 1, 25% on day 2 and 2% on day 3. Three laboratories received the samples beyond 48 hours.

Statistics for the evaluation were solely based on the results of the Belgian clinical laboratories. Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets were solely based 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 2022:

WBC 10^9/L

	Median	CV,%	N
FC/18847	9.78	3.5	48
FC/18848	5.92	3.0	48
FC/19104	8.41	2.3	46
FC/19105	37.22	2.2	44
FC/19570	8.45	1.9	46
FC/19571	7.03	1.9	46

Lymphocytes %
Haematology analyser

	Median	CV.%	N
FC/18847	26.9	3.4	47
FC/18848	37.6	3.0	47
FC/19104	23.7	4.4	45
FC/19105	84.6	3.8	42
FC/19570	22.3	5.0	45
FC/19571	34.3	1.9	45

Lymphocytes %
Flow cytometer

	Median	CV.%	N
FC/18847	26.7	8.0	45
FC/18848	36.9	9.6	45
FC/19104	22.3	10.0	45
FC/19105	85.6	2.9	45
FC/19570	21.0	12.0	45
FC/19571	32.6	8.6	45

CD3 %

	Median	CV.%	N
FC/18847	78.1	2.3	51
FC/18848	7.4	13.5	51
FC/19104	64.0	4.0	50
FC/19105	79.5	3.2	50
FC/19570	77.8	2.6	50
FC/19571	67.0	3.2	50

CD3 10^9/L

	Median	CV.%	N
FC/18847	1.689	6.4	48
FC/18848	1.779	6.6	48
FC/19104	1.577	4.7	47
FC/19105	2.395	13.9	45
FC/19570	1.450	6.1	46
FC/19571	1.602	6.3	46

CD4 %

	Median	CV.%	N
FC/18847	34.9	7.4	50
FC/18848	65.4	3.7	50
FC/19104	41.8	3.7	51
FC/19105	4.1	14.4	51
FC/19570	40.9	7.2	50
FC/19571	37.6	6.7	50

CD4 10^9/L

	Median	CV.%	N
FC/18847	0.920	7.9	48
FC/18848	1.460	7.5	48
FC/19104	0.845	6.4	47
FC/19105	1.327	17.3	45
FC/19570	0.755	10.3	46
FC/19571	0.900	10.3	46

CD8 %

	Median	CV.%	N
FC/18847	27.4	3.0	50
FC/18848	12.8	4.0	50
FC/19104	24.5	5.6	51
FC/19105	3.0	16.0	51
FC/19570	35.5	7.1	50
FC/19571	27.8	4.0	50

CD8 10^9/L

	Median	CV.%	N
FC/18847	0.719	6.9	48
FC/18848	0.286	6.7	48
FC/19104	0.493	9.2	47
FC/19105	0.942	16.6	45
FC/19570	0.658	8.9	46
FC/19571	0.668	6.0	46

CD19 %

	Median	CV.%	N
FC/18847	12.7	9.9	50
FC/18848	12.5	12.4	50
FC/19104	10.0	11.5	51
FC/19105	91.0	2.0	51
FC/19570	18.5	7.6	50
FC/19571	11.1	13.3	50

CD19 10^9/L

	Median	CV.%	N
FC/18847	0.331	14.5	48
FC/18848	0.280	10.0	48
FC/19104	0.202	14.7	47
FC/19105	28.823	6.1	43
FC/19570	0.343	12.1	46
FC/19571	0.267	16.1	46

NK %

	Median	CV.%	N
FC/18847	22	11.1	50
FC/18848	7.3	24.3	50
FC/19104	10.3	12.6	51
FC/19105	0.8	37.0	51
FC/19570	3.2	23.1	50
FC/19571	20.5	12.6	50

NK 10^9/L

	Median	CV.%	N
FC/18847	0.571	15.7	48
FC/18848	0.164	18.0	48
FC/19104	0.206	14.4	47
FC/19105	0.236	33.6	45
FC/19570	0.058	29.3	46
FC/19571	0.500	18.8	46

κ % B lymphocytes

	Median	CV.%	N
FC/18847	62.1	5.1	43
FC/18848	58.9	3.3	43
FC/19104	63.2	4.4	43
FC/19105	99.8	1.7	43
FC/19570	60.1	6.3	42
FC/19571	61.9	7.2	42

λ % B lymphocytes

	Median	CV.%	N
FC/18847	37.1	7.2	43
FC/18848	40.2	4.2	43
FC/19104	35.5	6.4	43
FC/19105	0.1	74.0	43
FC/19570	39.5	11.8	42
FC/19571	36.7	14.7	42

κ/λ ratio

	Median	CV.%	N
FC/18847	1.67	12.0	43
FC/18848	1.46	7.6	43
FC/19104	1.77	10.2	43
FC/19105	958	52.0	40
FC/19570	1.53	17.9	42
FC/19571	1.67	26.1	42

κ+λ % B lymphocytes

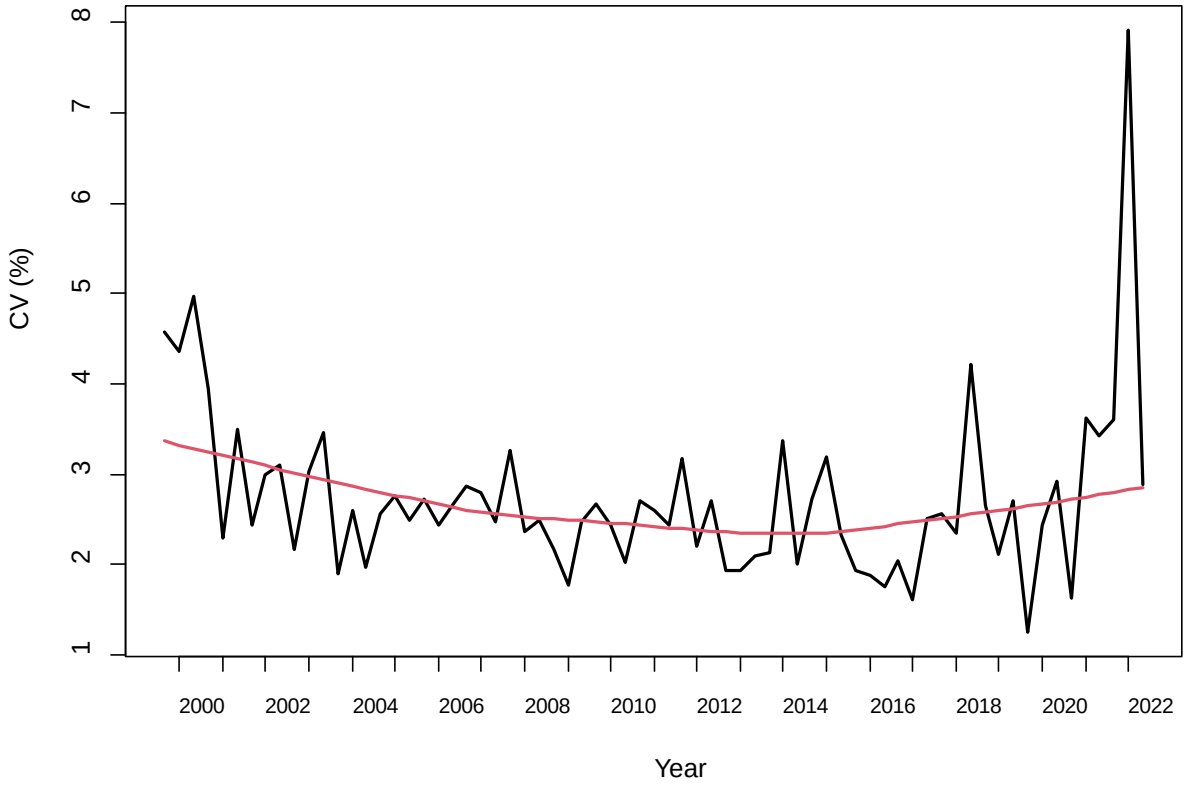
	Median	CV.%	N
FC/18847	99.6	1.0	43
FC/18848	99.8	0.7	43
FC/19104	99.7	1.1	43
FC/19105	100.0	1.5	43
FC/19570	99.7	1.6	42
FC/19571	99.6	1.3	42

Lymphosum %

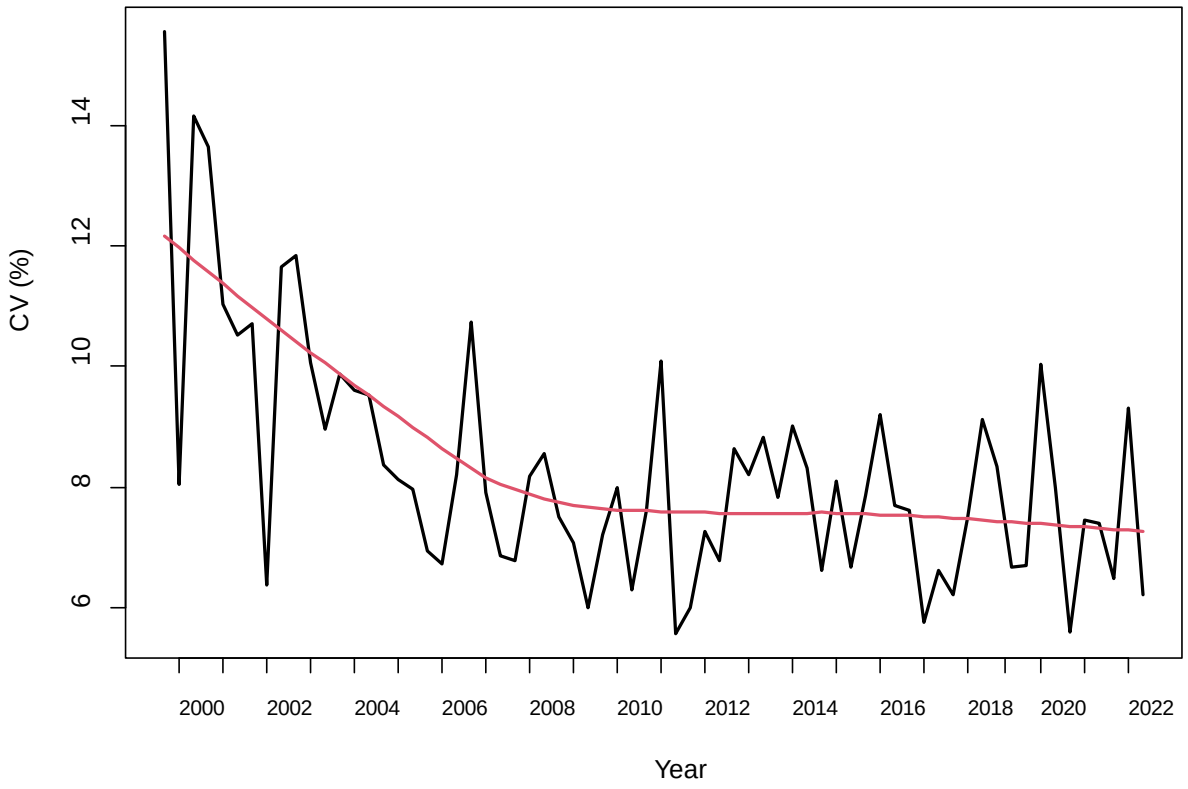
	Median	CV.%	N
FC/18847	98.8	1.9	50
FC/18848	99.5	0.6	50
FC/19104	99.3	1.1	51
FC/19105	99.5	0.4	51
FC/19570	99.5	0.6	50
FC/19571	99.2	1.6	50

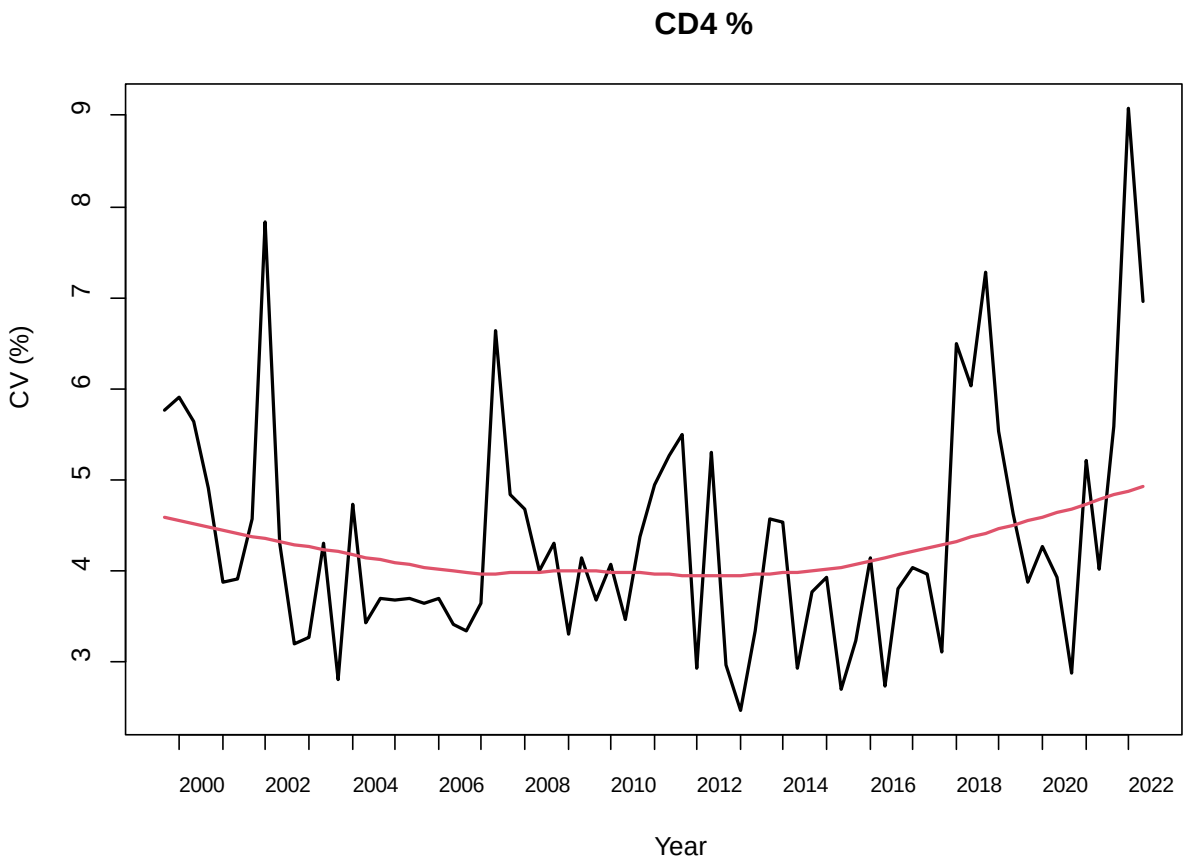
The following graphs show for the different parameters the evolution of the interlaboratory variability over the years. The black lines show the mean CV per survey. The red lines are a smoothed representation of the black lines and depict the evolution of the mean CV over time.

CD3 %



CD3




CD4 %
CV (%)
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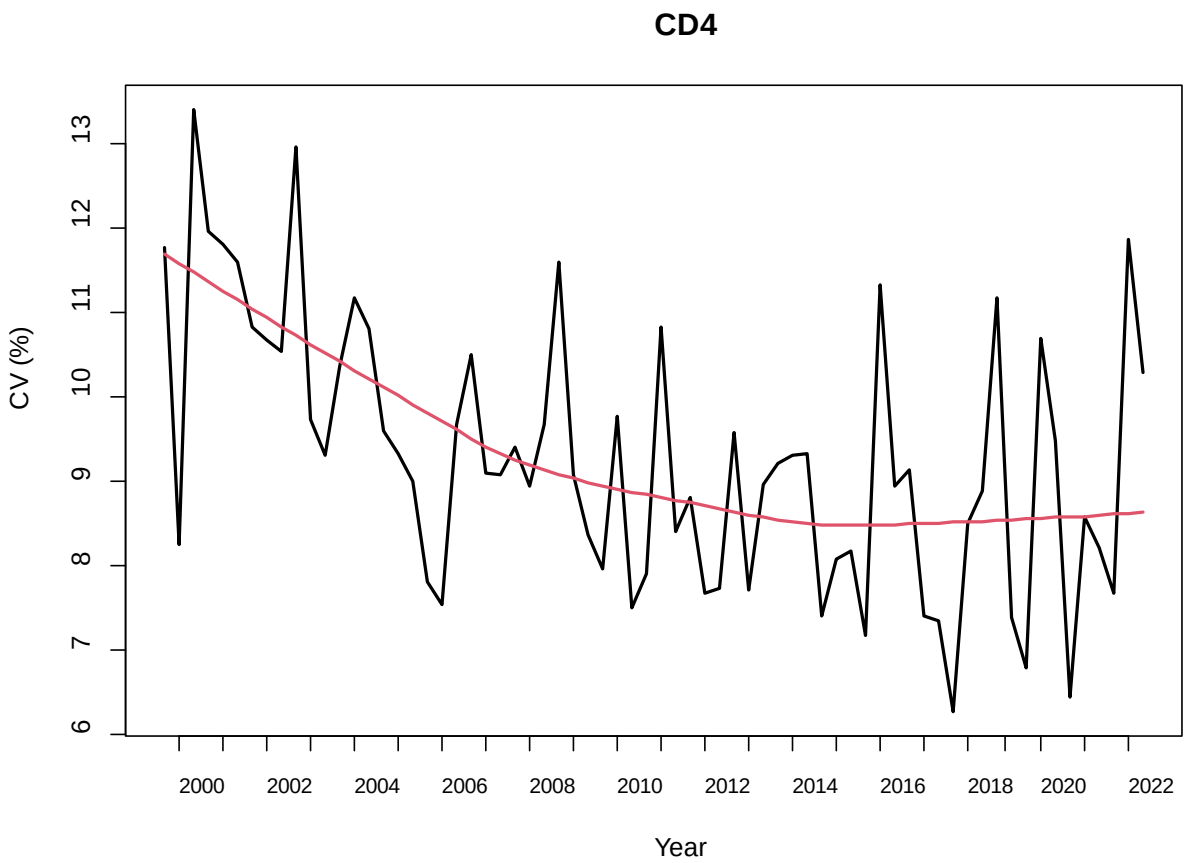
2018
2020
2022
Year


CD4
CV (%)
6
7
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11
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2006
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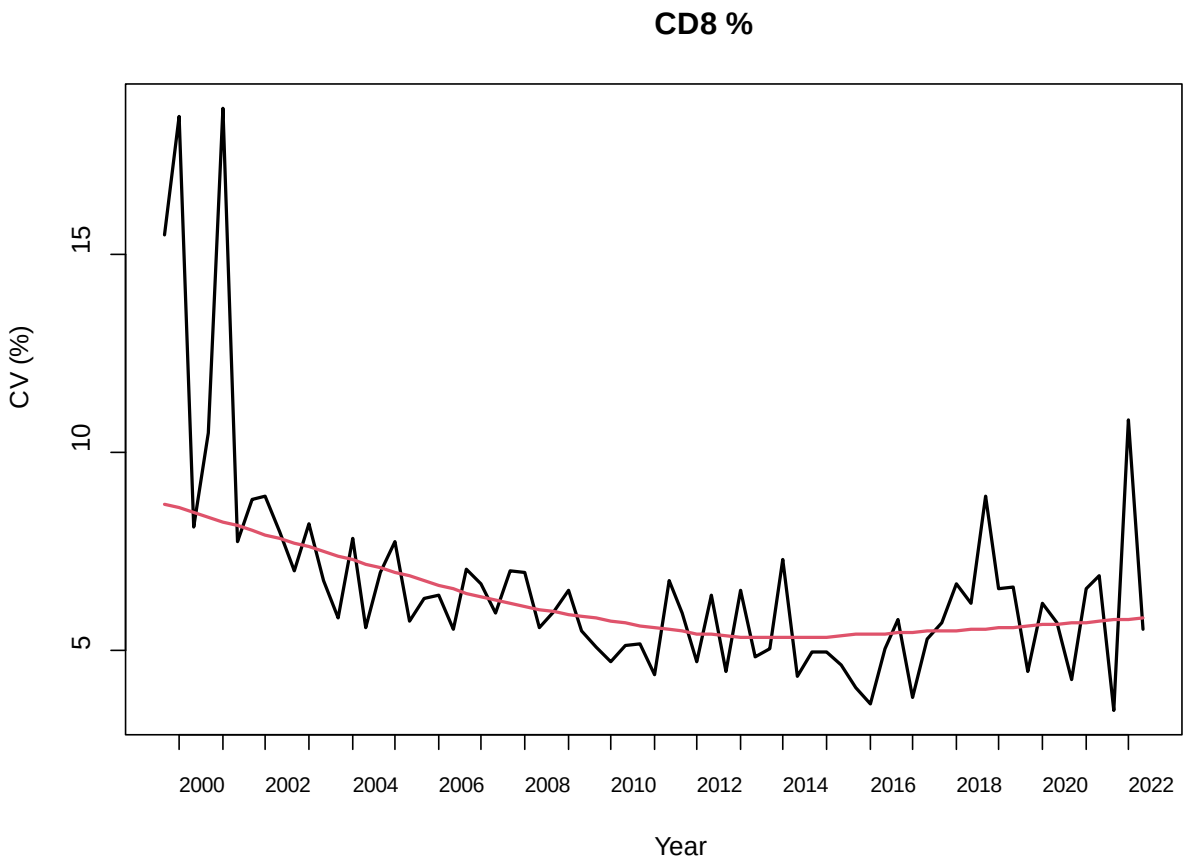
2016
2018
2020
2022
Year


CD8 %
CV (%)
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2000
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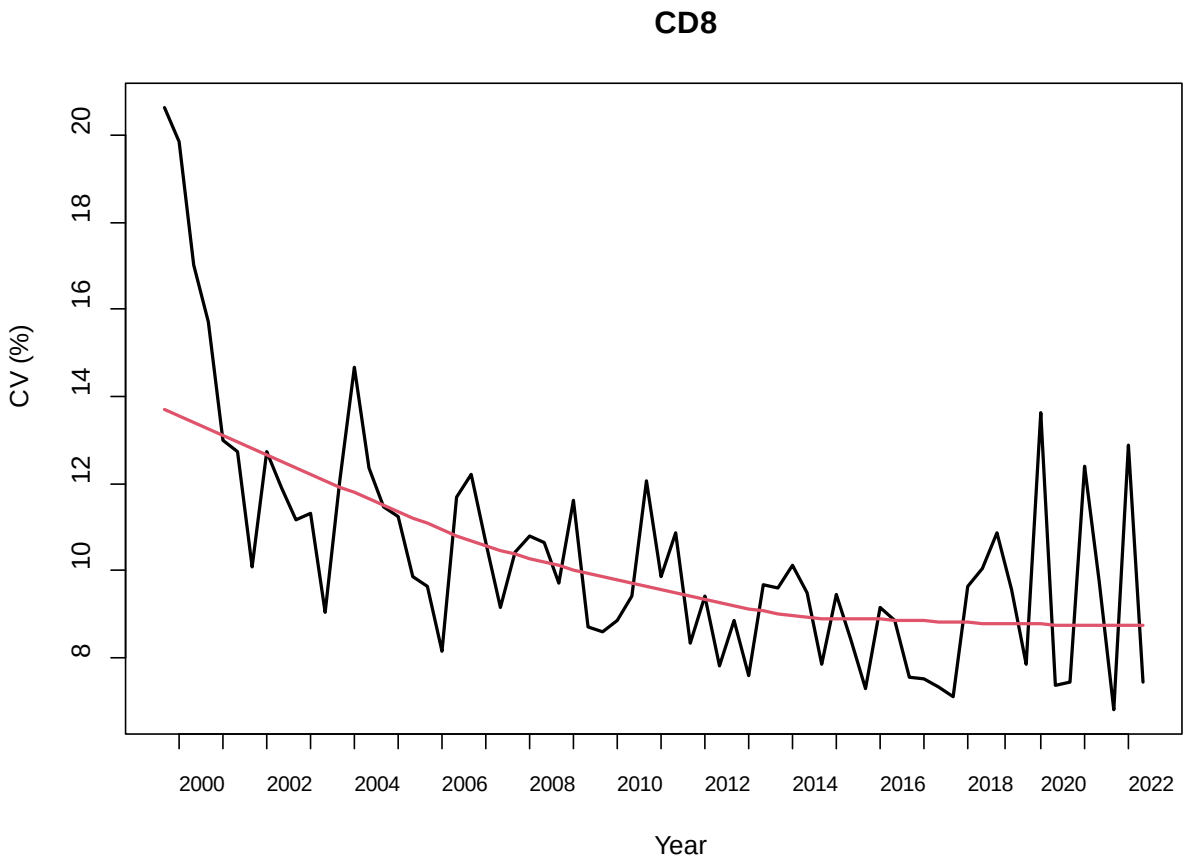
2020
2022
Year


CD8
CV (%)
8
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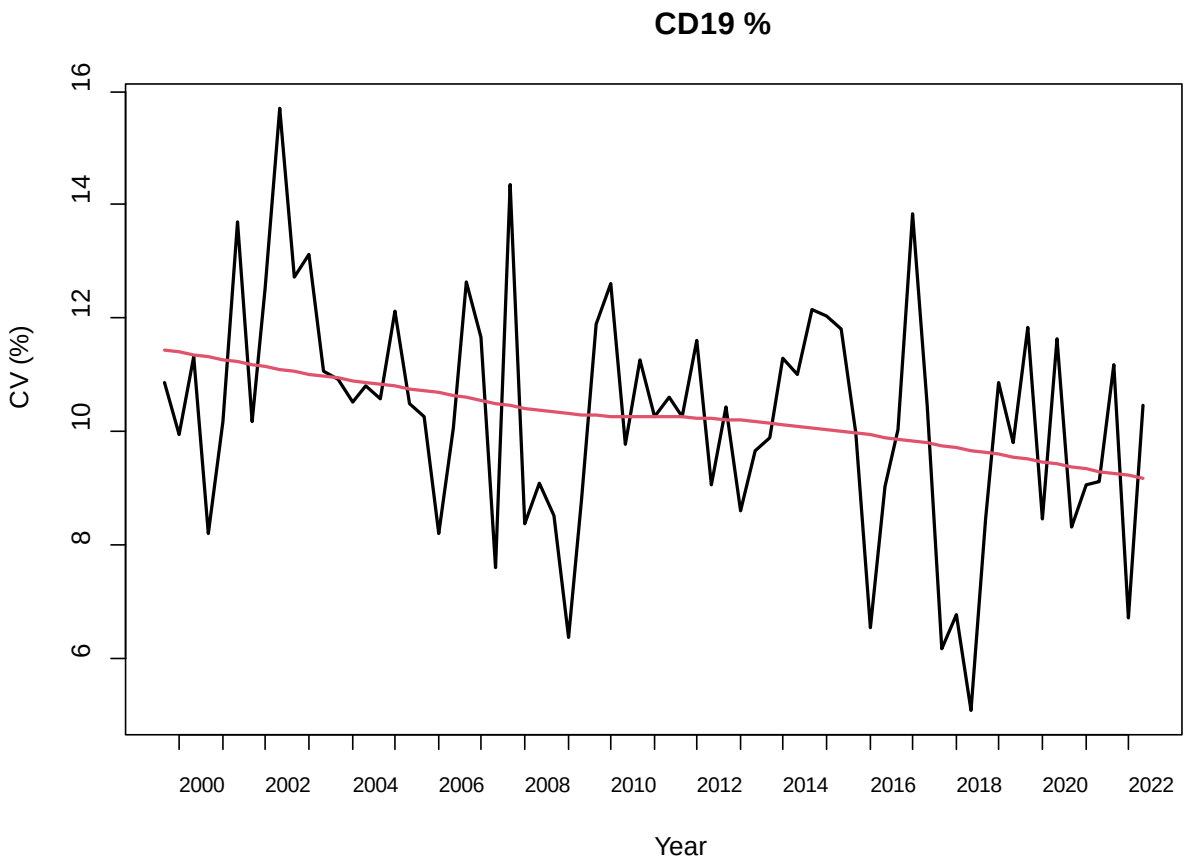
2020
2022
Year


CD19 %
CV (%)
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2012
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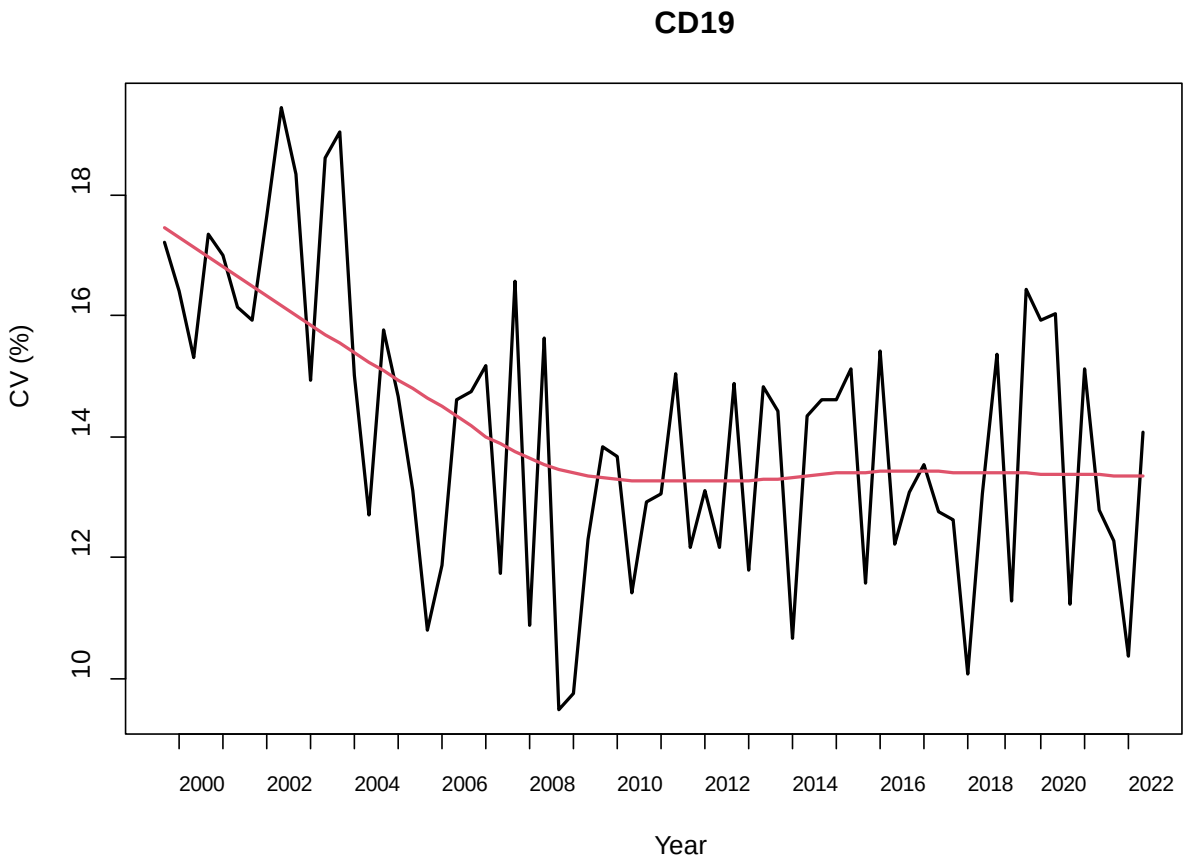
2016
2018
2020
2022
Year


CD19
CV (%)
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2000
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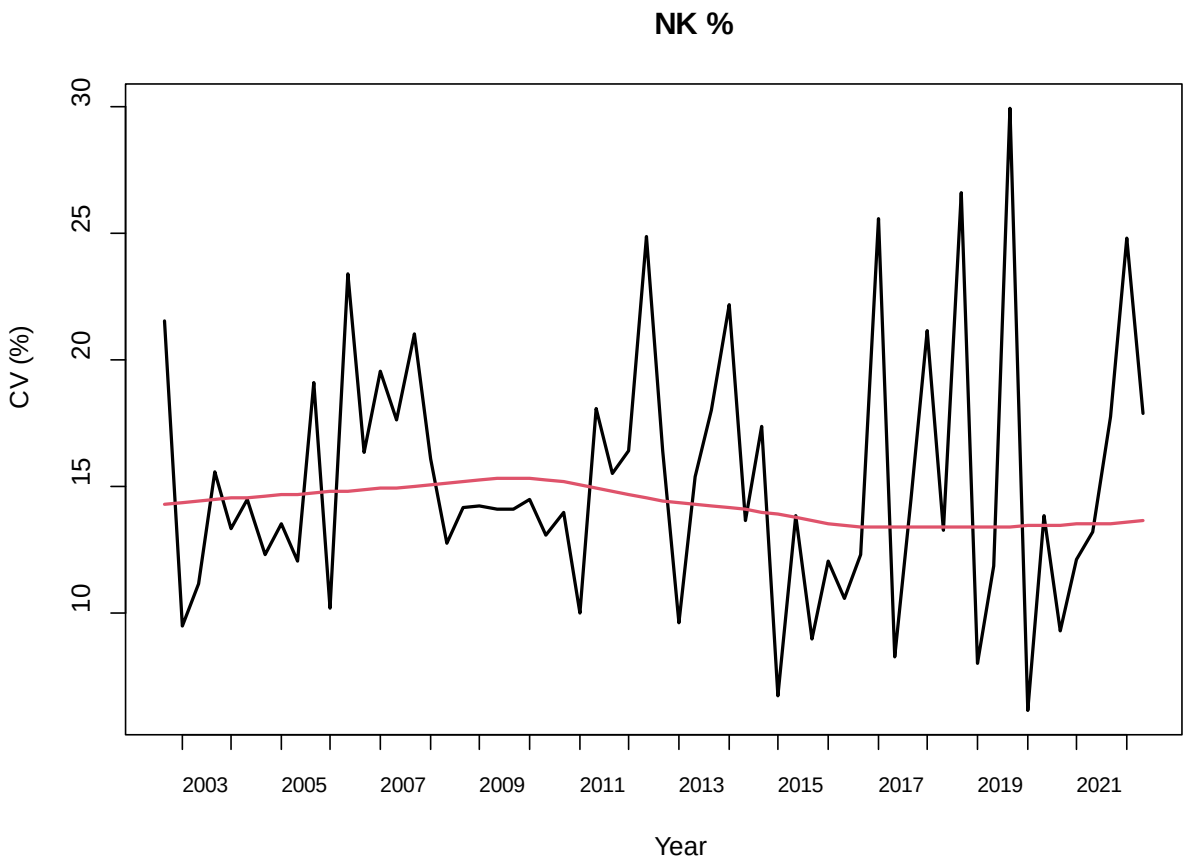
2022
Year


NK %
CV (%)
10
15
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25
30
2003
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2007
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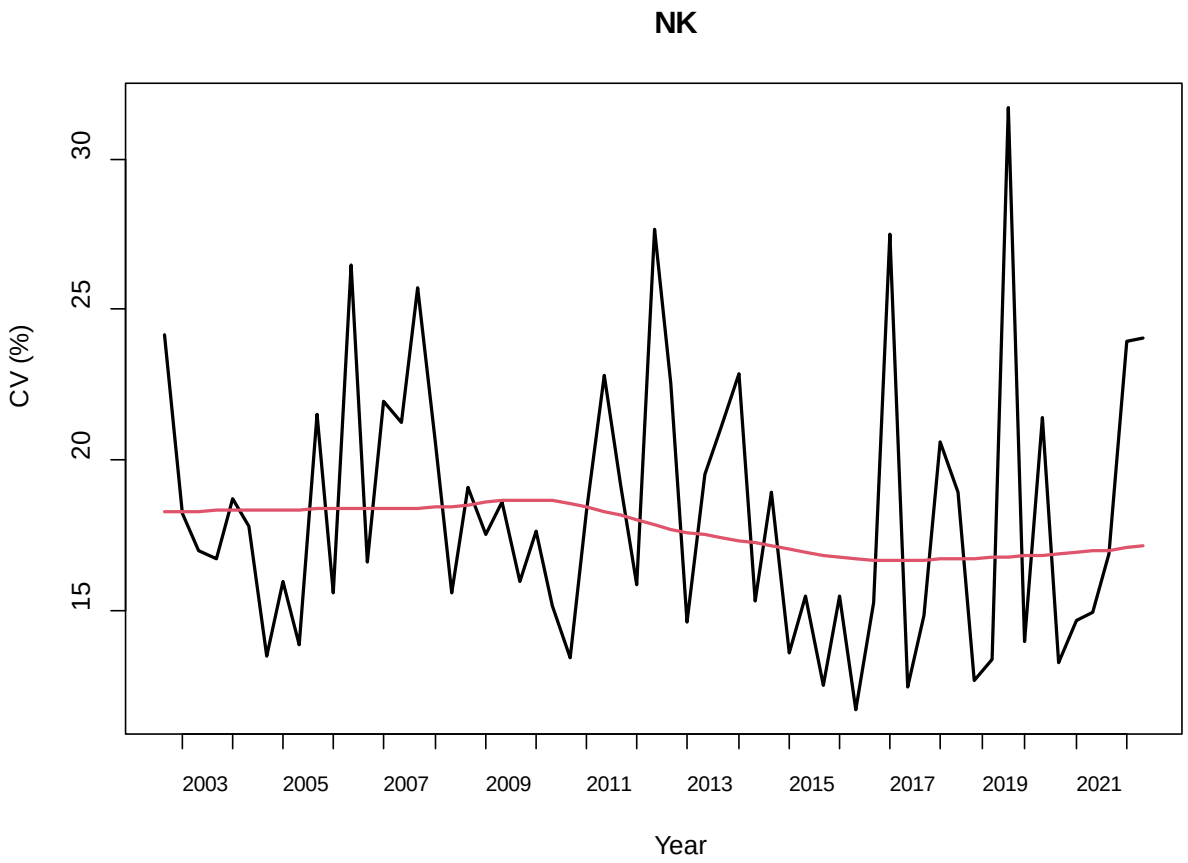
2013
2015
2017
2019
2021
Year


NK
CV (%)
15
20
25
30
2003
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2007
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2011
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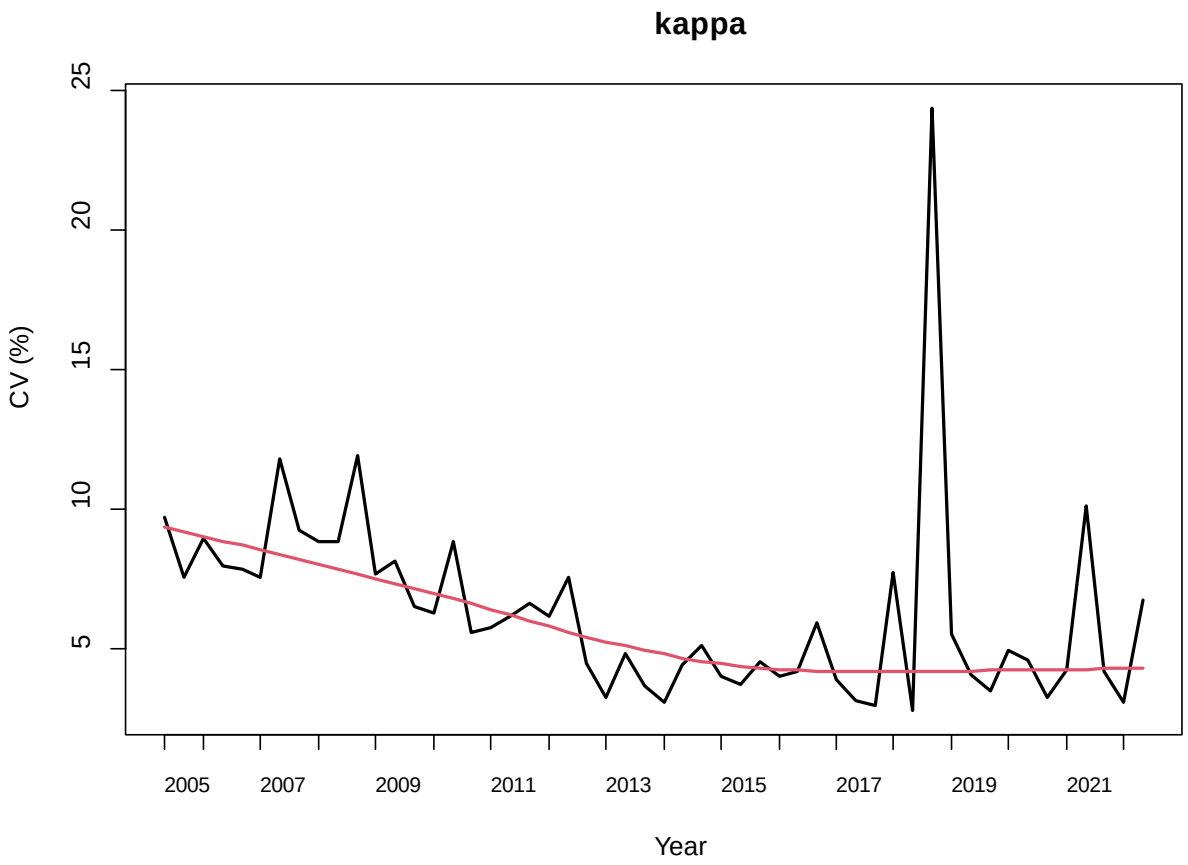
2015
2017
2019
2021
Year


kappa
CV (%)
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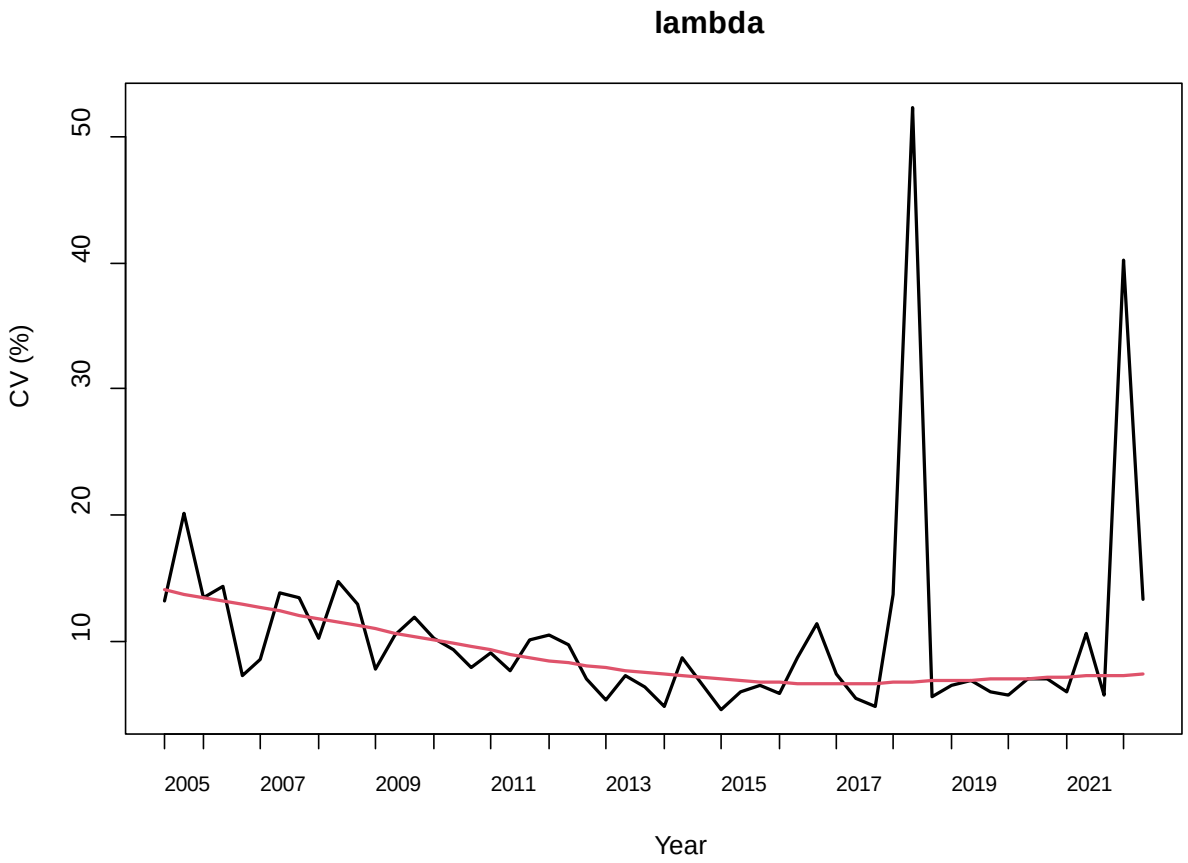
15
20
25
2005
2007
2009
2011
2013
2015
2017
2019
2021
Year


lambda
CV (%)
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2005
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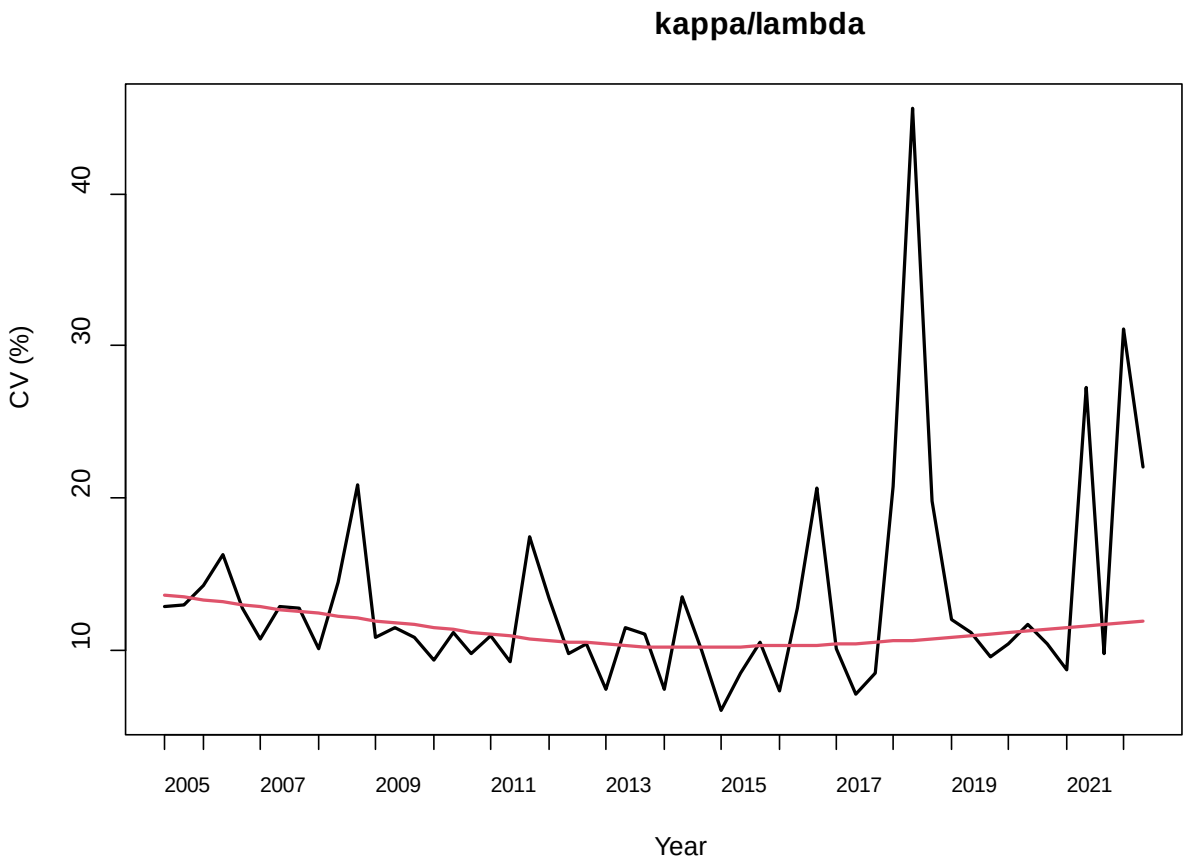
Year


kappa/lambda
CV (%)
10
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40
2005
2007
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2015
2017
2019
2021
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

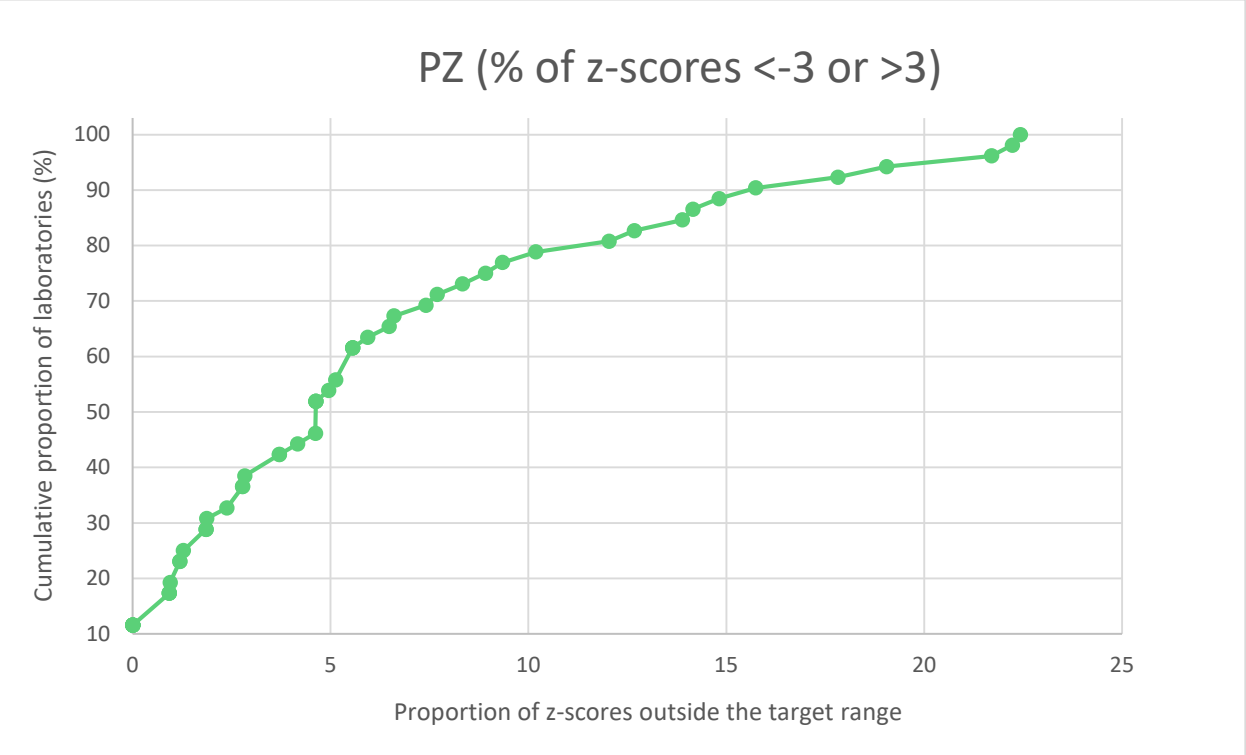
Z-scores reflect the performance of a laboratory with respect to its peer group. Z-scores <-3 or >3 (results falling beyond 3 SD from the median) are considered unacceptable.
The performance of the laboratories is evaluated by means of the percentage of unacceptable z-scores (P_Z, % of results falling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

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

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

The maximum of evaluated results per laboratory was 108.
This table shows a.o. that Belgian laboratories reported an average of 6.5% results beyond 3 SD and that 25% of laboratories got less than 1.7% of results beyond 3 SD in 2022.
The next table summarises for the different parameters the number of evaluated results and the percentage of results beyond 3 SD:

	2021		2022	
Parameter	**Number of evaluated results**	**% results >3 SD**	**Number of evaluated results**	**% results >3 SD**
Leukocytes 10^9/L	301	5.1	290	5.9
Lymphocytes % HA	292	8.0	283	5.7
Lymphocytes % FC	276	6.9	266	6.4
CD3 %	308	6.6	298	2.7
CD3 10^9/L	304	9.4	292	9.6
CD4 %	308	4.3	298	4.4
CD4 10^9/L	304	8.4	292	7.5
CD8 %	308	3.9	298	5.0
CD8 10^9/L	304	6.0	292	8.9
CD19 %	308	7.3	296	3.0
CD19 10^9/L	304	7.0	284	4.2
NK cells %	308	5.6	298	4.7
NK cells 10^9/L	304	9.7	292	7.2
κ % B lymphocytes	266	5.7	252	7.5
λ % B lymphocytes	266	6.5	252	7.9
κ/λ ratio	266	7.3	249	7.6
κ+λ % B lymphocytes	266	12.2	252	7.9
Lymphosum	308	4.3	298	6.4

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	286	12	10%	0%
CD4 10^9/L	286	12	8%	0%
CD8 10^9/L	286	12	10%	0%
CD19 10^9/L	278	11	5%	0%
NK cells 10^9/L	286	12	8%	0%

Parameter	Number of evaluated results		% results >3 SD	
	Lyse and wash	Lyse no wash	Lyse and wash	Lyse no wash
CD3 %	158	146	4%	1%
CD3 10^9/L	152	146	9%	12%
CD4 %	158	146	5%	5%
CD4 10^9/L	152	146	5%	11%
CD8 %	158	146	7%	4%
CD8 10^9/L	152	146	9%	10%
CD19 %	156	146	4%	1%
CD19 10^9/L	147	142	5%	5%
NK cells %	158	146	4%	5%
NK cells 10^9/L	152	146	7%	8%
Lymphosum	158	146	7%	5%

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	60	198	10%	8%
λ % B lymphocytes	59	196	10%	8%
κ/λ ratio	60	198	7%	8%
κ+λ % B lymphocytes	60	198	15%	6%

The following tables show 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 %	206	98	2%	3%
CD3 10^9/L	200	98	6%	18%
CD4 %	206	98	5%	5%
CD4 10^9/L	200	98	5%	13%
CD8 %	206	98	7%	2%
CD8 10^9/L	200	98	6%	17%
CD19 %	206	96	3%	2%
CD19 10^9/L	195	94	5%	5%
NK cells %	206	98	5%	4%
NK cells 10^9/L	200	98	6%	10%
Lymphosum	206	98	7%	5%

2. CD34+ STEM CELL ENUMERATION

2.1. Surveys

A triannual external quality assessment scheme for CD34+ stem cell enumeration is operational in Belgium since 2011. Each survey, participating laboratories are sent one or two fresh umbilical cord blood samples collected into heparin or citrate**-**phosphate-dextrose. The participants are asked to perform flow cytometric CD34+ stem cell enumeration and to indicate the date of receipt, the date of acquisition, and to provide details of the type of flow cytometer, the sample preparation technique, the source of antibodies, the gating strategy, and the data analysis software used.

In 2022, two surveys were conducted, in May (FC/18849) and November (FC/19307, FC/19308). The survey initially planned in February could not be carried out due to a lack of samples,

Twenty-one Belgian clinical laboratories participated in these surveys.

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

2.2. Methodology of the Belgian clinical laboratories Survey 2022/3 (n=21)

Fourteen laboratories (67%) used a single platform approach for determining the absolute CD34+ cell count. Of these laboratories, 8 used Trucount technology (BD Biosciences), 4 Flow-Count or Stem-count beads (Beckman-Coulter) and one Perfect-Count Microspheres (Cytognos). 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 FACSCanto II	7
Beckman-Coulter Navios	6
BD Biosciences FACSLyric	6
Miltenyi Biotec MACSQuant analyzer	1
Beckman Coulter AQUIOS CL	1

Sample preparation

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

The following table summarises the lysing reagents used:

Lysing reagent	Number of laboratories
Ammonium chloride (NH_4Cl)	6
BD Biosciences Ammonium chloride lysing solution	4
BD Biosciences Pharm Lyse	3
Beckman-Coulter VersaLyse Lysing Solution	2
Beckman-Coulter Ammonium chloride	2
BD Biosciences FACS Lysing Solution	1
Cytognos Quicklysis	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

With 3 exceptions (BD Biosciences ProCount Kit (n=1) and BD Biosciences Stem Cell Enumeration Kit (n=2)), all participants applied the ISHAGE (International Society of Hematotherapy and Graft Engineering) gating protocol.

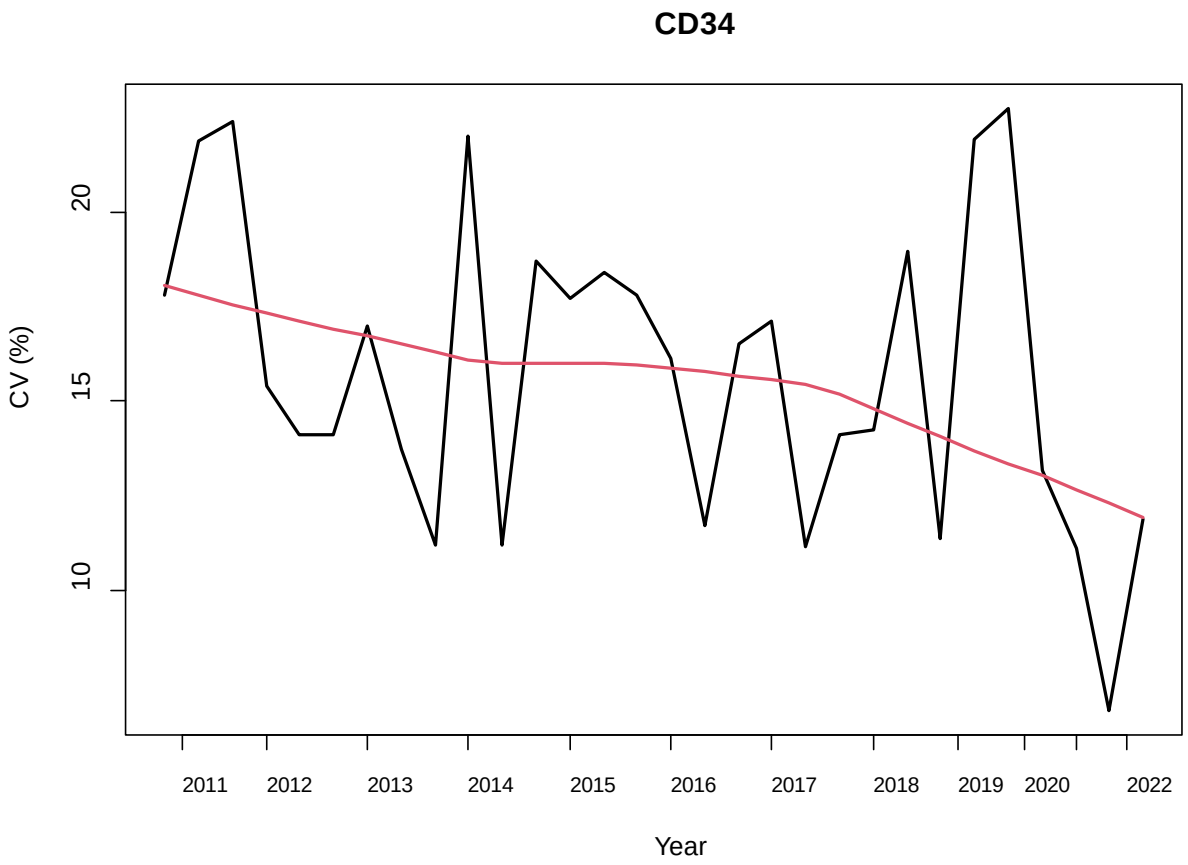
2.3. Results

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 2022:

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/µL	CV %	N
FC/18849	0.200	9.4	22	11.9	9.5	22
FC/19307	0.140	13.2	21	8.1	11.9	21
FC/19308	0.580	6.4	21	33.8	7.4	21

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.

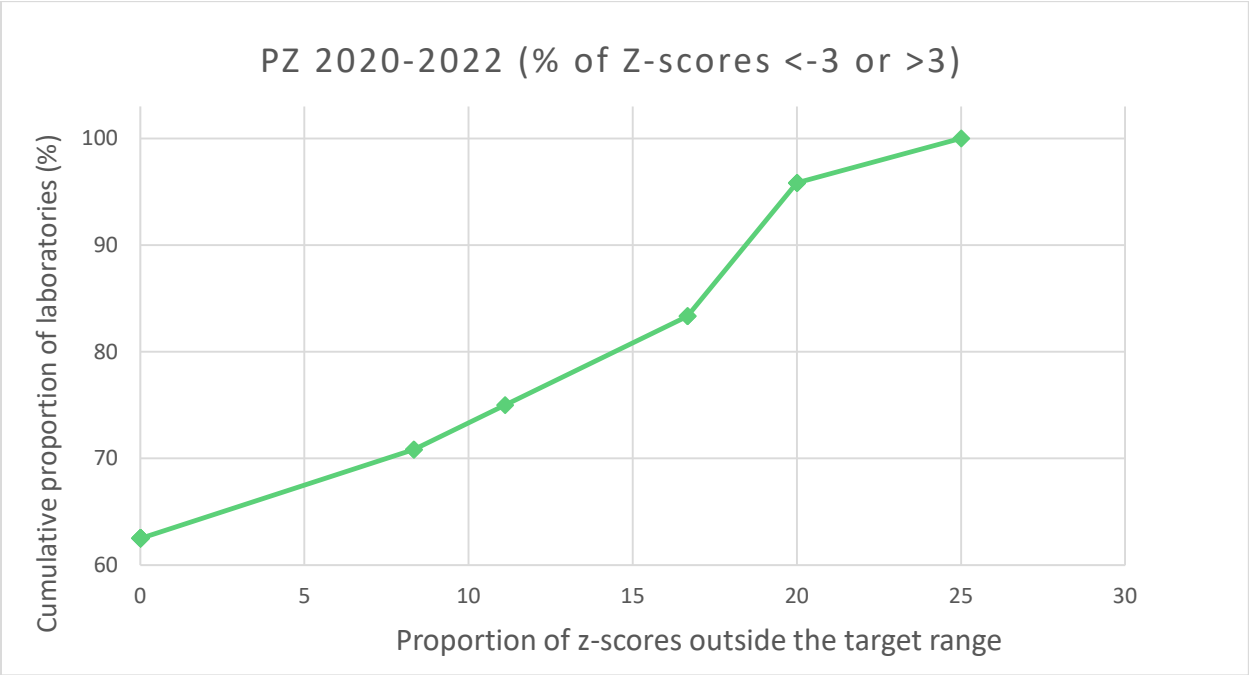


2.4. P_Z evaluation

The performance of the laboratories was also examined by means of the P_Z evaluation.
Given the very limited number of results available per year (2020: n=4, 2021: n=2, 2022: n=6), 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.

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 during the period 2020-2022: 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
2020-2022	24	6.1 ± 8.7	0	0	12.5	20.0	20.0	23.8	0 – 25.0

During the period 2020-2022, the maximum of evaluated results per laboratory was 12.
The table shows that Belgian laboratories reported an average of 6.1% results beyond 3 SD. In addition, fifteen laboratories (63%) reported no results beyond 3 SD during this period.

END