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

2021

Sciensano/Flow cytometry/80-E

Biological health risks
Quality of laboratories
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Lobna Bouacida Digitally signed by Lobna Bouacida

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TABLE OF CONTENTS

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 2021, surveys were conducted in February (FC/17921, FC/17922), May (FC/18215, FC/18216) and November (FC/18731 and FC/18732).

52 Belgian clinical laboratories participated in these surveys.

1.2. Methodology of the Belgian clinical laboratories Survey 2021/3 (n=52)

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	36
Beckman Coulter UniCel DxH 800	6
Siemens Advia 2120	3
Sysmex XE 2100/XE 5000	2
Abbott Cell-Dyn Ruby	1
Not mentioned	4

Flow cytometer	Number of participants
BD Biosciences FACSCanto II	17
Beckman Coulter Navios	16
BD Biosciences FACSLyric	13
Beckman Coulter Cytomics FC 500	3
BD Biosciences FACSCanto	1
BD Biosciences FACSVia	1
Beckman Coulter DxFLEX	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. Except for two laboratories, they all use commercial bead material (70% daily, 18% weekly and 12% per batch).

The following table summarises the bead material used:

Bead material	Number of laboratories
BD Biosciences, cytometer Setup and Tracking beads (CST beads)	27
Beckman-Coulter Flow-Check Fluorospheres	11
Beckman-Coulter Flow-Check Pro Fluorospheres	7
BD Biosciences 7-color setup beads	4
Beckman-Coulter Flow-Set Fluorospheres	1

73% 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
Beckman-Coulter IMMUNO-TROL Cells	11
Streck CD-Chex Plus	11
BD Biosciences Multi-Check Control	9
R&D Systems StatusFlow	2
BD Biosciences Multi-Check CD4 Low Control	2
Streck CD-Chex Plus BC	2
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

51 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	9
Ammonium chloride (NH_4Cl)	5
Beckman-Coulter Optilyse C	5
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	5	5	5	4	3
5 colours	3	3	3	3	1
6 colours	26	26	26	26	26
7 colours	4	4	4	4	4
8 colours	10	10	10	10	10
10 colours	2	2	2	2	2

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, 42% of the participants used CD56 alone and 58% used the combination of CD16 and CD56.

All laboratories that have mentioned their gating technique (n=51) 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	12
100% CD45 positive cells[5,6] + lymphosum	10
Lymphosum + CD3 consistency check	6
100% CD45 positive cells[5,6]	2
Not mentioned	1

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 (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	13	21		**34**
Washing/RBC lysing before incubation with anti-κ/anti-λ reagents	3	7	1	**11**
Total	16	28	1	**45**

73% of the participants used polyclonal anti-κ/anti-λ reagents.
Except for five laboratories, all 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

86% (2021/2) to 92% (2021/1) of the Belgian clinical laboratories mentioning the day of receipt got the blood samples on day 1 and 8% (2021/1) to 14% (2021/2) received the blood samples on day 2 (day 0: send-out of blood samples).

71% (2021/2) to 82% (2021/1) of the Belgian clinical laboratories indicating the day of sample testing performed the analyses on day 1 and 15% (2021/3) to 23% (2021/2) on day 2 (day 0: send-out of blood samples).

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 laboratories were asked to submit their results over the internet using the url: https://qml.wiv-isp.be (toolkit). All participants returned their results this way.

The following tables show the medians and coefficients of variation obtained for the different parameters on the samples sent in 2021:

WBC 10^9/L

	Median	CV,%	N
FC/17921	7.84	2.8	49
FC/17922	7.34	2.6	49
FC/18215	8.60	2.5	47
FC/18216	5.98	2.5	47
FC/18731	10.70	3.7	45
FC/18732	7.36	3.1	46

Lymphocytes %
Haematology analyser

	Median	CV,%	N
FC/17921	37.7	2.0	46
FC/17922	32.7	2.9	46
FC/18215	27.3	2.7	47
FC/18216	21.5	5.7	47
FC/18731	15.7	4.7	46
FC/18732	41.7	2.3	46

Lymphocytes %
Flow cytometer

	Median	CV,%	N
FC/17921	37.4	4.7	46
FC/17922	32.4	6.2	46
FC/18215	27.3	6.8	47
FC/18216	21.4	10.4	47
FC/18731	15.4	9.1	45
FC/18732	40.3	6.9	45

CD3 %

	Median	CV,%	N
FC/17921	76.8	1.5	51
FC/17922	63.5	1.7	51
FC/18215	67.6	3.7	51
FC/18216	69.0	3.5	51
FC/18731	62.2	4.0	52
FC/18732	78.8	2.8	52

CD3 10^9/L

	Median	CV,%	N
FC/17921	2.290	5.5	49
FC/17922	1.531	5.7	49
FC/18215	1.578	7.1	47
FC/18216	0.868	7.8	47
FC/18731	1.046	9.2	47
FC/18732	2.402	5.6	47

CD4 %

	Median	CV,%	N
FC/17921	57.2	2.6	51
FC/17922	35.3	3.1	51
FC/18215	44.2	6.2	51
FC/18216	47.3	4.2	51
FC/18731	39.4	4.9	52
FC/18732	68.0	3.2	52

CD4 10^9/L

	Median	CV,%	N
FC/17921	1.726	6.9	49
FC/17922	0.850	5.9	49
FC/18215	1.037	7.9	47
FC/18216	0.600	9.3	47
FC/18731	0.660	10.5	47
FC/18732	2.055	5.9	47

CD8 %

	Median	CV,%	N
FC/17921	18.0	5.2	51
FC/17922	26.4	3.4	51
FC/18215	19.3	8.1	51
FC/18216	19.0	5.1	51
FC/18731	20.1	5.2	52
FC/18732	10.3	8.6	52

CD8 10^9/L

	Median	CV,%	N
FC/17921	0.521	8.0	49
FC/17922	0.640	6.9	49
FC/18215	0.447	12.0	47
FC/18216	0.240	12.8	47
FC/18731	0.340	11.3	47
FC/18732	0.318	8.1	47

CD19 %

	Median	CV,%	N
FC/17921	12.6	5.9	51
FC/17922	11.0	10.8	51
FC/18215	10.0	8.9	51
FC/18216	12.0	9.3	51
FC/18731	11.1	8.7	52
FC/18732	15.5	9.5	52

CD19 10^9/L

	Median	CV,%	N
FC/17921	0.370	8.8	49
FC/17922	0.260	13.7	49
FC/18215	0.235	16.5	47
FC/18216	0.151	13.7	47
FC/18731	0.183	11.3	47
FC/18732	0.470	14.2	47

NK %

	Median	CV,%	N
FC/17921	10.0	11.2	51
FC/17922	24.7	7.4	51
FC/18215	21.0	10.2	51
FC/18216	18.0	14.0	51
FC/18731	24.8	9.5	52
FC/18732	4.4	16.8	52

NK 10^9/L

	Median	CV,%	N
FC/17921	0.298	14.9	49
FC/17922	0.600	11.6	49
FC/18215	0.471	17.0	47
FC/18216	0.235	12.3	47
FC/18731	0.410	11.6	47
FC/18732	0.132	18.2	47

κ % B lymphocytes

	Median	CV,%	N
FC/17921	66.3	2.2	45
FC/17922	62.2	4.2	44
FC/18215	56.0	3.6	45
FC/18216	58.1	5.0	45
FC/18731	63.8	4.3	44
FC/18732	28.0	15.9	43

λ % B lymphocytes

	Median	CV,%	N
FC/17921	33.0	4.3	45
FC/17922	36.1	9.6	44
FC/18215	43.2	5.5	45
FC/18216	41.1	6.3	45
FC/18731	36.0	7.8	44
FC/18732	69.8	13.3	43

κ/λ ratio

	Median	CV,%	N
FC/17921	2.00	7.2	45
FC/17922	1.75	13.5	44
FC/18215	1.29	6.3	45
FC/18216	1.40	11.1	45
FC/18731	1.78	11.2	44
FC/18732	0.41	43.3	43

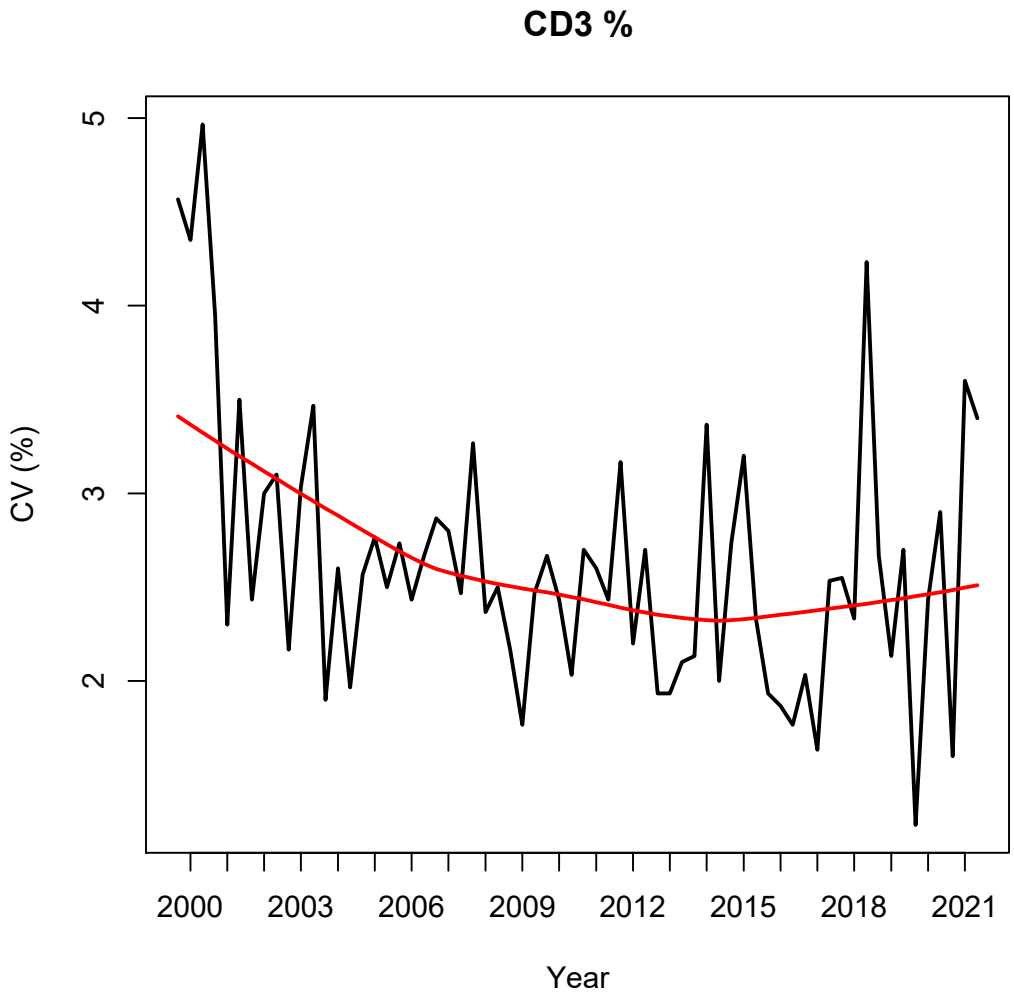
κ+λ % B lymphocytes

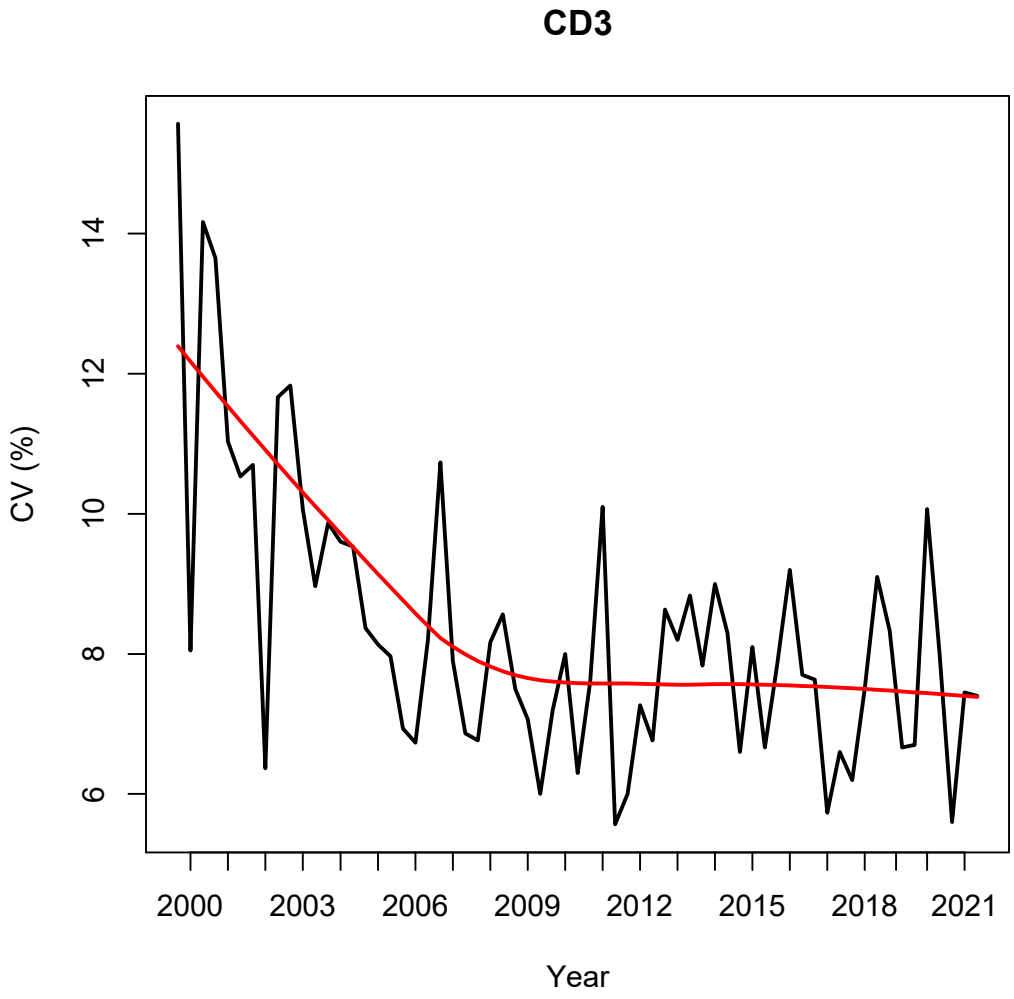
	Median	CV,%	N
FC/17921	99.7	0.7	45
FC/17922	99.8	0.7	44
FC/18215	99.4	0.9	45
FC/18216	99.8	1.0	45
FC/18731	99.8	0.6	44
FC/18732	99.7	1.3	43

Lymphosum %

	Median	CV,%	N
FC/17921	99.4	0.7	51
FC/17922	99.1	1.1	51
FC/18215	98.0	1.5	51
FC/18216	98.0	1.5	51
FC/18731	98.2	2.0	52
FC/18732	99.3	1.0	52

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
CV (%)
6
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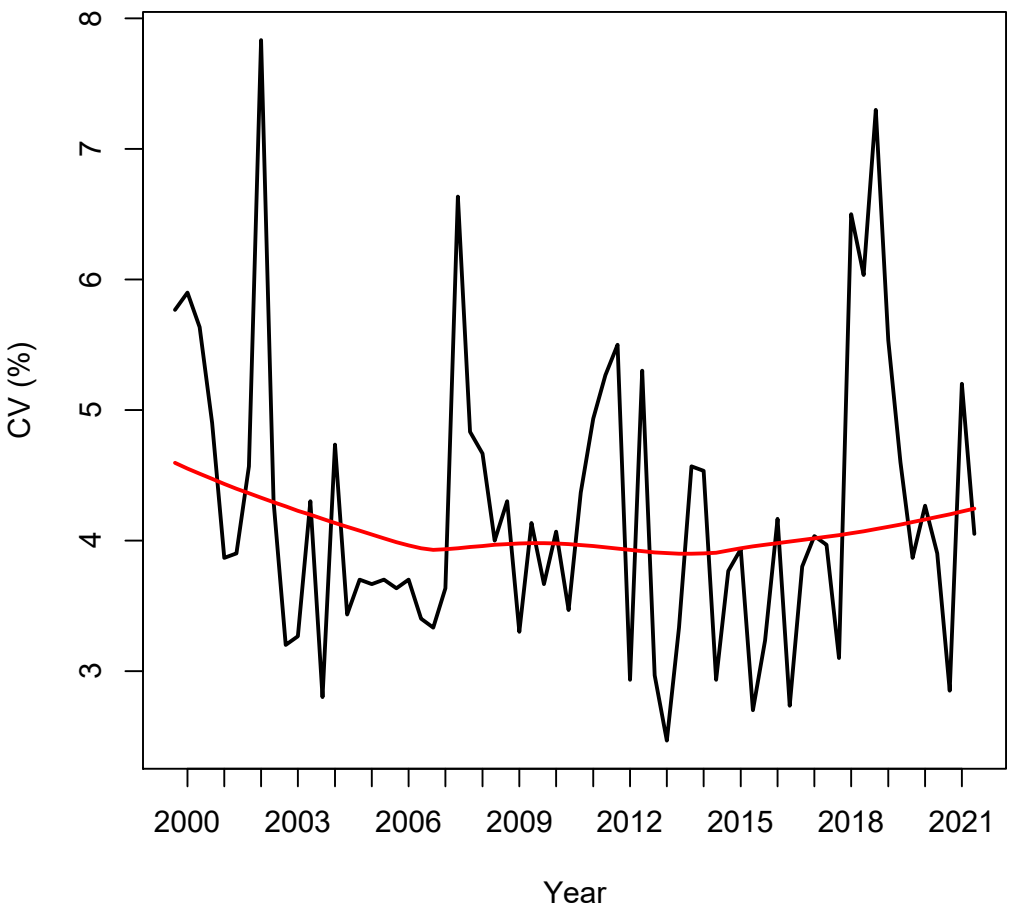
2015
2018
2021
Year


CD4 %
CV (%)
3
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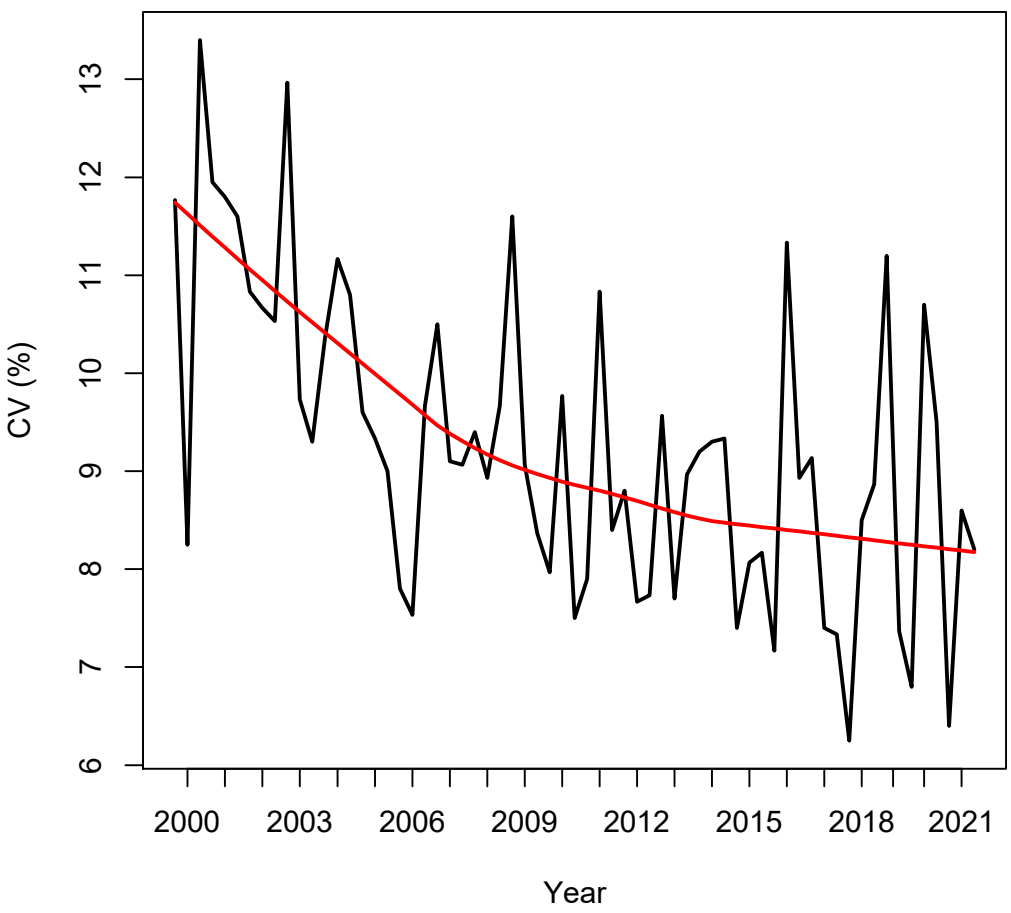
2018
2021
Year


CD4
CV (%)
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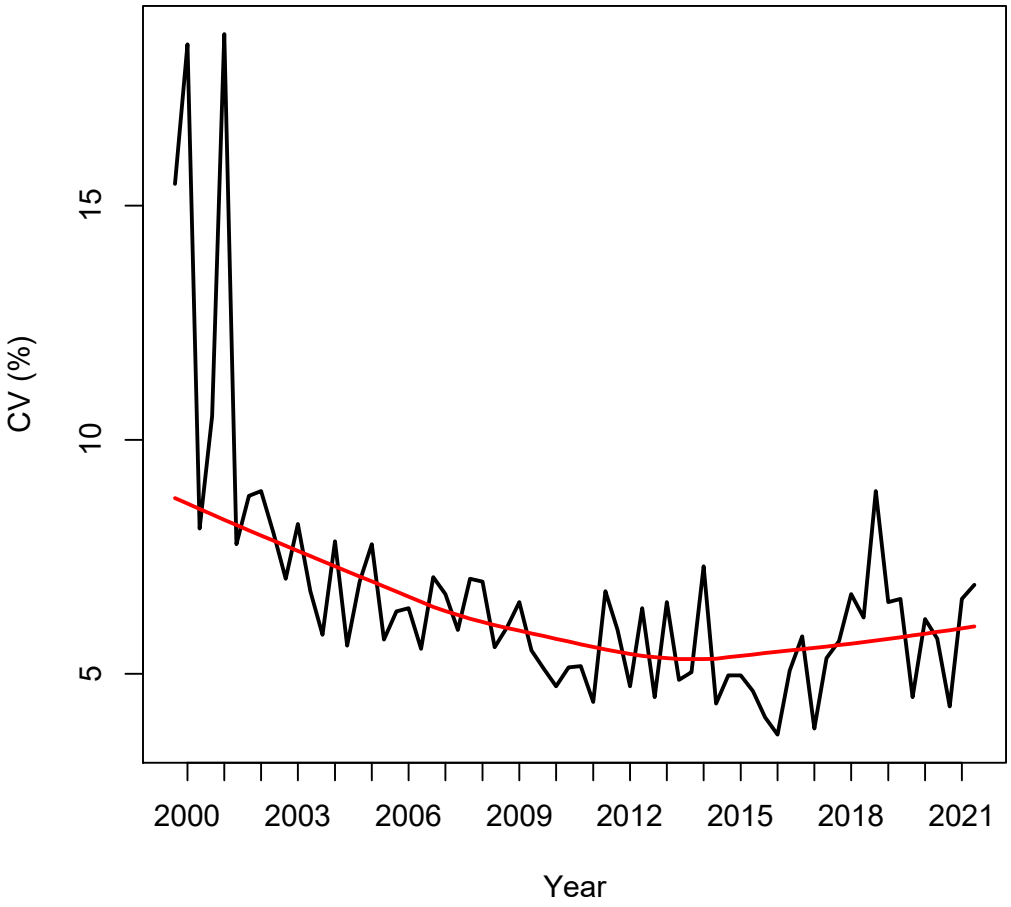
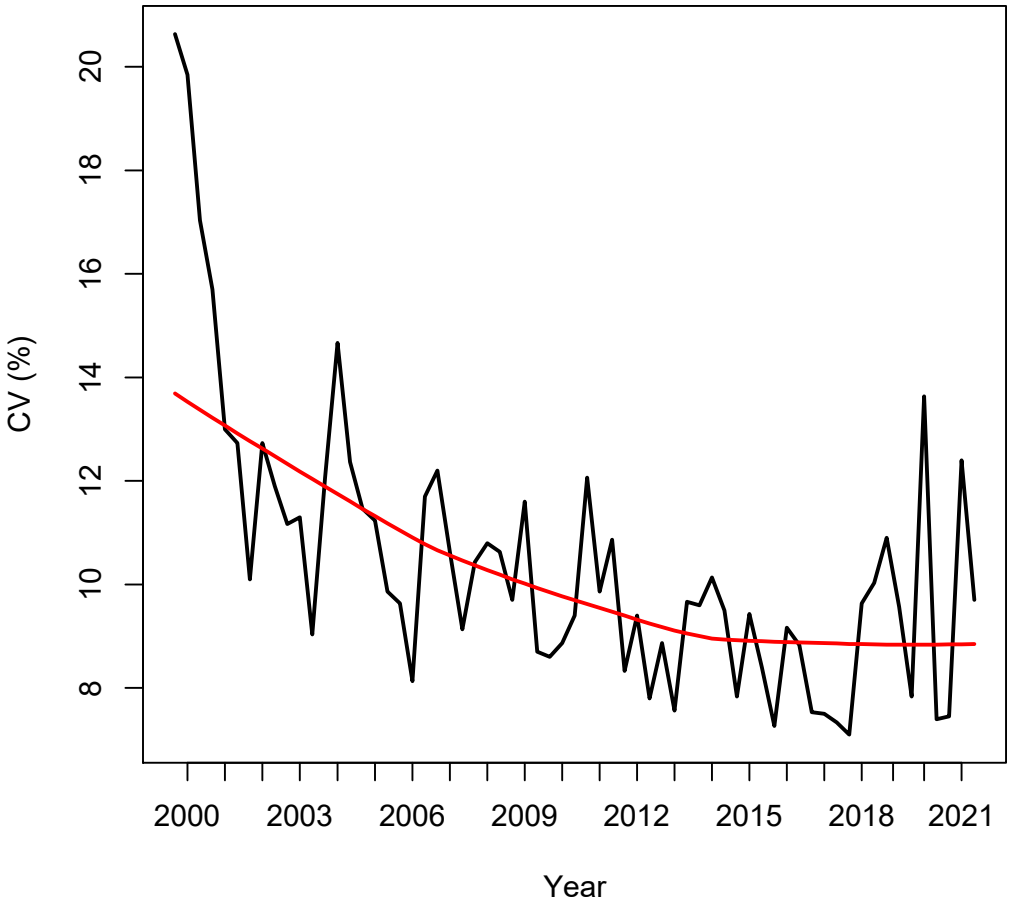
2018
2021
Year

CD8 %




CD8
CV (%)
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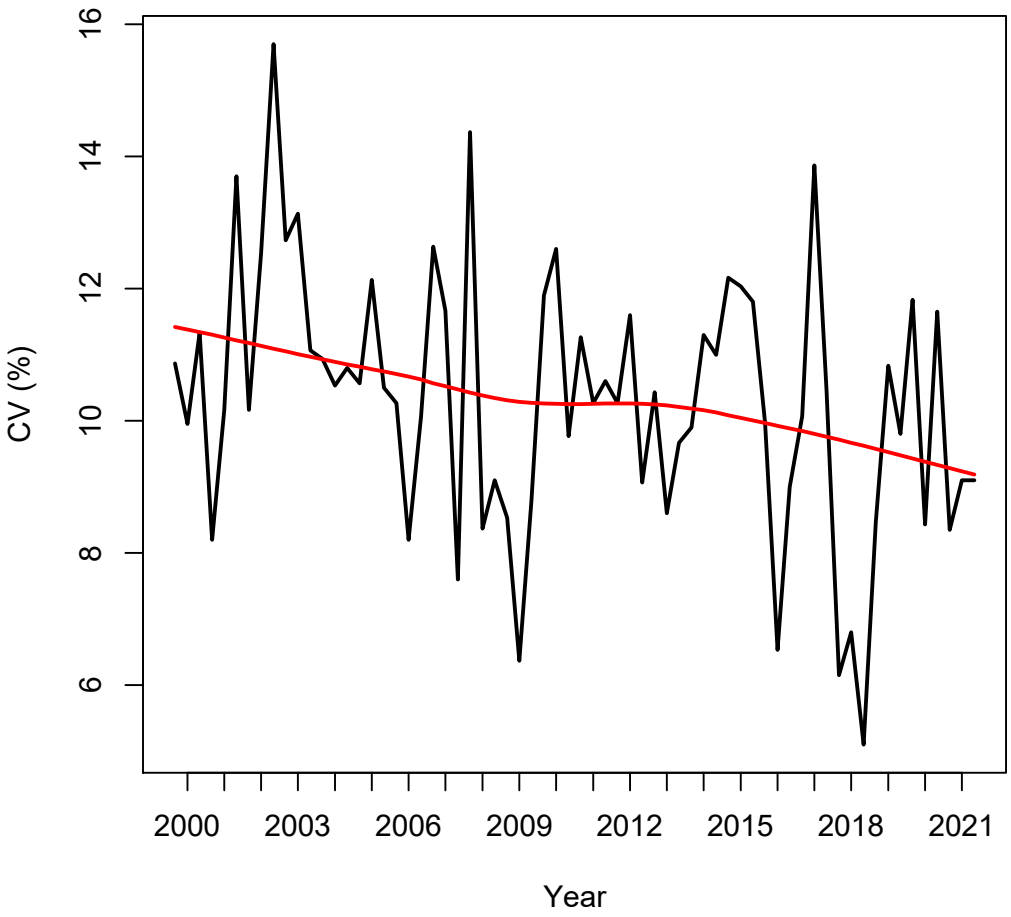
2021
Year


CD19 %
CV (%)
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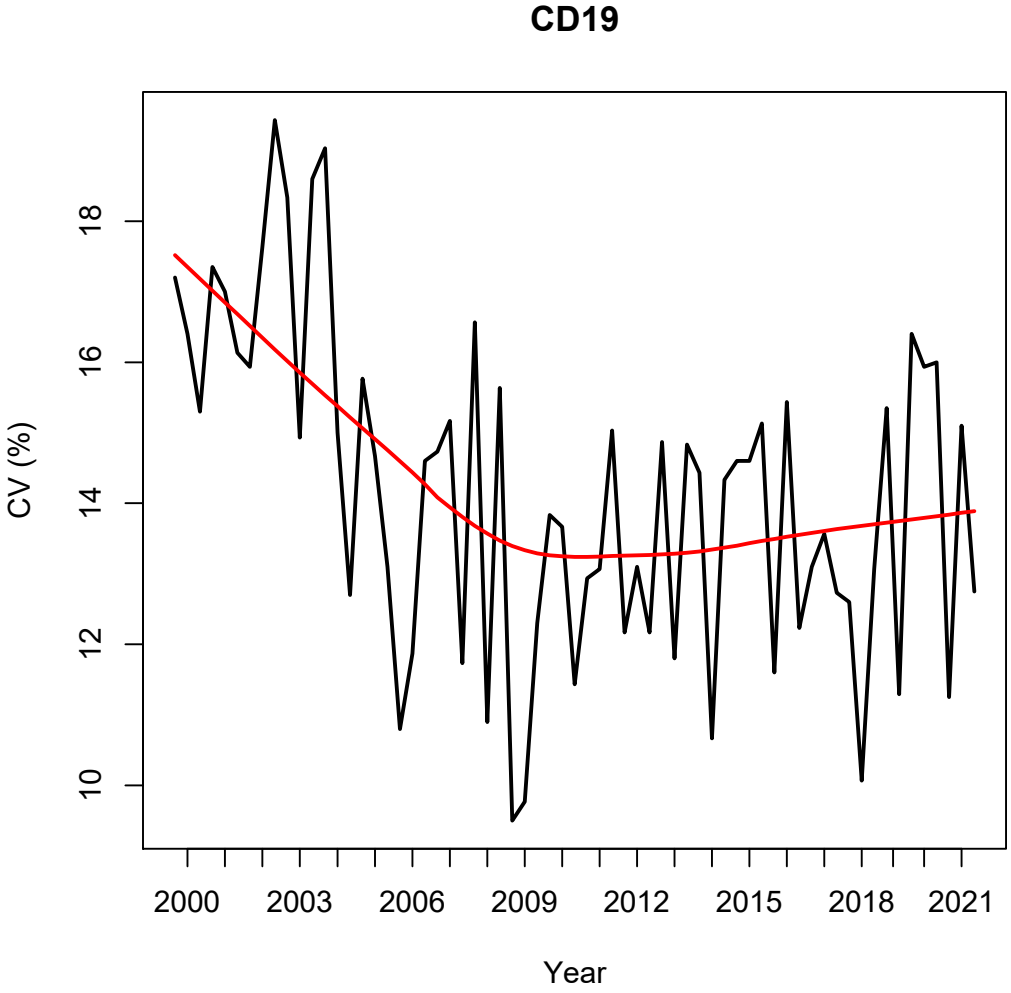
2018
2021
Year


CD19
CV (%)
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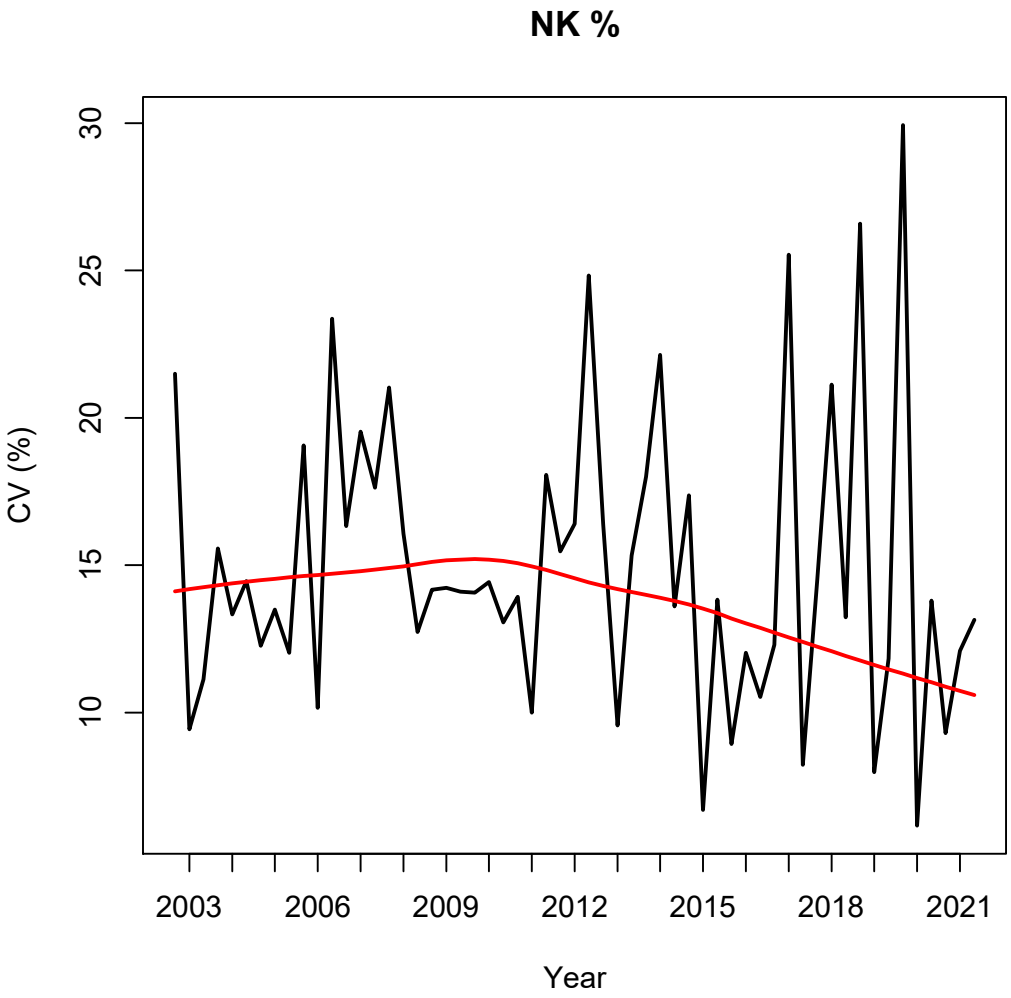
2018
2021
Year


NK %
CV (%)
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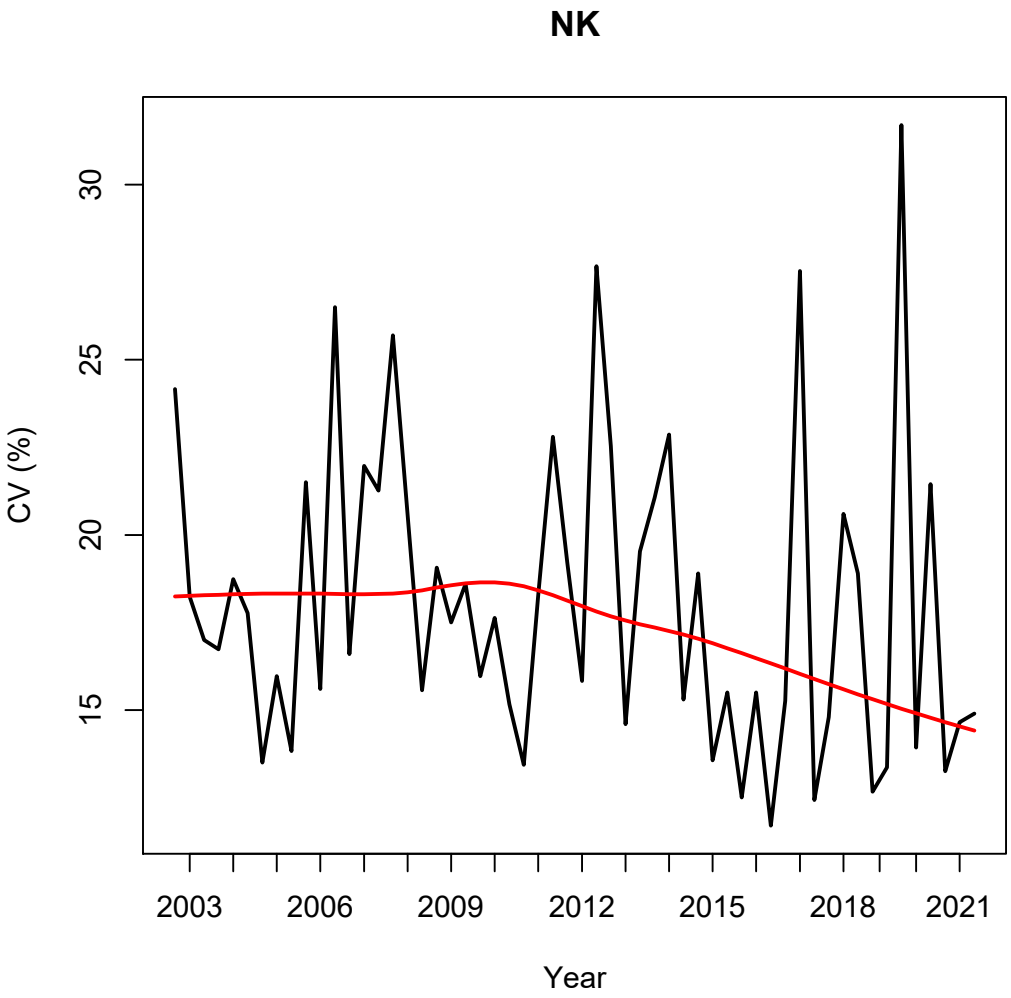
2018
2021
Year


NK
CV (%)
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25
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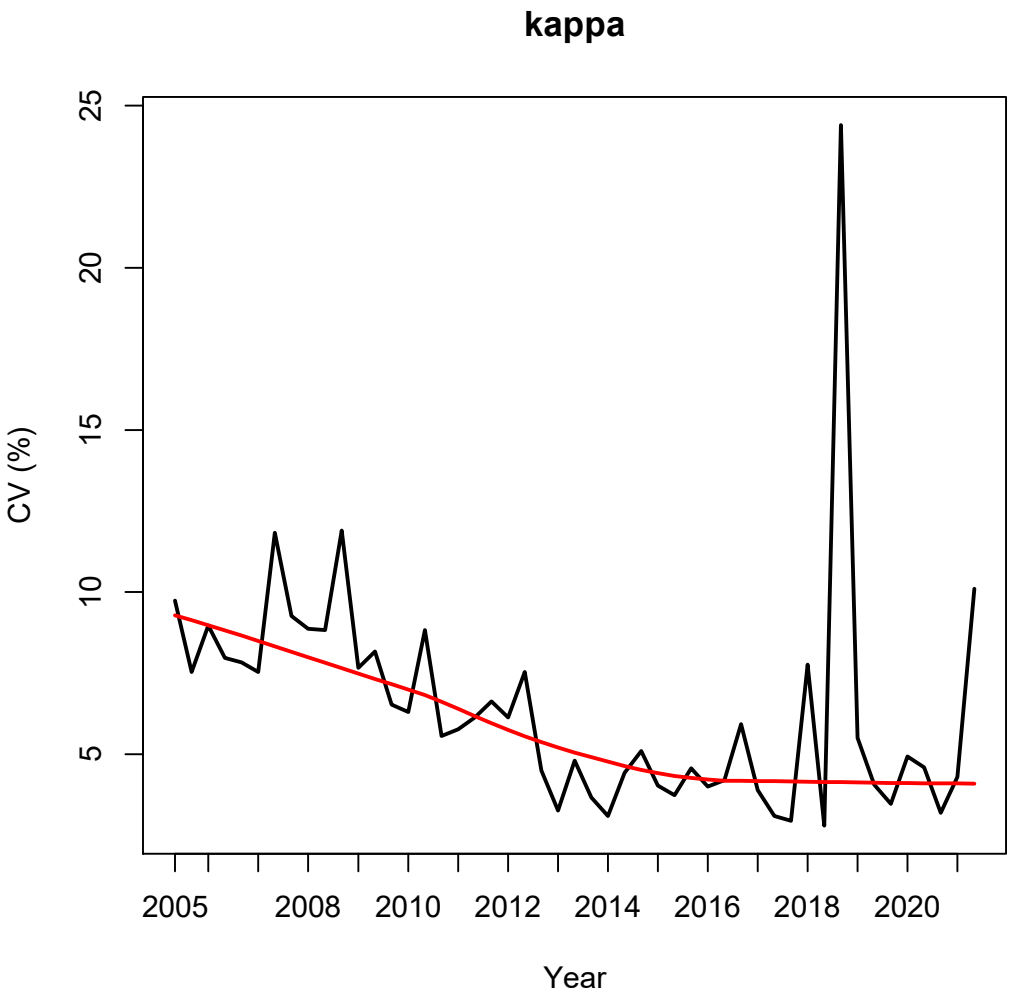
2021
Year


kappa
CV (%)
25
20
15
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5
2005
2008
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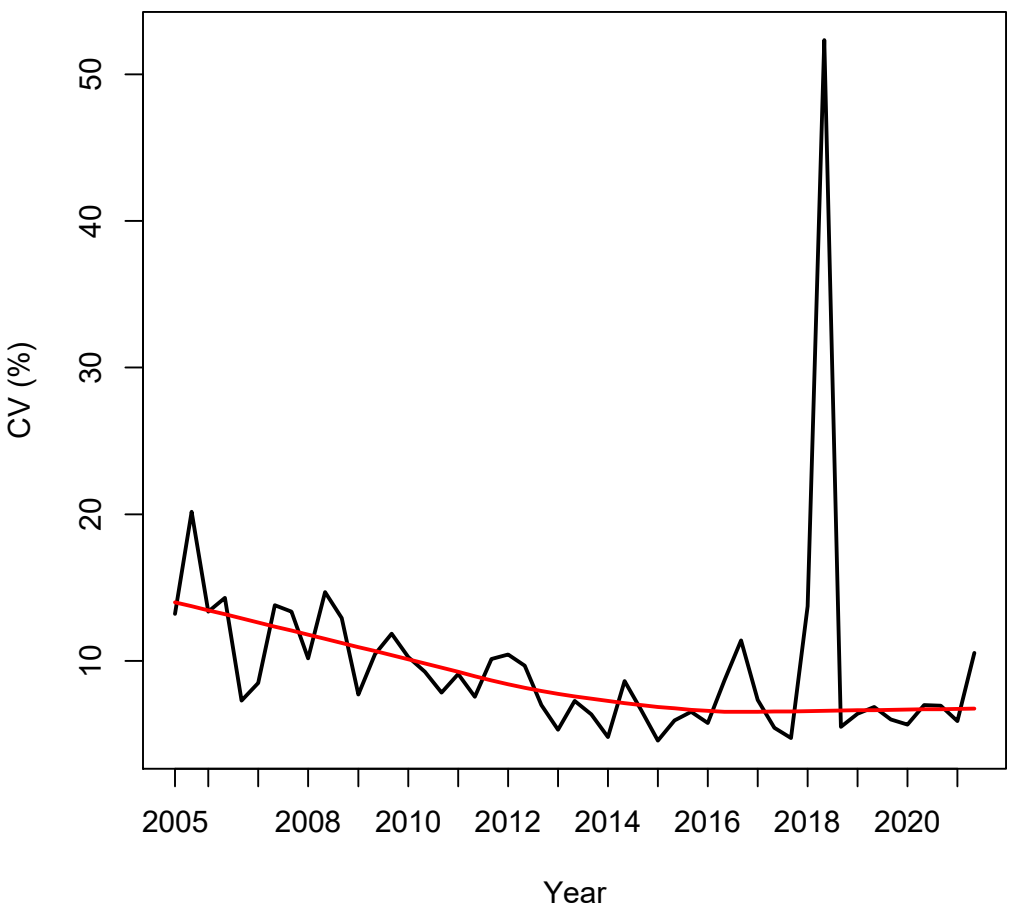
2012
2014
2016
2018
2020
Year


lambda
CV (%)
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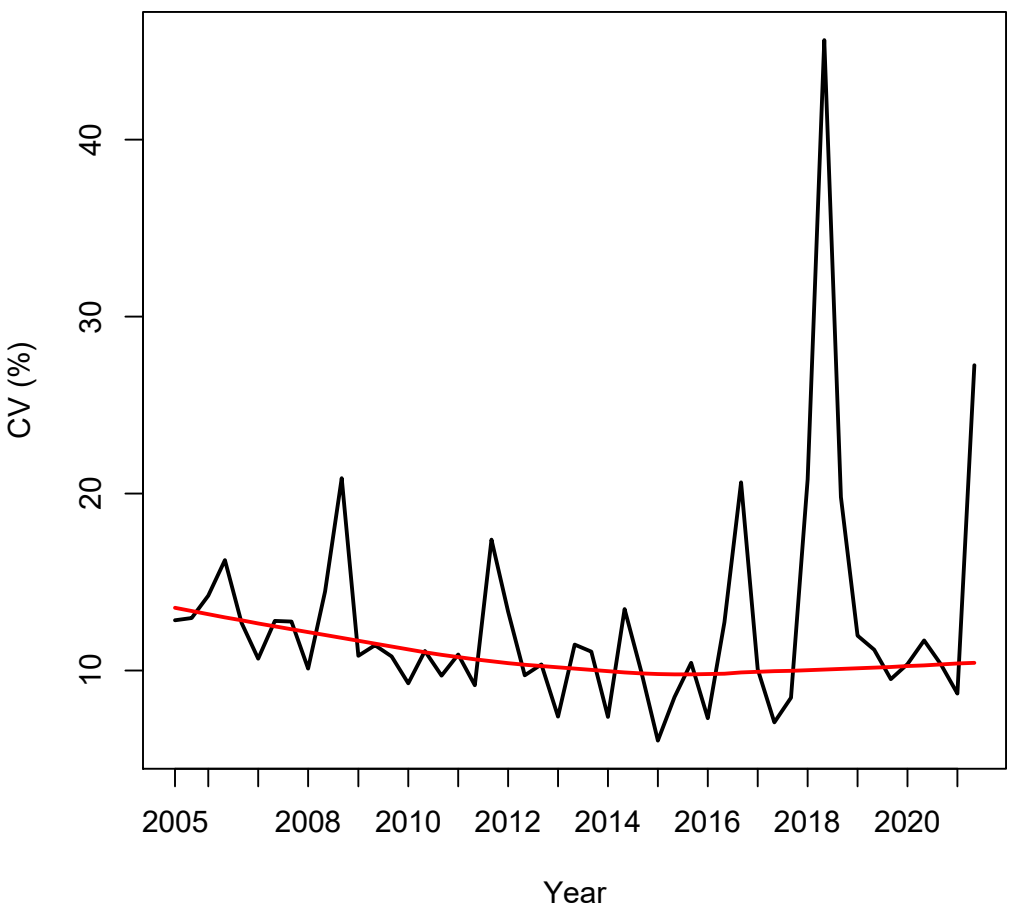
2020
Year


kappa/lambda
CV (%)
10
20
30
40
2005
2008
2010
2012
2014
2016
2018
2020
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

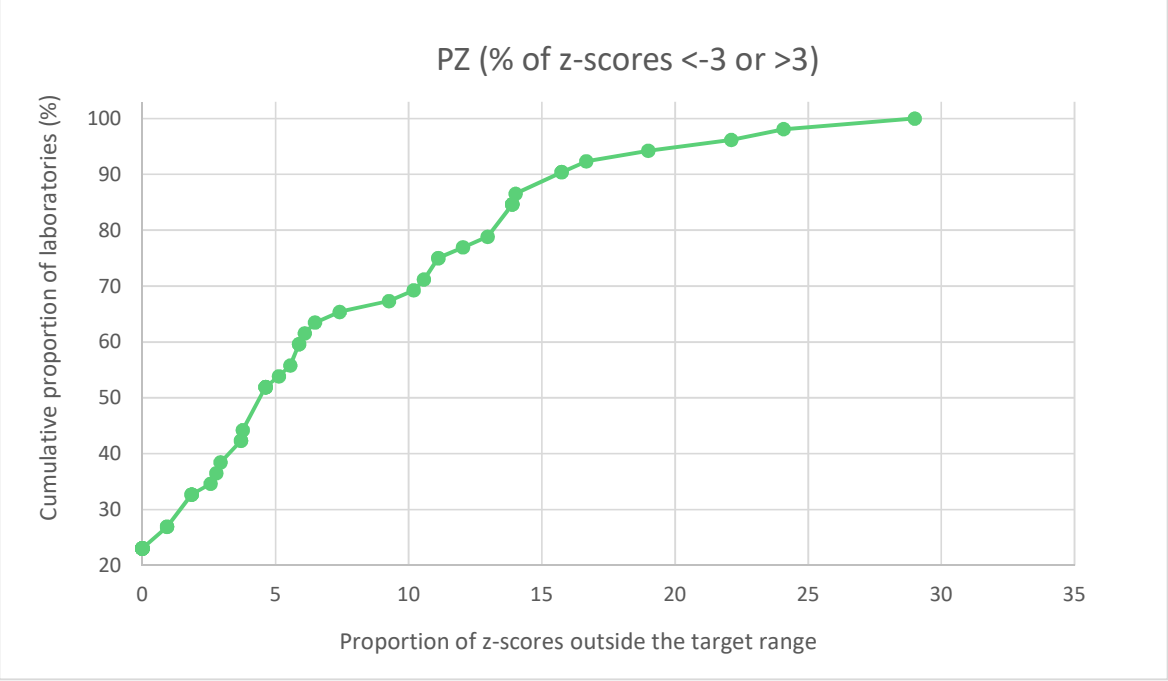
Z-scores reflect the performance of a laboratory with respect to its peer group. Z-scores <-3 or >3 (results falling beyond 3 SD from the median) are considered unacceptable.
The performance of the laboratories is evaluated by means of the percentage of unacceptable z-scores (P_Z, % of results falling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

The maximum of evaluated results per laboratory was 108.
This table shows a.o. that Belgian laboratories reported an average of 6.9% results beyond 3 SD and that 25% of laboratories got less than 0.9% of results beyond 3 SD in 2021.
The next table summarises for the different parameters the number of evaluated results and the percentage of results beyond 3 SD:

	2020		2021	
Parameter	**Number of evaluated results**	**% results >3 SD**	**Number of evaluated results**	**% results >3 SD**
Leukocytes 10^9/L	390	7.9	301	5.1
Lymphocytes % HA	353	6.8	292	8.0
Lymphocytes % FC	384	6.0	276	6.9
CD3 %	402	5.0	308	6.6
CD3 10^9/L	395	10.1	304	9.4
CD4 %	402	5.7	308	4.3
CD4 10^9/L	395	13.4	304	8.4
CD8 %	402	2.5	308	3.9
CD8 10^9/L	395	10.4	304	6.0
CD19 %	402	6.5	308	7.3
CD19 10^9/L	395	11.4	304	7.0
NK cells %	402	5.7	308	5.6
NK cells 10^9/L	395	13.7	304	9.7
κ % B lymphocytes	351	8.3	266	5.7
λ % B lymphocytes	351	7.7	266	6.5
κ/λ ratio	351	4.3	266	7.3
κ+λ % B lymphocytes	351	19.1	266	12.2
Lymphosum	402	7.7	308	4.3

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	292	12	10%	0%
CD4 10^9/L	292	12	9%	0%
CD8 10^9/L	292	12	8%	0%
CD19 10^9/L	292	12	8%	0%
NK cells 10^9/L	292	12	11%	0%

Parameter	Number of evaluated results		% results >3 SD	
	Lyse and wash	Lyse no wash	Lyse and wash	Lyse no wash
CD3 %	150	158	9%	4%
CD3 10^9/L	146	158	11%	9%
CD4 %	150	158	8%	1%
CD4 10^9/L	146	158	12%	6%
CD8 %	150	158	7%	2%
CD8 10^9/L	146	158	12%	3%
CD19 %	150	158	11%	4%
CD19 10^9/L	146	158	10%	6%
NK cells %	158	150	10%	2%
NK cells 10^9/L	146	158	12%	9%
Lymphosum	150	158	5%	4%

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	79	187	6%	5%
λ % B lymphocytes	79	187	8%	6%
κ/λ ratio	79	187	9%	6%
κ+λ % B lymphocytes	79	187	13%	12%

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 %	210	98	6%	7%
CD3 10^9/L	206	98	7%	15%
CD4 %	210	98	4%	4%
CD4 10^9/L	206	98	6%	14%
CD8 %	210	98	3%	8%
CD8 10^9/L	206	98	4%	13%
CD19 %	210	98	8%	6%
CD19 10^9/L	206	98	8%	7%
NK cells %	210	98	5%	8%
NK cells 10^9/L	206	98	9%	13%
Lymphosum	210	98	5%	3%

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 2021, only one survey could be conducted, in February (**FC/17923**). Due to a lack of samples, the surveys initially planned in May and November could not be carried out.

Twenty-two Belgian clinical laboratories participated in this survey.

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).

The laboratories were asked to submit their results over the internet using the url: https://qml.wiv-isp.be (toolkit). All participants returned their results this way.

2.2. Methodology of the Belgian clinical laboratories Survey 2021/1 (n=22)

Fifteen laboratories (68%) used a single platform approach for determining the absolute CD34+ cell count. Of these laboratories, 10 used Trucount technology (BD Biosciences) and 4 Flow-Count or Stem-count beads (Beckman-Coulter). 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	9
Beckman-Coulter Navios	7
BD Biosciences FACSLyric	5
Miltenyi Biotec MACSQuant analyzer	1

Sample preparation

Ten participants used a sample volume of 50 µL and ten a sample volume of 100 µL. Two participants used other volumes: 25 µL for one and 30 µL for the other. 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)	7
BD Biosciences Pharm Lyse	5
Beckman-Coulter VersaLyse	4
BD Biosciences Ammonium chloride lysing solution	3
Beckman-Coulter Ammonium chloride	2
Qiagen EL-buffer	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.

Viability

91% (n=20) of the laboratories evaluated CD34+ cell viability using 7-Aminoactinomycin D.

Gating strategy

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

2.3. Results

All apart from one of the laboratories received the samples on day 1 or 2. Nineteen laboratories (86%) received the samples on day 1 and three (14%) received them on day 2. The laboratory which received the samples later did not respond to the survey.

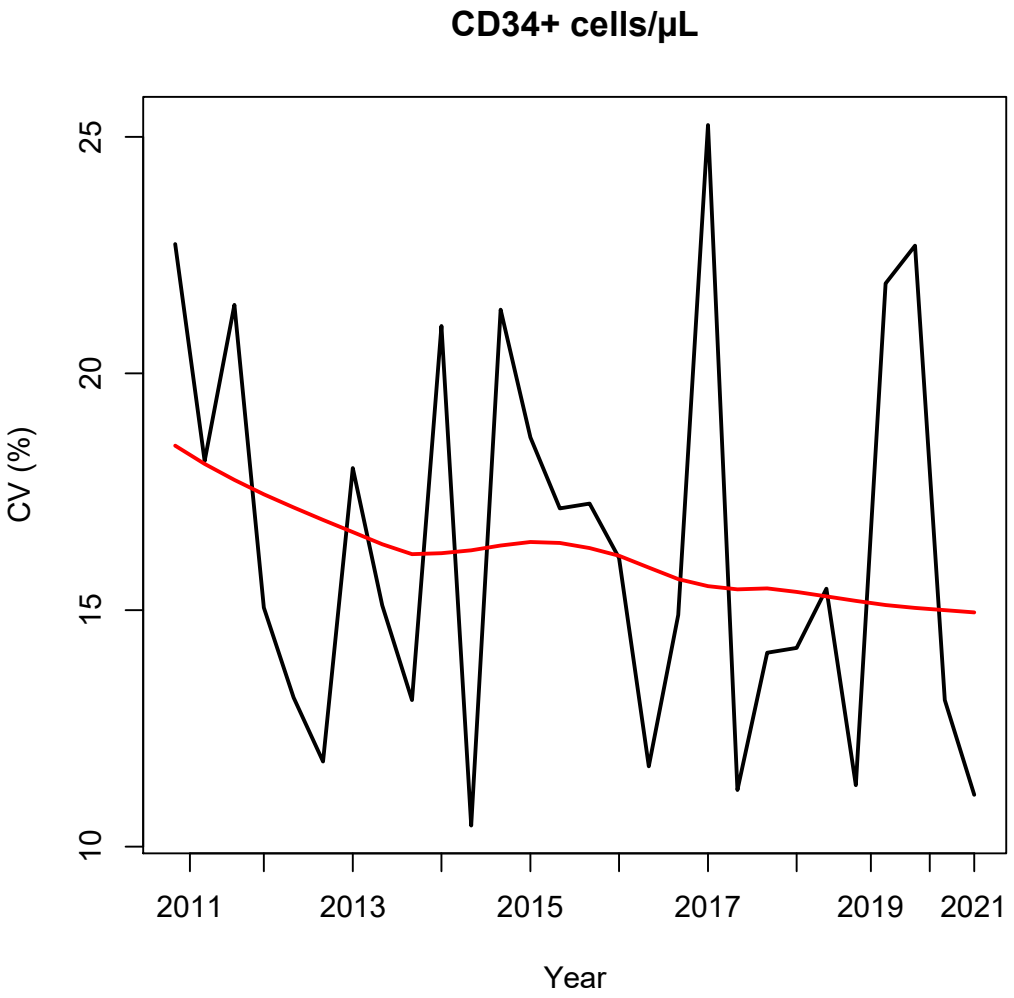
73% of the laboratories (n=16) performed the analyses on day 1, and 27% on day 2 (n=6).

Statistics for the evaluation were solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2.

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 2021:

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/μL	CV %	N
FC/17923	0.492	13.1	22	60.0	11.1	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.

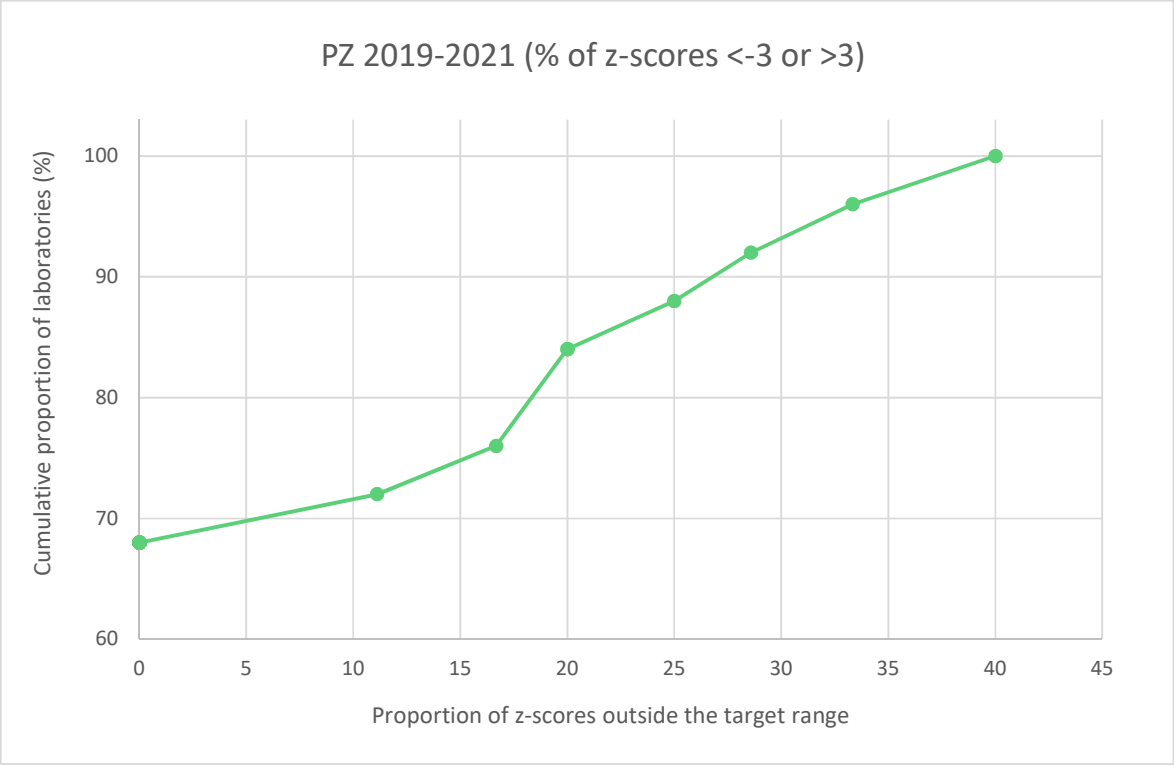


2.4. P_Z evaluation

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 (2019: n=4, 2020: n=4, 2021: n=2), 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 2019-2021: 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
2019-2021	25	7.8 ± 12.7	0	0	16.7	27.1	32.4	38.4	0 – 40.0

During the period 2019-2021, the maximum of evaluated results per laboratory was 10.
The table shows that Belgian laboratories reported an average of 7.8% results beyond 3 SD. In addition, seventeen laboratories (68%) reported no results beyond 3 SD during this period.

The following tables show the percentage of results beyond 3 SD according to the methodology and the monitoring.

Parameter	Commercial control material usage			
	Number of evaluated results		% results >3 SD	
	YES	NO	YES	NO
% viable CD34+ cells within total WBC	89	22	3%	32%
Absolute viable CD34+ cell count (cells/µL)	88	21	2%	10%

Parameter	Viability staining			
	Number of evaluated results		% results >3 SD	
	YES	NO	YES	NO
% viable CD34+ cells within total WBC	100	11	9%	9%
Absolute viable CD34+ cell count (cells/µL)	99	10	4%	0%

Parameter	ISHAGE Protocol Gating Strategy			
	Number of evaluated results		% results >3 SD	
	YES	NO	YES	NO
% viable CD34+ cells within total WBC	98	13	5%	38%
Absolute viable CD34+ cell count (cells/µL)	96	13	3%	8%

END