EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
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EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

PROVISIONAL GLOBAL ANNUAL REPORT

FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS

CD34+ STEM CELL ENUMERATION

2019

Sciensano/Flow cytometry/72-E

Expertise and service provision
Quality of laboratories
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Authorization to release the report: by Lobna Bouacida, scheme coordinator, on 12/06/2020

Digitally signed by Lobna Bouacida
Date: 2020.06.12 13:35:05 +02'00'

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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 3 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 2019, surveys were conducted in February (FC/16260, FC/16261 and FC/16262), May (FC/16508, FC/16509 and FC/16510) and October (FC/16941, FC/16942 and FC/16943).

1 Canadian, 1 Latvian and 52 Belgian clinical laboratories participated in these surveys.

1.2. Methodology of the Belgian clinical laboratories Survey 2019/3 (n=52)

Nine laboratories (17%) used a single platform approach for determining the absolute lymphocyte subset counts. Of these laboratories, 7 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	25
Sysmex XE 2100/XE 5000	7
Beckman Coulter UniCel DxH 800	7
Siemens Advia 2120	6
Abbott Cell-Dyn Sapphire	2
Sysmex XT 4000i	1
Sysmex XS 1000i	1
Abbott Cell-Dyn Ruby	1
Not mentioned	2

Flow cytometer	Number of participants
BD Biosciences FACSCanto II	21
Beckman Coulter Navios	14
Beckman Coulter Cytomics FC 500	9
BD Biosciences FACSCalibur	2
BD Biosciences FACSLyric	2
BD Biosciences FACSCanto	1
BD Biosciences FACSVia	1
Not mentioned	2

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 (62% daily, 24% weekly, 10% per batch, 2% 3 times a week and 2% 2 times a week).

The following table summarises the bead material used:

Bead material	Number of laboratories
BD Biosciences, cytometer Setup and Tracking beads (CST beads)	24
Beckman-Coulter Flow-Check Fluorospheres	11
Beckman-Coulter Flow-Check Pro Fluorospheres	8
BD Biosciences 7-color setup beads	4
BD Biosciences Calibrite 3 (+ APC beads)	2
Beckman-Coulter Flow-Set Fluorospheres	1

75% 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
Beckman-Coulter IMMUNO-TROL Cells	15
BD Biosciences Multi-Check Control	9
Streck CD-Chex Plus	7
R&D Systems StatusFlow	4
BD Biosciences Multi-Check CD4 Low Control	2
Streck CD-Chex Plus CD4 Low, Normal	1
Streck CD-Chex Plus BC	1

One participant mentioned using in addition a home-made control material, which is a normal blood sample.

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

50 laboratories applied the whole blood lysis technique. The other two did not give any indication of their technique. Among those who responded, 56% used a lyse no wash procedure.

The following table summarises the lysing reagents used:

Lysing reagent	Number of laboratories
BD Biosciences FACS Lysing Solution	24
Beckman-Coulter VersaLyse	12
Ammonium chloride (NH_4Cl)	5
BD Biosciences Pharm Lyse	4
Beckman-Coulter Optilyse C	3
Beckman-Coulter Immunoprep reagent system	2

Most laboratories used 6-colour combination (n=48, responding laboratories).

	Number of participants				
	$CD3^+$	$CD4^+$	$CD8^+$	$CD19^+$	NK
4 colours	7	7	7	6	6
5 colours	6	6	6	5	5
6 colours	23	23	23	23	23
8 colours	7	7	7	7	6
10 colours	5	5	5	5	5

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)*

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, 40% of the participants used CD56 alone and 60% used the combination of CD16 and CD56.

All laboratories that have mentioned their gating technique (n=50) used CD45 as gating agent.

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

Sample quality control assessment	Number
100% CD45 positive cells[5,6] + lymphosum + CD3 consistency check	15
Lymphosum	15
100% CD45 positive cells[5,6] + lymphosum	11
Lymphosum + CD3 consistency check	7
100% CD45 positive cells[5,6]	2
Not mentioned	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 (30%) or more (70%) 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	10	21		**31**
Washing/RBC lysing before incubation with anti-κ/anti-λ reagents	3	8	1	**12**
Total	13	29	1	**43**

72% of the participants used polyclonal anti-κ/anti-λ reagents.
Except for one laboratory, all used anti-κ and anti-λ antibodies in combination with CD19 in one tube.
88% of the participants used CD19/SSC gating and 12% 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

9% (2019/1) to 86% (2019/2) of the Belgian clinical laboratories mentioning the day of receipt got the blood samples on day 1 and 4% (2019/1) to 14% (2019/2) received the blood samples on day 2 (day 0: send-out of blood samples).

7% (2019/1) to 76% (2019/2) of the Belgian clinical laboratories indicating the day of sample testing performed the analyses on day 1 and 4% (2019/1) to 21% (2019/3) on day 2 (day 0: send-out of blood samples).

For the first survey 2019/1, owing to a problem with the carrier, the majority of the laboratories (87%) received the samples on day 3 instead of day 1.
It was therefore decided not to evaluate the laboratories for the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets.

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 2019:

WBC 10^9/L

	Median	CV,%	N
FC/16508	5.30	2.5	44
FC/16509	5.31	2.7	44
FC/16510	5.37	2.9	44
FC/16941	9.40	3.0	48
FC/16942	6.03	4.7	48
FC/16943	9.38	3.6	49

Lymphocytes %
Haematology analyser

	Median	CV,%	N
FC/16508	31.6	4.7	44
FC/16509	32.0	4.2	44
FC/16510	31.8	6.3	44
FC/16941	27.6	4.0	46
FC/16942	32.8	3.4	46
FC/16943	27.7	3.3	47

Lymphocytes %
Flow cytometer

	Median	CV,%	N
FC/16260	37.9	10.8	41
FC/16261	37.5	11.7	41
FC/16262	37.8	11.1	41
FC/16508	29.7	8.2	40
FC/16509	29.5	13.3	40
FC/16510	29.8	11.4	40
FC/16941	27.1	6.5	42
FC/16942	32.3	8.0	42
FC/16943	27.3	7.7	43

CD3 %

	Median	CV,%	N
FC/16260	80.0	1.9	46
FC/16261	80.2	2.5	46
FC/16262	80.8	3.6	46
FC/16508	73.1	2.5	49
FC/16509	73.5	2.1	49
FC/16510	73.3	1.8	49
FC/16941	64.7	2.5	51
FC/16942	74.0	2.7	51
FC/16943	64.8	2.9	52

CD3 10^9/L

	Median	CV,%	N
FC/16508	1.234	6.4	45
FC/16509	1.250	9.7	45
FC/16510	1.244	8.9	45
FC/16941	1.694	5.4	48
FC/16942	1.440	7.9	48
FC/16943	1.705	6.7	49

CD4 %

	Median	CV,%	N
FC/16260	45.0	6.9	46
FC/16261	44.6	6.3	46
FC/16262	45.0	8.7	46
FC/16508	24.0	5.2	49
FC/16509	24.2	4.6	49
FC/16510	24.1	6.8	49
FC/16941	36.9	5.8	51
FC/16942	52.4	4.0	51
FC/16943	36.8	4.0	52

CD4 10^9/L

	Median	CV,%	N
FC/16508	0.413	10.4	45
FC/16509	0.410	9.4	45
FC/16510	0.409	13.8	45
FC/16941	0.963	6.9	48
FC/16942	1.035	9.1	48
FC/16943	0.965	6.1	49

CD8 %

	Median	CV,%	N
FC/16260	34.1	8.2	46
FC/16261	33.4	10.2	46
FC/16262	33.9	8.3	46
FC/16508	41.0	5.8	49
FC/16509	40.5	6.9	49
FC/16510	40.6	6.9	49
FC/16941	26.0	7.1	51
FC/16942	14.0	7.4	51
FC/16943	26.4	5.3	52

CD8 10^9/L

	Median	CV,%	N
FC/16508	0.680	8.2	45
FC/16509	0.657	11.3	45
FC/16510	0.678	13.2	45
FC/16941	0.681	9.6	48
FC/16942	0.269	11.0	48
FC/16943	0.692	8.1	49

CD19 %

	Median	CV,%	N
FC/16260	15.4	6.5	46
FC/16261	15.0	9.3	46
FC/16262	14.6	9.6	46
FC/16508	5.2	9.1	49
FC/16509	5.3	11.1	49
FC/16510	5.4	12.3	49
FC/16941	14.5	8.8	51
FC/16942	12.2	9.7	51
FC/16943	14.3	10.9	52

CD19 10^9/L

	Median	CV,%	N
FC/16508	0.087	15.3	45
FC/16509	0.088	14.3	45
FC/16510	0.090	16.4	45
FC/16941	0.380	11.3	48
FC/16942	0.240	10.5	48
FC/16943	0.367	12.1	49

NK %

	Median	CV,%	N
FC/16260	3.4	23.5	46
FC/16261	3.2	28.1	46
FC/16262	3.2	28.1	46
FC/16508	19.9	8.6	49
FC/16509	20.2	7.6	49
FC/16510	20.2	7.8	49
FC/16941	19.6	10.6	51
FC/16942	12.2	15.9	51
FC/16943	19.7	9.0	52

NK 10^9/L

	Median	CV,%	N
FC/16508	0.340	13.1	45
FC/16509	0.340	12.0	45
FC/16510	0.343	12.9	45
FC/16941	0.510	12.5	48
FC/16942	0.242	15.3	48
FC/16943	0.510	12.3	49

κ % B lymphocytes

	Median	CV,%	N
FC/16260	64.0	28.5	36
FC/16261	62.0	24.0	36
FC/16262	63.6	20.7	36
FC/16508	59.2	5.9	43
FC/16509	58.2	5.7	43
FC/16510	58.5	4.9	43
FC/16941	61.7	4.8	45
FC/16942	59.8	2.6	45
FC/16943	61.9	4.8	46

λ % B lymphocytes

	Median	CV,%	N
FC/16260	42.0	4.3	36
FC/16261	39.6	8.2	36
FC/16262	41.3	4.0	36
FC/16508	40.0	4.7	43
FC/16509	40.9	7.9	43
FC/16510	40.1	6.6	43
FC/16941	36.8	8.8	45
FC/16942	39.0	3.8	45
FC/16943	36.9	8.0	46

κ/λ ratio

	Median	CV,%	N
FC/16260	1.77	18.6	36
FC/16261	1.64	25.3	36
FC/16262	1.67	15.5	36
FC/16508	1.48	11.0	43
FC/16509	1.40	13.2	43
FC/16510	1.46	11.7	43
FC/16941	1.69	14.0	45
FC/16942	1.54	6.2	45
FC/16943	1.67	13.3	46

κ+λ % B lymphocytes

	Median	CV,%	N
FC/16260	100.2	10.6	36
FC/16261	100.3	6.4	36
FC/16262	99.5	8.4	36
FC/16508	99.6	1.1	42
FC/16509	99.7	1.6	42
FC/16510	99.4	1.8	42
FC/16941	99.6	1.3	45
FC/16942	99.5	1.0	45
FC/16943	99.5	1.3	46

Lymphosum %

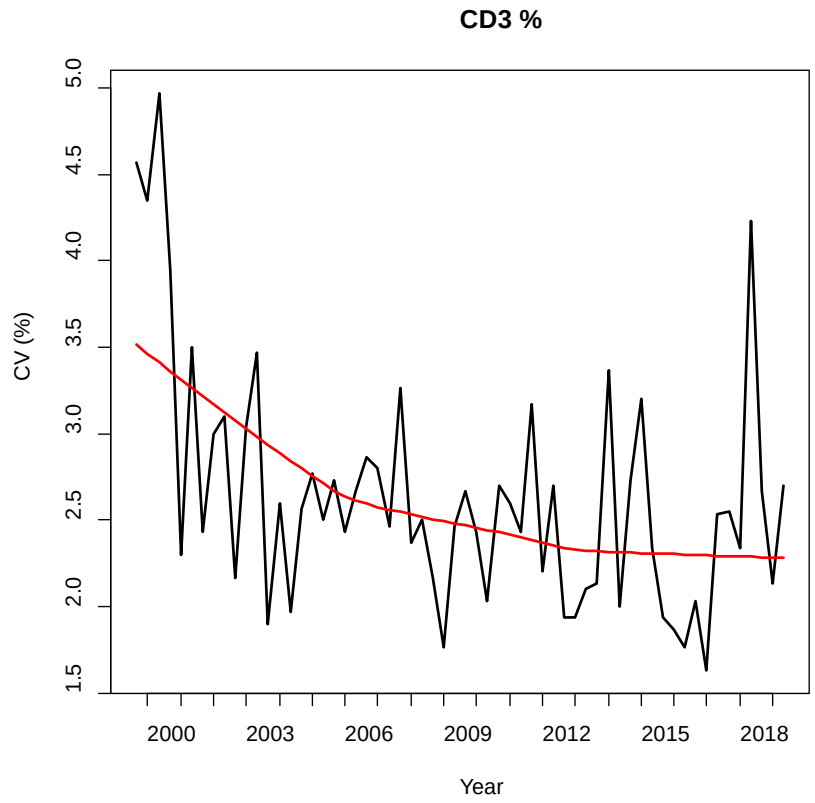
	Median	CV,%	N
FC/16260	99.2	0.9	46
FC/16261	99.1	1.1	46
FC/16262	99.0	1.0	46
FC/16508	99.1	0.8	49
FC/16509	99.2	1.0	49
FC/16510	99.1	0.6	49
FC/16941	99.0	1.1	51
FC/16942	98.9	1.0	51
FC/16943	99.3	1.3	52

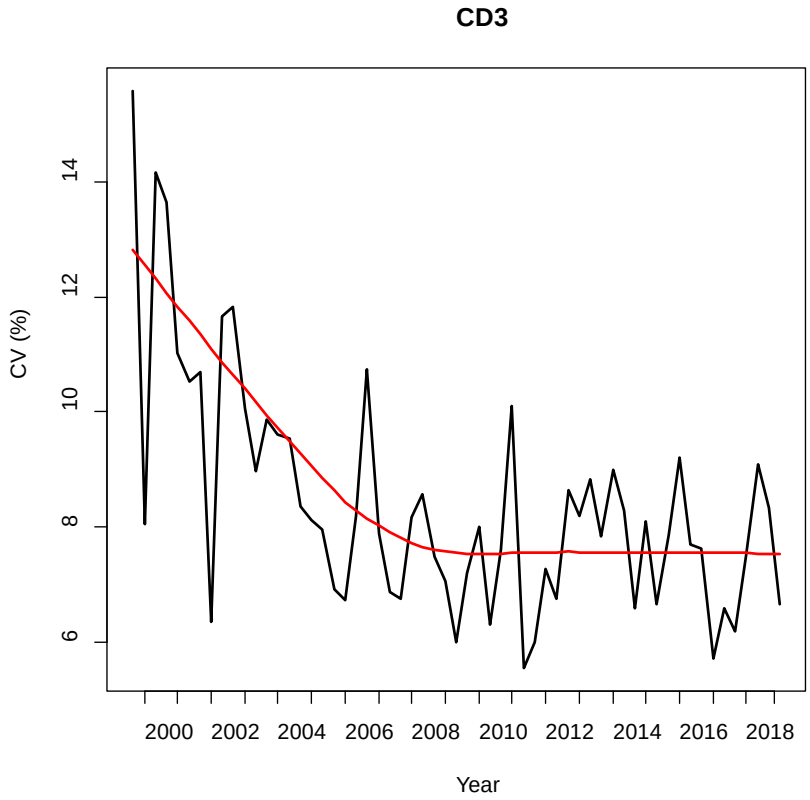
The CVs for the WBC count ranged between 2.5 and 4.7%. The CVs for the % lymphocytes ranged from 3.3 to 6.3% for the % lymphocytes obtained with haematology analysers and ranged from 6.5 to 13.3% for the % lymphocytes obtained with flow cytometers.

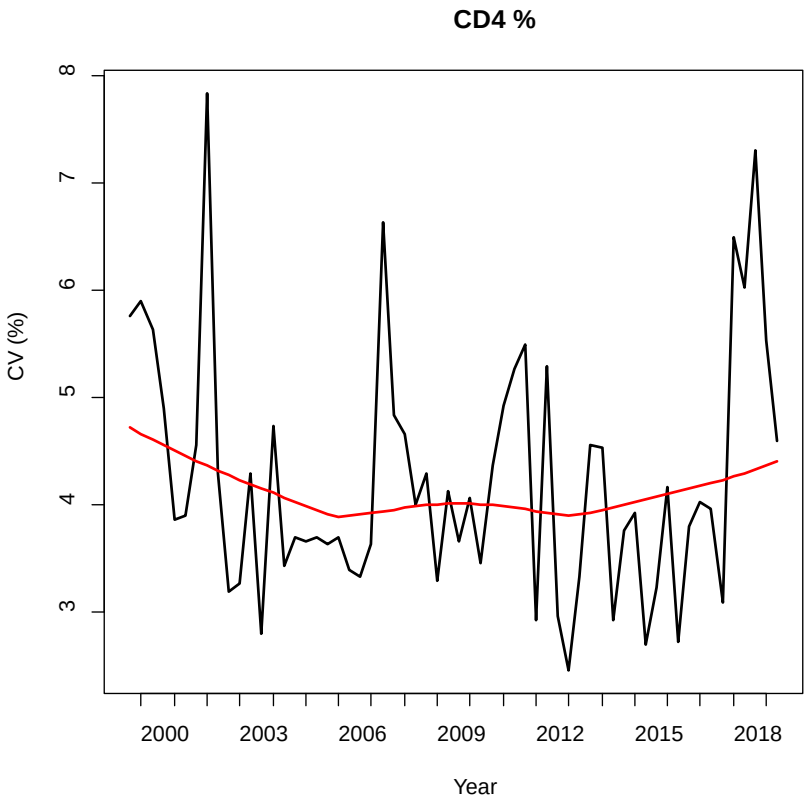
For the different lymphocyte subsets, the average between-laboratory variability was 2.5, 5.8, 7.3, 9.7 and 15.5% for the % of CD3+, CD4+, CD8+, CD19+, and NK cells, respectively. The average CVs of the absolute values were higher and amounted to 7.5, 9.3, 10.2, 13.3 and 13.0% for CD3+, CD4+, CD8+, CD19+, and NK cells, respectively. The overall CVs were larger for small subsets.

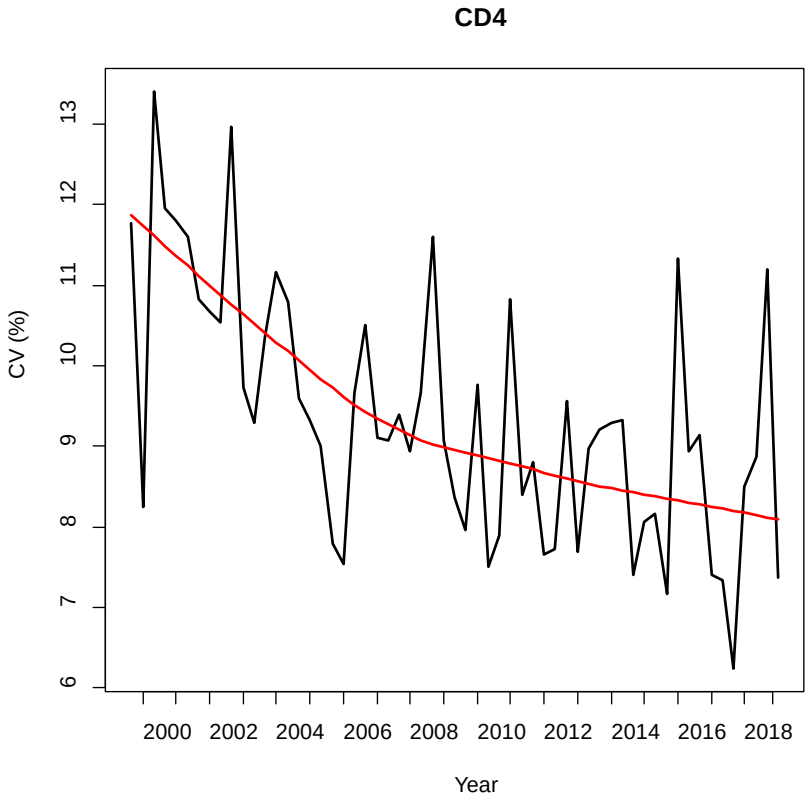
For the percentages of κ and λ chain expressing B cells and the κ/λ ratio, the average CVs were 11.3, 6.3 and 14.3%, respectively.

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.

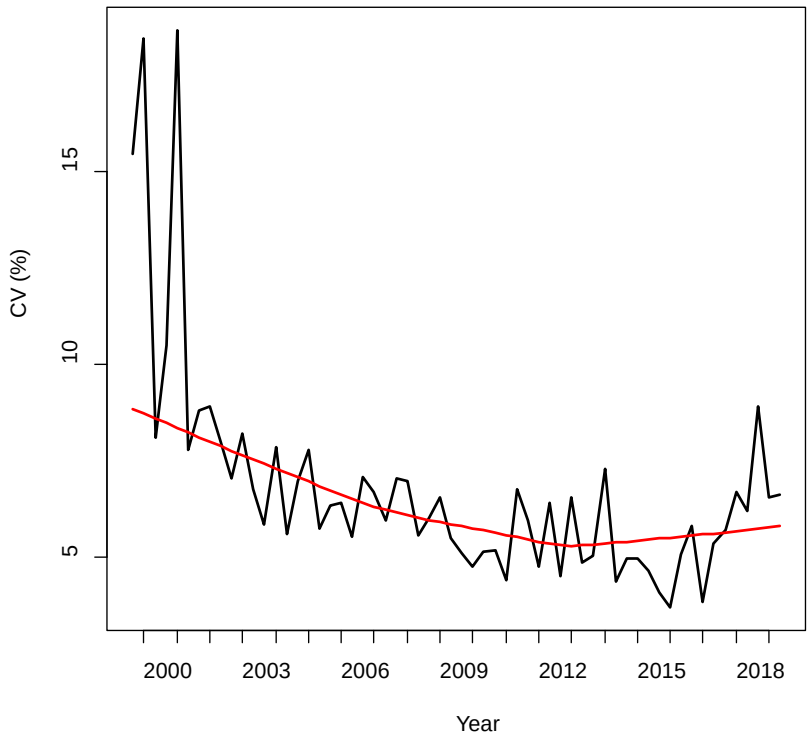

CD3 %
CV (%)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
2000
2003
2006
2009
2012
2015
2018
Year


CD3
CV (%)
6
8
10
12
14
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Year

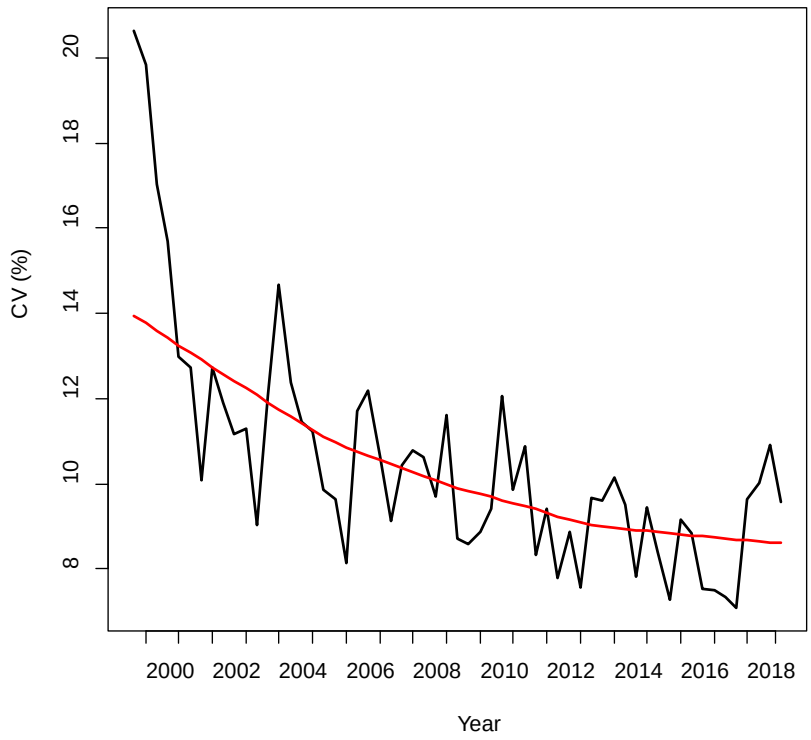

CD4 %
CV (%)
3
4
5
6
7
8
2000
2003
2006
2009
2012
2015
2018
Year

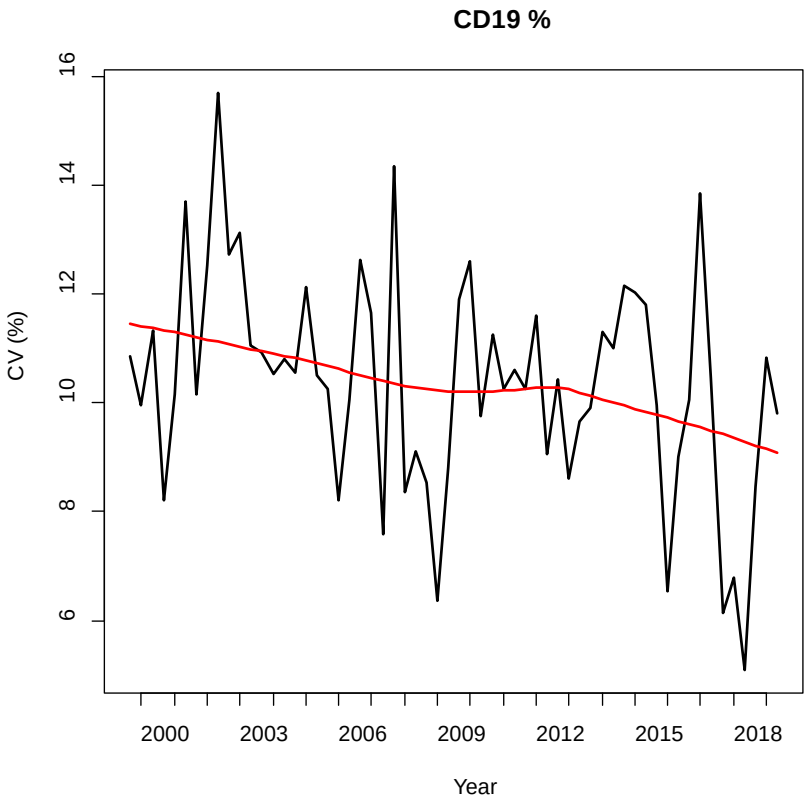

CD4
CV (%)
6
7
8
9
10
11
12
13
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Year

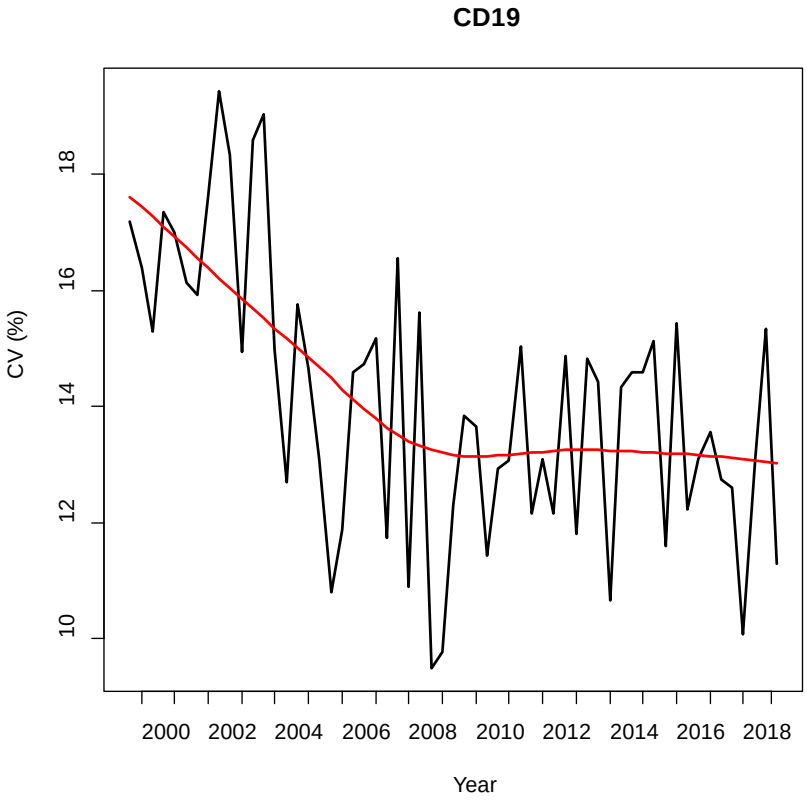
CD8 %

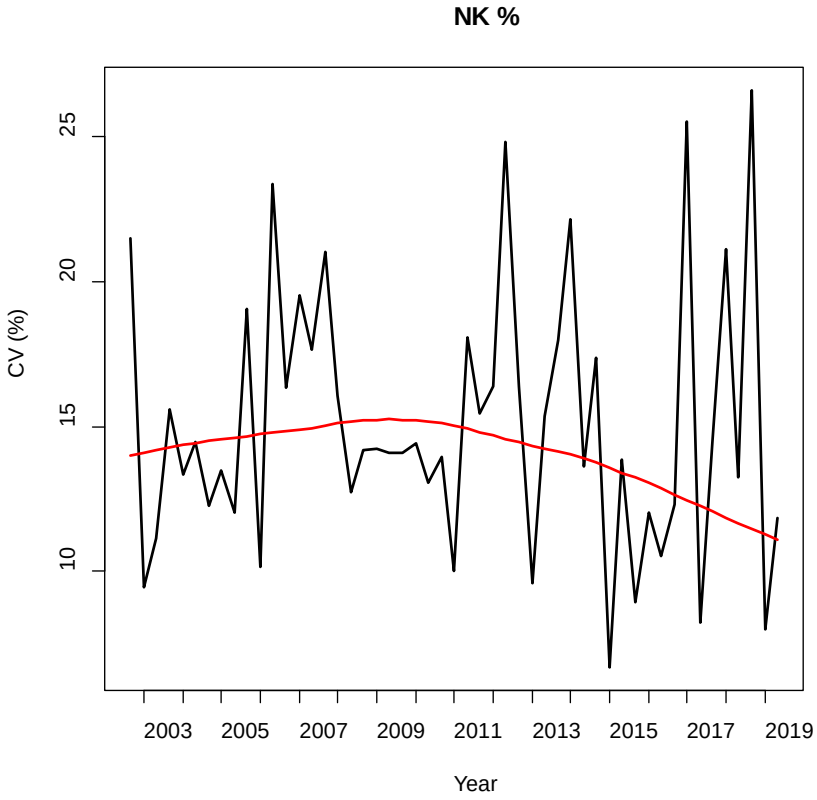

CV (%)
5
10
15
2000
2003
2006
2009
2012
2015
2018
Year

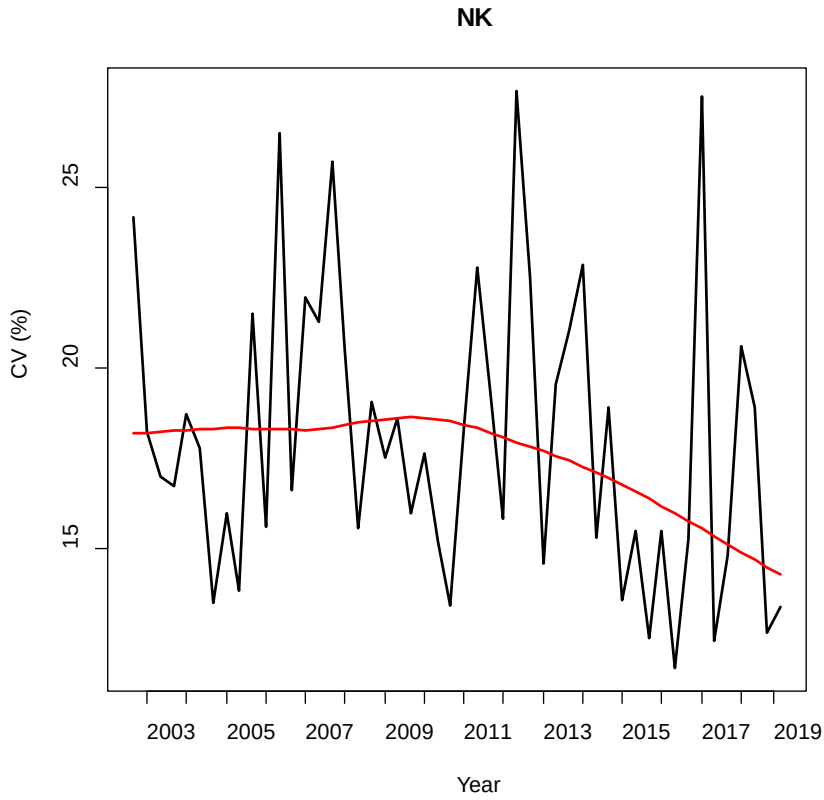
CD8

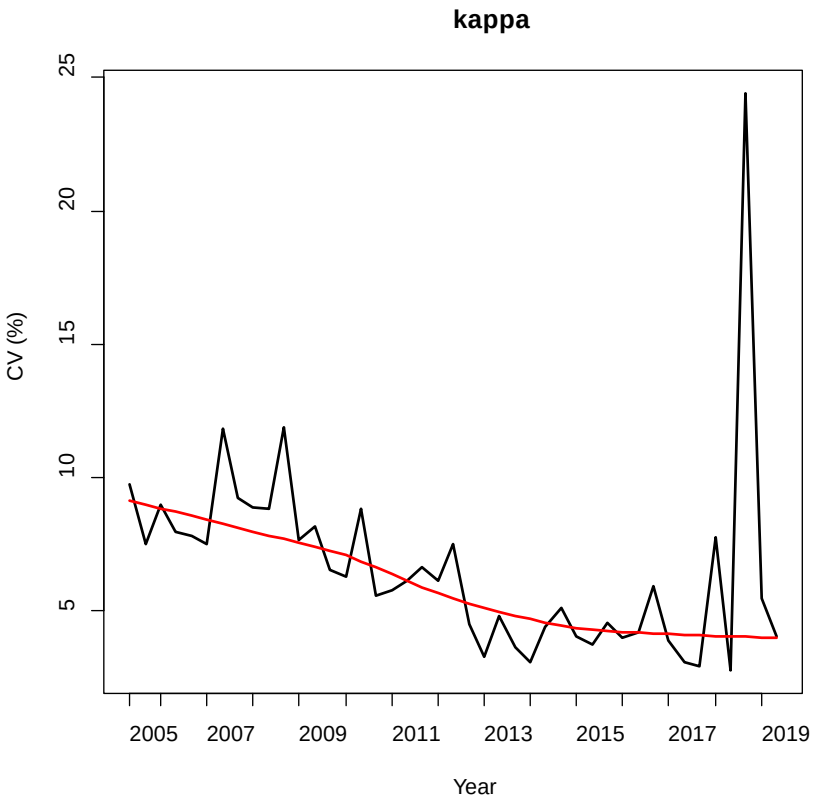

CV (%)
8
10
12
14
16
18
20
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Year

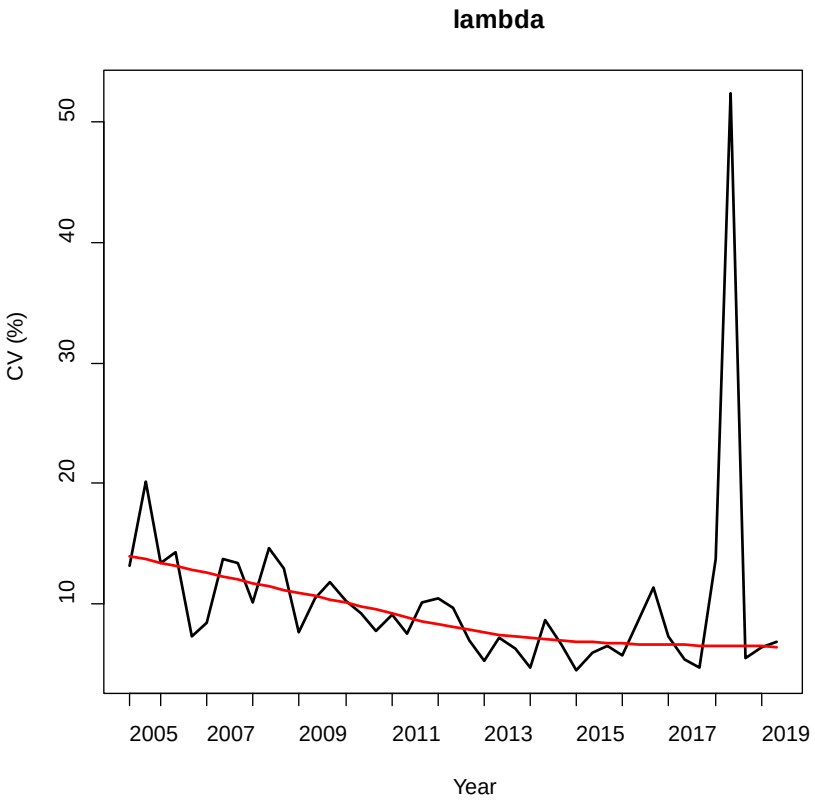

CD19 %
CV (%)
6
8
10
12
14
16
2000
2003
2006
2009
2012
2015
2018
Year

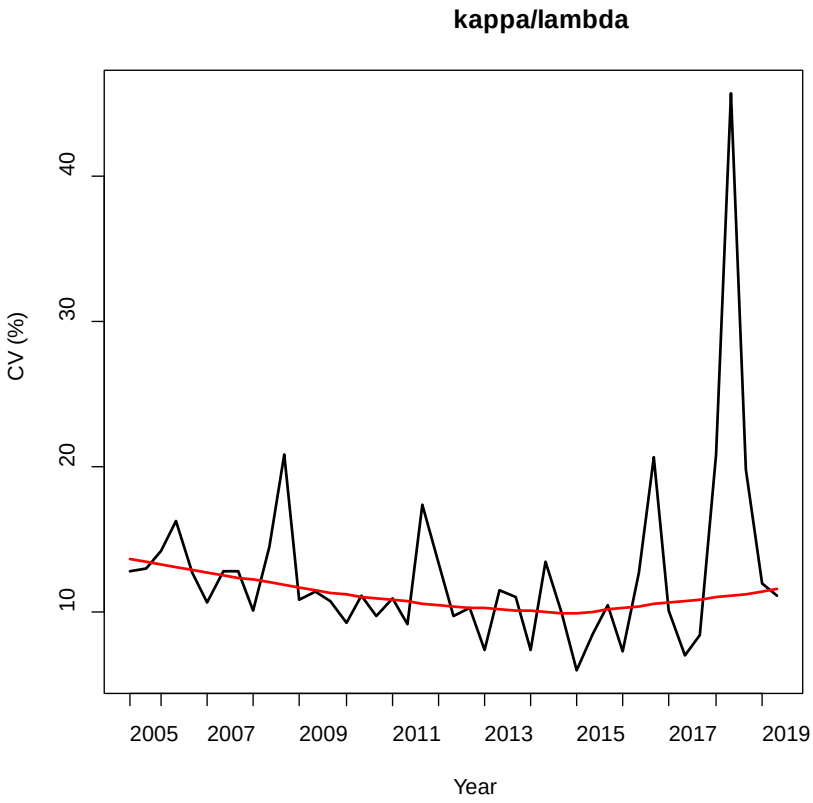

CD19
CV (%)
10
12
14
16
18
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Year


NK %
CV (%)
10
15
20
25
2003
2005
2007
2009
2011
2013
2015
2017
2019
Year


NK
CV (%)
15
20
25
2003
2005
2007
2009
2011
2013
2015
2017
2019
Year


kappa
CV (%)
5
10
15
20
25
2005
2007
2009
2011
2013
2015
2017
2019
Year


lambda
CV (%)
10
20
30
40
50
2005
2007
2009
2011
2013
2015
2017
2019
Year


kappa/lambda
CV (%)
10
20
30
40
2005
2007
2009
2011
2013
2015
2017
2019
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.

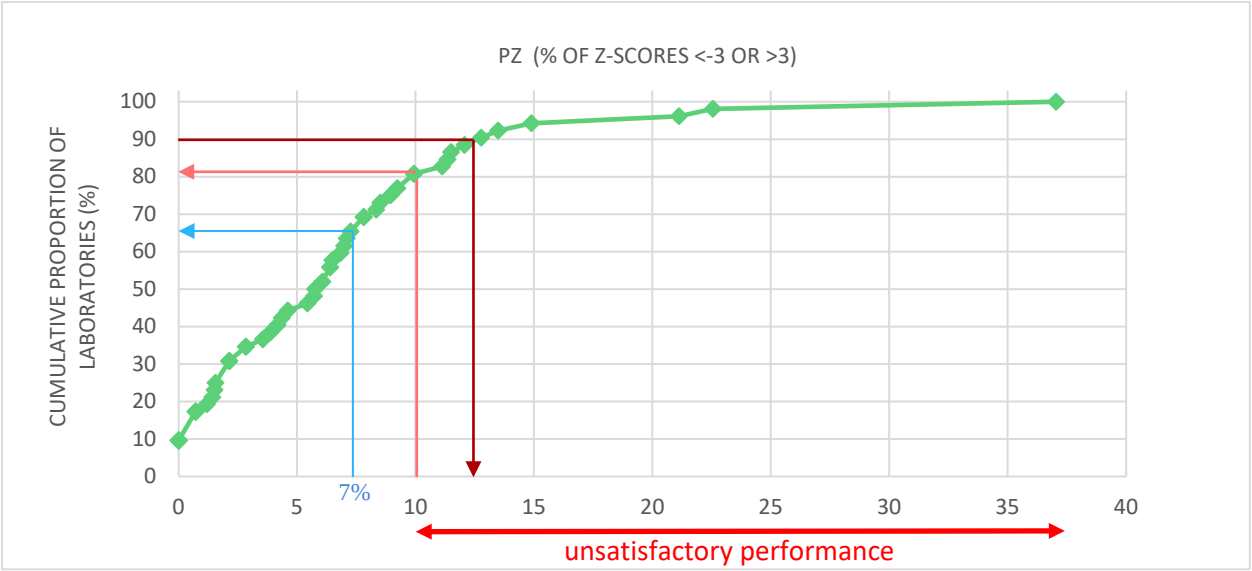
The performance of the laboratories is evaluated by means of the percentage of unacceptable z-scores (P_Z, % of results falling beyond 3 SD from the median) obtained in the course of 1 year.

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

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.



Let us take the example of a laboratory that obtained 10 z-scores <-3 or >3 out of 141 results in the year 2019. Its P_Z score is 10/141 = 7%. This laboratory ranks in the 65% best performing Belgian laboratories.

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

The graph shows that about 19% of the laboratories obtained a P_Z > 10%.

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 graph above also shows that less than 10% of the Belgian laboratories obtained P_Z scores greater than about 13%. The list of these laboratories is transmitted to the inspectors.

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
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

The maximum of evaluated results per laboratory was 141.
This table shows a.o. that Belgian laboratories reported an average of 6.7% results beyond 3 SD and that 25% of laboratories got less than 2% of results beyond 3 SD in 2019.

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

	2018		2019	
Parameter	**Number of evaluated results**	**% results >3 SD**	**Number of evaluated results**	**% results >3 SD**
Leukocytes 10^9/L	318	5.8	292	5.8
Lymphocytes % HA	307	6.3	286	4.9
Lymphocytes % FC	273	7.5	370	7.3
CD3 %	326	4.1	439	4.3
CD3 10^9/L	326	4.7	298	3.7
CD4 %	326	5.3	439	7.3
CD4 10^9/L	326	6.3	298	7.0
CD8 %	326	3.4	439	1.8
CD8 10^9/L	326	5.9	298	4.4
CD19 %	326	4.1	439	6.2
CD19 10^9/L	326	4.7	298	8.4
NK cells %	326	3.4	439	3.9
NK cells 10^9/L	326	7.8	298	4.7
κ % B lymphocytes	282	6.9	373	4.3
λ % B lymphocytes	282	4.7	373	21.4
κ/λ ratio	279	5.9	373	10.7
κ+λ % B lymphocytes	282	17.8	370	7.0
Lymphosum	326	8.1	439	5.7

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	271	27	3.0	11.1
CD4 10^9/L	271	27	6.6	11.1
CD8 10^9/L	271	27	4.1	7.4
CD19 10^9/L	271	27	7.7	14.8
NK cells 10^9/L	271	27	4.8	3.7

Parameter	**Number of evaluated results**		**% results >3 SD**	
	Lyse and wash	**Lyse no wash**	**Lyse and wash**	**Lyse no wash**
CD3 %	178	261	6.7	2.7
CD3 10^9/L	124	174	2.4	4.6
CD4 %	178	261	11.2	4.6
CD4 10^9/L	124	174	8.1	6.3
CD8 %	178	261	2.8	1.1
CD8 10^9/L	124	174	3.2	5.2
CD19 %	178	261	11.2	2.7
CD19 10^9/L	124	174	12.1	5.7
NK cells %	178	261	6.2	2.3
NK cells 10^9/L	124	174	4.8	4.6
Lymphosum	178	261	6.2	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	123	250	3.3	4.8
λ % B lymphocytes	123	250	22.0	21.2
κ/λ ratio	123	250	9.8	11.2
κ+λ % B lymphocytes	123	250	4.2	8.4

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

Parameter	Commercial Control Material			
	Number of evaluated results		% results >3 SD	
	TRUE	FALSE	TRUE	FALSE
CD3 %	307	132	4%	5%
CD3 10^9/L	208	90	5%	1%
CD4 %	307	132	7%	8%
CD4 10^9/L	208	90	5%	11%
CD8 %	307	132	2%	2%
CD8 10^9/L	208	90	4%	6%
CD19 %	307	132	5%	8%
CD19 10^9/L	208	90	10%	4%
NK cells %	307	132	4%	3%
NK cells 10^9/L	208	90	5%	3%
Lymphosum	307	132	6%	5%

Parameter	Commercial Bead Material			
	Number of evaluated results		% results >3 SD	
	TRUE	FALSE	TRUE	FALSE
CD3 %	397	42	4%	7%
CD3 10^9/L	271	27	3%	7%
CD4 %	397	42	7%	10%
CD4 10^9/L	271	27	7%	11%
CD8 %	397	42	2%	2%
CD8 10^9/L	271	27	4%	4%
CD19 %	397	42	6%	12%
CD19 10^9/L	271	27	7%	19%
NK cells %	397	42	4%	7%
NK cells 10^9/L	271	27	4%	11%
Lymphosum	397	42	5%	17%

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 2019, only two surveys were conducted, in February (**FC/16265**) and May (**FC/16494**). Due to a lack of availability of samples, the survey initially planned in November could not be carried out.

Twenty-four 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).

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 2019/2 (n=24)

Sixteen laboratories (67%) used a single platform approach for determining the absolute CD34+ cell count. Of these laboratories, 9 used Trucount technology (BD Biosciences), 5 Flow-Count or Stem-count beads (Beckman-Coulter) and 1 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	14
Beckman-Coulter Navios	7
Beckman-Coulter Cytomics FC 500	2
Miltenyi Biotec MACSQuant analyzer	1

Monitoring of flow cytometer performance

Except for one laboratory, all participants mentioned monitoring the performance of their flow cytometer, for which they all use commercial bead material. In addition, 91% of them also make use of commercial control material.

Sample preparation

Twelve participants used a sample volume of 50 µL and 10 participants 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
Beckman-Coulter Ammonium chloride	3
BD Biosciences Ammonium chloride lysing solution	2
BD Biosciences FACS Lyse	1
Qiagen EL-buffer	1
Cytognos Quicklysis	1

Monoclonal antibodies

All but 2 laboratories (PC5.5/PE-Cy5.5, APC) used a phycoerythrin (PE)-conjugated CD34 monoclonal antibody. All but 5 participants (Horizon V500 (n=3), Krome Orange, VioBlue) used a fluorescein isothiocyanate (FITC)-conjugated CD45 monoclonal antibody.

Viability

88% of the laboratories evaluated CD34+ cell viability using 7-AAD (7-Aminoactinomycin D, n=20) or TO-PRO-3 (n=1).

Gating strategy

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

2.3. Results

83% (2019/1) to 88% (2019/2) of the participants mentioning the day of receipt got the blood samples on day 1 and 8% (2019/1) to 13% (2019/2) received the blood samples on day 2 (day 0: send-out of blood samples).

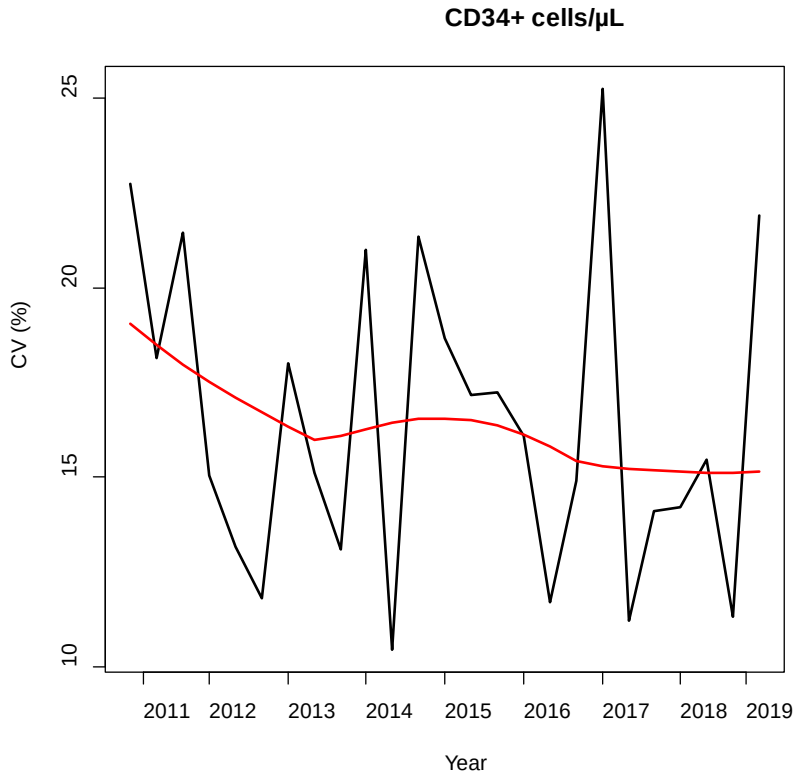
79% (2019/2) to 80% (2019/1) of the participants indicating the day of sample testing performed the analyses on day 1 and 8% (survey 2019/1) to 13% (survey 2019/2) on day 2 (day 0: send-out of blood samples).

Statistics for the evaluation were solely based on the results of the Belgian clinical laboratories 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 2019:

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/µL	CV %	N
FC/16265	0.325	14.3	21	18.2	11.3	21
FC/16494	0.118	17.0	22	5.5	21.9	22

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.

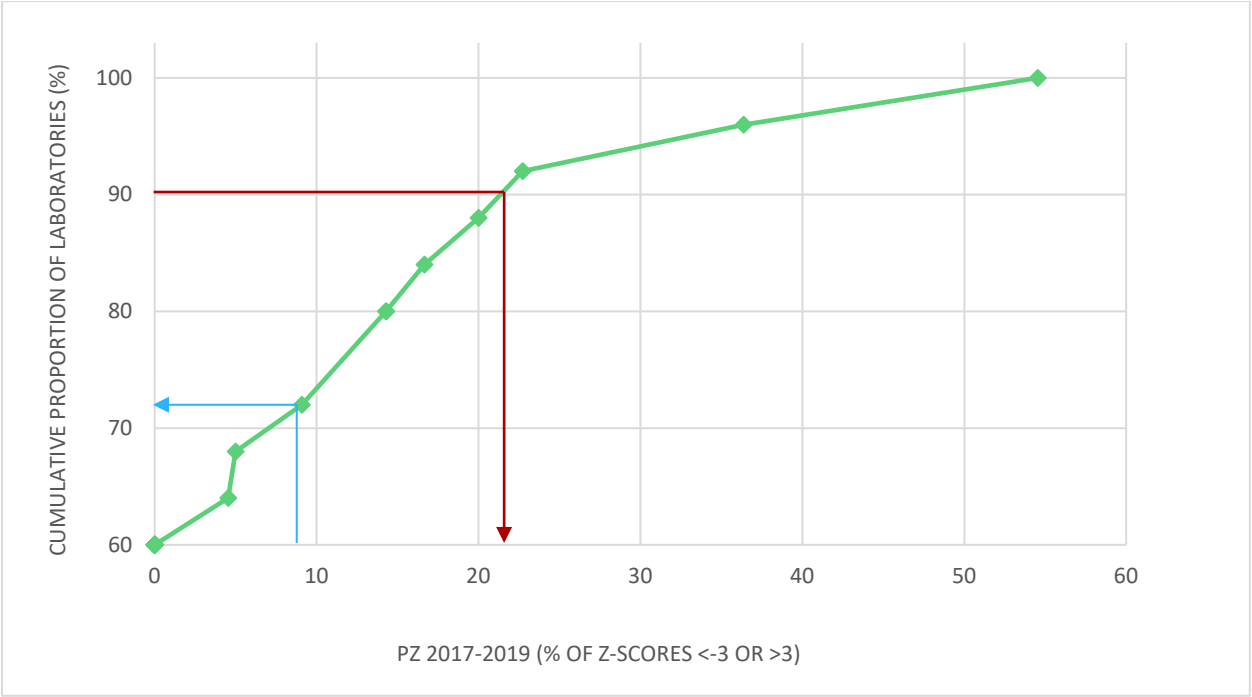


2.4. P_Z evaluation

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 (2017: n=10, 2018: n=8, 2019: n=4), 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.



Let us take the example of a laboratory that obtained 2 z-scores <-3 or >3 out of 22 results in the period 2017-2019. Its Pz score is 2/22 = 9%. This laboratory ranks in the 72% best performing Belgian laboratories.

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 2017-2019: 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
2017-2019	25	7,9 ± 13,6	0	0	14.3	21.6	33.6	50.2	0 – 54.6

During the period 2017-2019, the maximum of evaluated results per laboratory was 22.
The table shows that Belgian laboratories reported an average of 7.9% results beyond 3 SD. In addition, fifteen laboratories (60%) 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			
	Number of evaluated results		% results >3 SD	
	TRUE	FALSE	TRUE	FALSE
% viable CD34+ cells within total WBC	199	53	5%	23%
Absolute viable CD34+ cell count (cells/µL)	199	54	5%	19%

Parameter	ViabilityStaining			
	Number of evaluated results		% results >3 SD	
	TRUE	FALSE	TRUE	FALSE
% viable CD34+ cells within total WBC	218	34	6%	21%
Absolute viable CD34+ cell count (cells/µL)	219	34	5%	26%

Parameter	ISHAGE Protocol Gating Strategy			
	Number of evaluated results		% results >3 SD	
	TRUE	FALSE	TRUE	FALSE
% viable CD34+ cells within total WBC	220	32	6%	25%
Absolute viable CD34+ cell count (cells/µL)	220	33	5%	21%

END