BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
COMMITTEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

DEFINITIVE GLOBAL REPORT

FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS

SURVEY 2024/1

Sciensano/Flow cytometry/89-E

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

www.sciensano.be

ISSN: 2294-3498

COMMITTEE OF EXPERTS

Sciensano					
Secretariat		PHONE:	02/642.55.22	FAX:	02/642.56.45
		e-mail:	QL_secretariat@sciensano.be		
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		
Experts	**Institute**				
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A draft version of this report was submitted to the experts EQA Flow Cytometry on: 18/03/2024
This report was discussed at the meeting of the committee of experts EQA Flow Cytometry on: 28/03/2024

Authorization of the report: by Lobna Bouacida, scheme coordinator

Date of publication: 08/04/2024

All the reports are also available on our webpage:

- NL: https://www.sciensano.be/nl/kwaliteit-van-laboratoria
- FR: https://www.sciensano.be/fr/qualite-des-laboratoires

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INTERPRETATION OF THE INDIVIDUAL REPORT

Besides this global report, an individual report is at your disposal via toolkit.

Below you can find information to help you interpreting this report.

The position of your quantitative results is presented on the one hand in comparison with the results from all the participants and on the other hand in comparison with the results of the laboratories using your method.

Following information is provided:

- Your result (R)
- Your method
- Global median (M_G):
  central value of the results obtained by all laboratories (all methods together).
- Global standard deviation (SD_G):
  measure of the spread of the results obtained by all the laboratories (all methods together).
- Global median of your method (M_M):
  central value of the results obtained by the laboratories using your method.
- Standard deviation of your method (SD_M):
  measure of the spread of the results obtained by the laboratories using your method.
- The coefficient of variation CV (expressed in %) for all laboratories and for the laboratories using your method:
  $CV_M = (SD_M / M_M) * 100$ (%) and $CV_g = (SD_G / M_G) * 100$ (%).
- Z score:
  difference between your result and the median of your method (expressed as a number of SD): **$Z_M = (R – M_M) / SD_M$ and $Z_G = (R – M_G) / SD_G$**.
  The result is flagged when **$|Z_M| > 3$**.
- U score:
  relative deviation of your result from the median of your method (expressed in %):
  $U_m = ((R – M_M) / M_M) * 100$ (%) and $U_G = ((R – M_G) / M_G) * 100$ (%).
  The result is flagged when **$|U_M| > d$**, where "d" is a parameter-dependent fixed limit, namely the percentage maximal deviation from the method median.
- A graphical interpretation of the position of your result (R), towards the results of all the participants as well as the results of the participants using your method, based on the method of Tukey, for each parameter and for each analyzed sample.

  R : your result

  $M_{M/G}$: median

  $H_{M/G}$: percentiles 25 en 75

  $I_{M/G}$: internal limits (M ± 2.7 SD)

  $O_{M/G}$: external limits (M ± 4.7 SD)

The global graph and the one of your method are presented on the same scale, which allows you to compare them. These graphs give you a rough estimation of the position of your result (R) with respect to the medians ($M_{M/G}$).

More information can be found in the brochures available on our website (only in Dutch and French):
Klinische gezondheid | EKE klinische biologie | sciensano.be

- Algemene informatiebrochure EKE
- Statistische methoden gebruikt voor EKE
- Verwerking van gecensureerde waarden

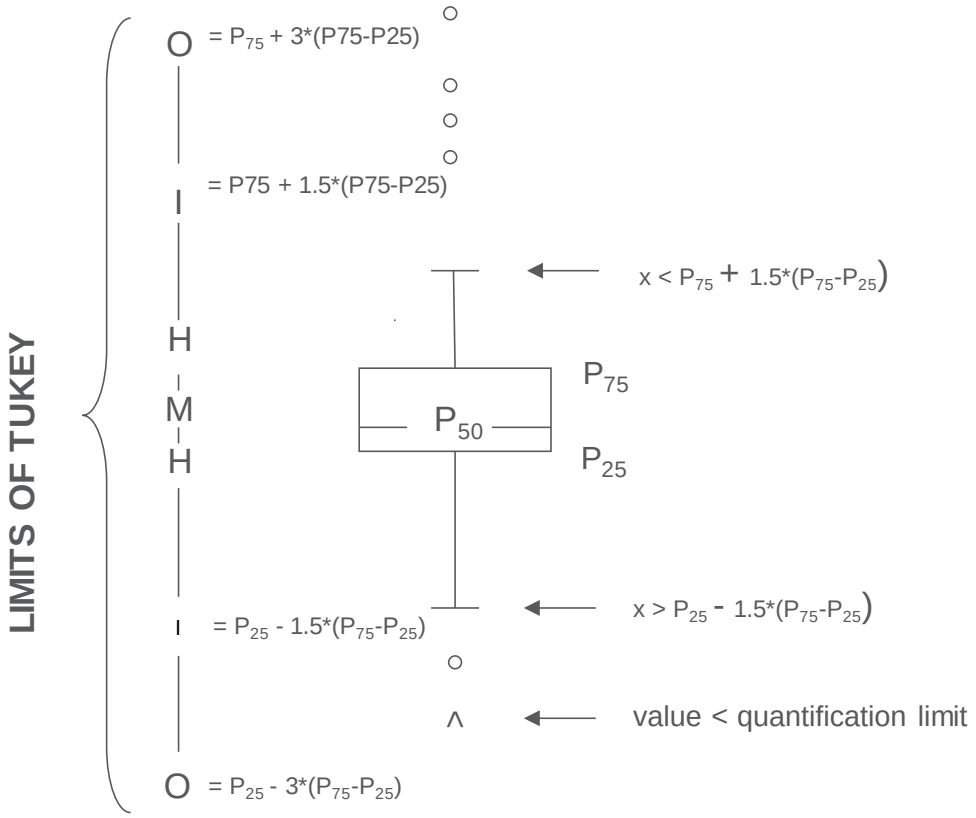
Santé clinique | EEQ biologie clinique | sciensano.be

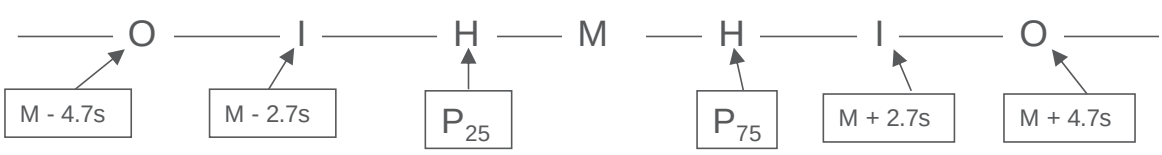
- Brochure d'information générale EEQ
- Méthodes statistiques appliquées à l'EEQ
- Traitement des valeurs censurées

Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 6 participants:

- a rectangle ranging from percentile 25 (**P_{25}**) to percentile 75 (**P_{75}**)
- a central line representing the median of the results (**P_{50}**)
- a lower limit showing the smallest value **$x > P_{25} - 1.5 * (P_{75} - P_{25})$**
- an upper limit representing the largest value **$x < P_{75} + 1.5 * (P_{75} - P_{25})$**
- all points outside this interval are represented by a dot.





Corresponding limits in case of normal distribution

SAMPLE MATERIAL

Two blood samples (FC/20511 and FC/20512) collected on K2EDTA were sent to the laboratories.

These two samples were collected from two healthy and voluntary blood donors and distributed into aliquots at Sciensano.

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).

Homogeneity was confirmed based on white blood cells determination.

Control analysis on the day of collection and distribution yielded the following results (UZ Brussel):

FC/20511

	%	10^9/L
Leukocytes		7.4
Lymphocytes	25.3	
$CD3^+$ cells	76.5	1.43
$CD4^+CD3^+$ cells	41.4	0.78
$CD8^+CD3^+$ cells	36.9	0.69
$CD19^+$ cells	17.6	0.33
NK cells	5.4	0.10
κ % B lymphocytes	59.9	
λ % B lymphocytes	40.1	
κ/λ ratio	1.49	

FC/20512

	%	10^9/L
Leukocytes		8.4
Lymphocytes	15.8	
$CD3^+$ cells	70.7	0.94
$CD4^+CD3^+$ cells	46.8	0.62
$CD8^+CD3^+$ cells	22.1	0.29
$CD19^+$ cells	12.8	0.17
NK cells	16.4	0.22
κ % B lymphocytes	63.3	
λ % B lymphocytes	36.5	
κ/λ ratio	1.73	

PARTICIPATION

Forty-nine Belgian clinical laboratories participated in the survey 2024/1 (send-out of blood samples on February 26, 2024 (day 0)).

RESULTS

All the Belgian laboratories received the samples on day 1 or 2: 90% on day 1 and 10% on day 2.

Most of the laboratories conducted the analyses promptly, with 80% performing them on day 1 and 18% on day 2. One laboratory (2%) completed the analyses on day 3.

Given that the samples are fresh and unstabilized, it is crucial to initiate sample testing immediately upon receipt.

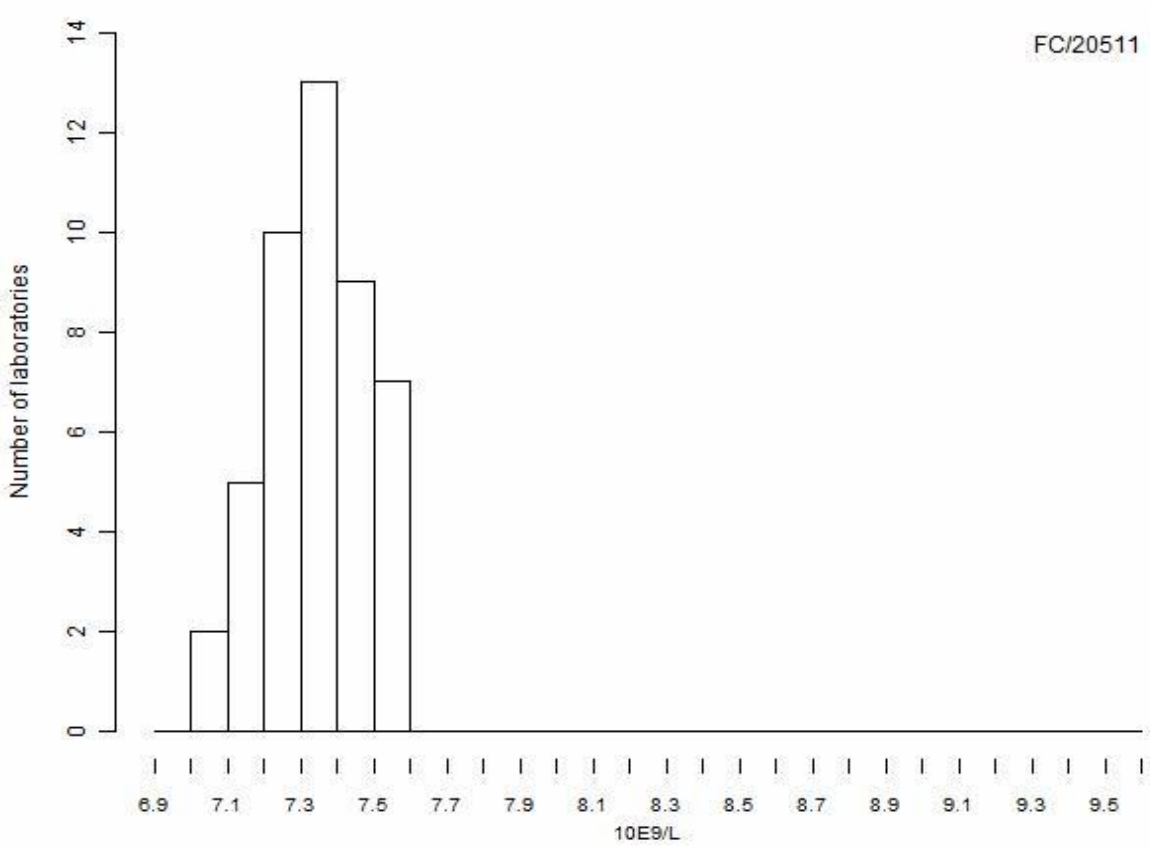
The evaluation statistics are derived solely from the results obtained by the Belgian clinical laboratories (n=49).

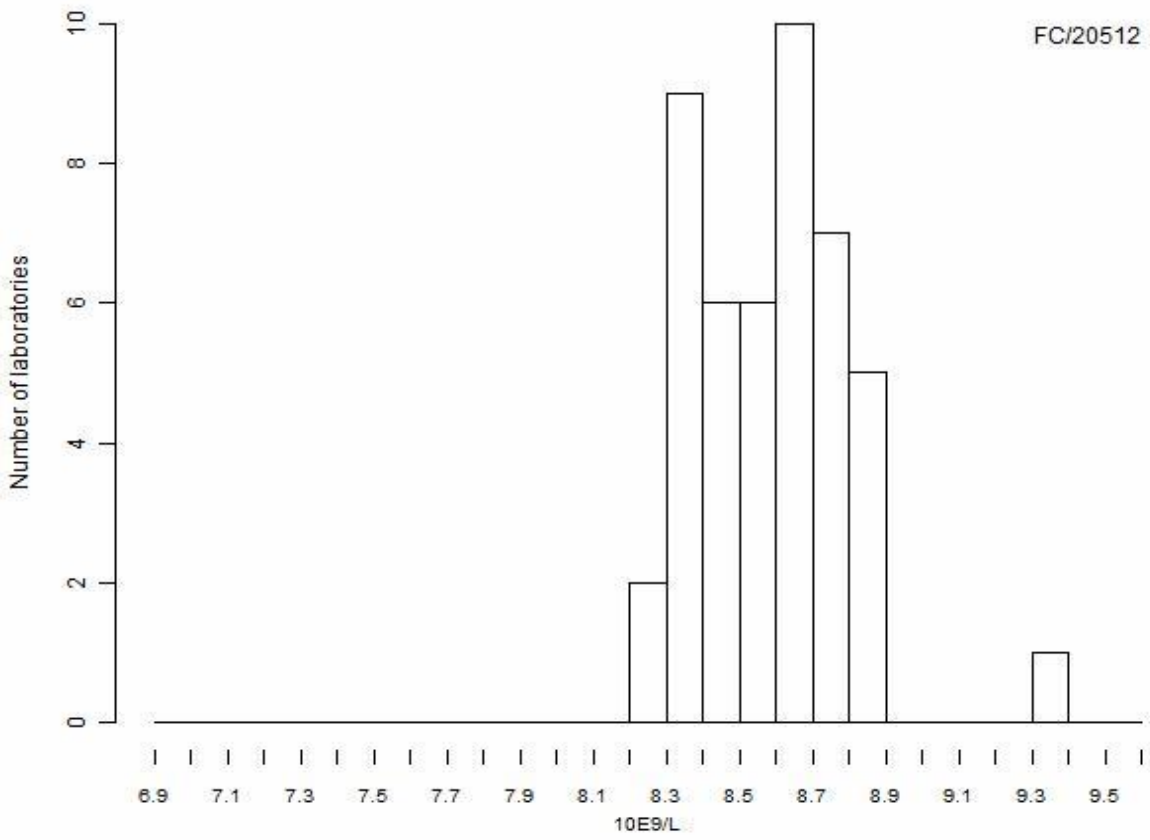
The following tables show the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/20511 and FC/20512:

FC/20511	Median	SD	CV,%	N
WBC 10E9/L	7.37	0.11	1.6	48
Lympho% haematology analyser	25.6	0.7	2.6	46
Lympho% flow cytometer	25.0	1.4	5.6	42
CD3 %	78.1	1.9	2.5	49
CD3 10E9/L	1.480	0.092	6.2	49
CD4 %	39.3	2.5	6.4	49
CD4 10E9/L	0.740	0.070	9.4	49
CD8 %	36.9	2.9	7.8	49
CD8 10E9/L	0.700	0.060	8.6	49
CD19 %	16.2	1.9	11.9	49
CD19 10E9/L	0.303	0.042	13.9	49
NKcells %	5.0	1.1	22.2	49
NKcells 10E9/L	0.094	0.029	30.7	49
Kappa % B lymphocytes	62.6	7.0	11.2	39
Lambda % B lymphocytes	36.0	8.2	22.8	39
Kappa/lambda	1.78	0.64	35.8	39
Sum K+L % B lymphocytes	99.9	0.8	0.8	39
Lymphosum %	99.5	0.7	0.7	49

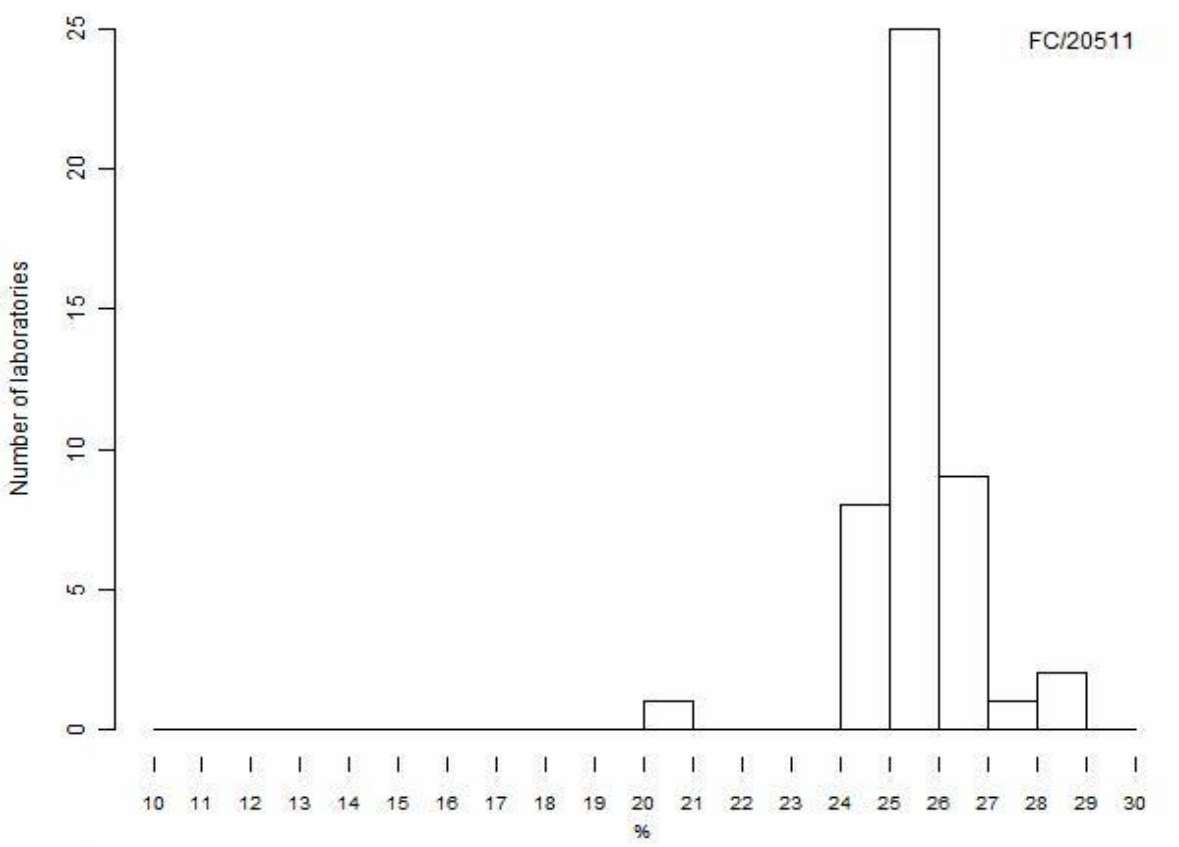
FC/20512	Median	SD	CV,%	N
WBC 10E9/L	8.60	0.23	2.7	48
Lympho% haematology analyser	16.0	0.9	5.6	46
Lympho% flow cytometer	15.4	1.0	6.7	42
CD3 %	73.3	3.0	4.1	49
CD3 10E9/L	1.008	0.100	9.9	49
CD4 %	47.6	2.1	4.4	49
CD4 10E9/L	0.664	0.081	12.1	49
CD8 %	22.4	1.0	4.3	49
CD8 10E9/L	0.310	0.024	7.9	49
CD19 %	10.2	1.5	14.5	49
CD19 10E9/L	0.145	0.028	19.4	49
NKcells %	15.6	1.7	10.9	49
NKcells 10E9/L	0.213	0.034	16.0	49
Kappa % B lymphocytes	61.9	2.0	3.2	40
Lambda % B lymphocytes	37.5	2.3	6.1	40
Kappa/lambda	1.65	0.14	8.8	40
Sum K+L % B lymphocytes	99.9	0.5	0.5	40
Lymphosum %	99.0	1.3	1.3	49

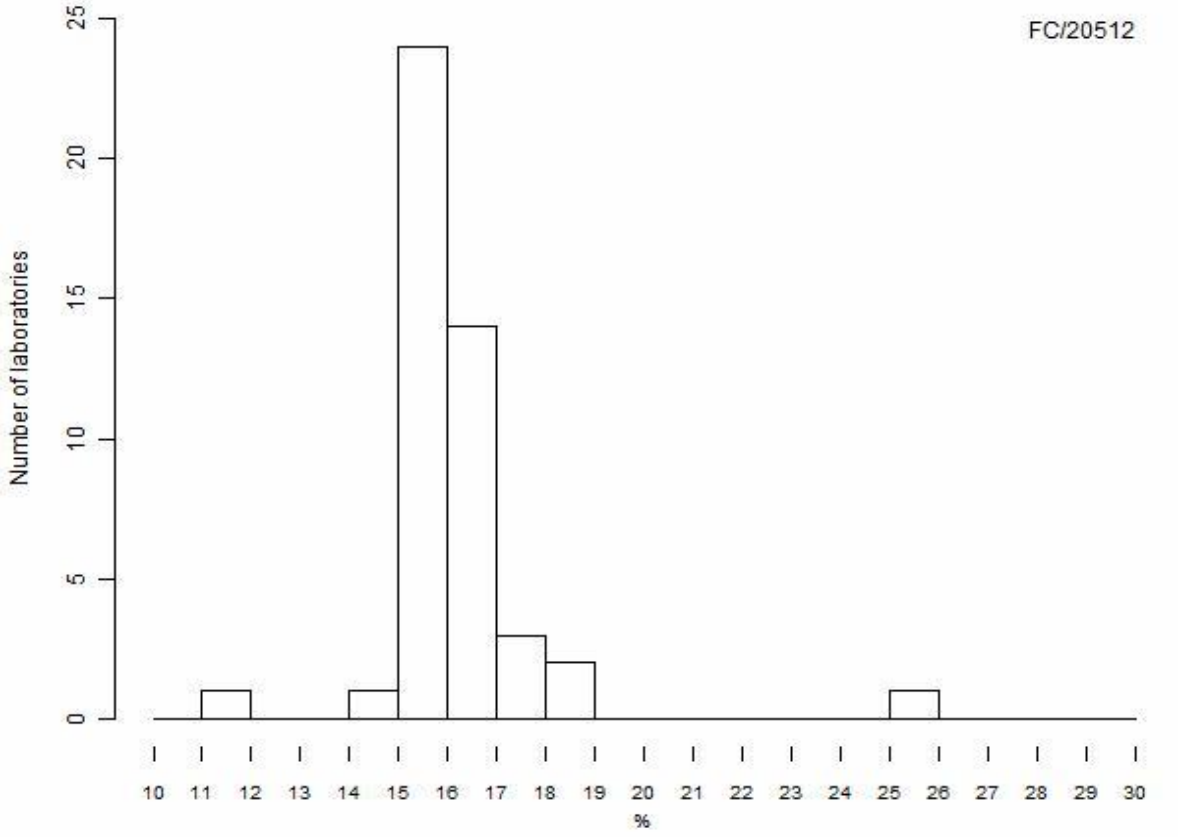
WBC 10E9/L


FC/20511
Number of laboratories
0
2
4
6
8
10
12
14
6.9
7.1
7.3
7.5
7.7
7.9
8.1
8.3
8.5
8.7
8.9
9.1
9.3
9.5
10E9/L

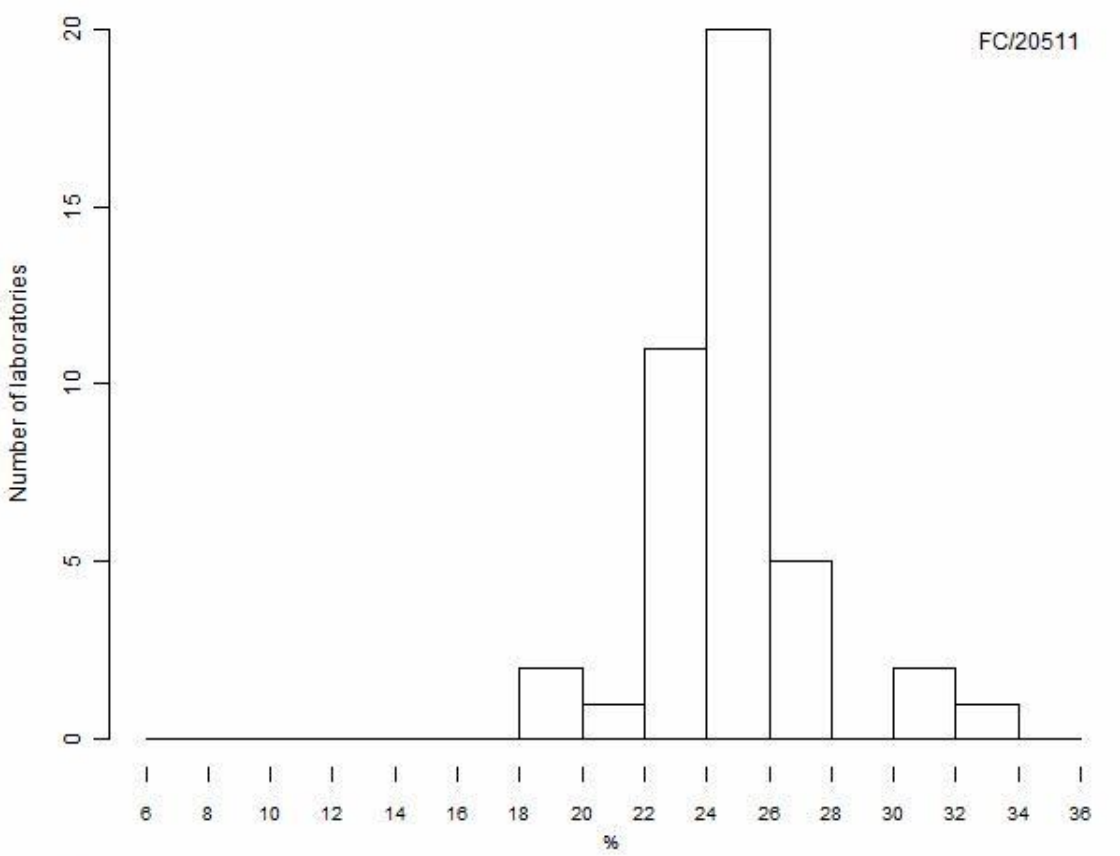

FC/20512
Number of laboratories
0
2
4
6
8
10
6.9
7.1
7.3
7.5
7.7
7.9
8.1
8.3
8.5
8.7
8.9
9.1
9.3
9.5
10E9/L

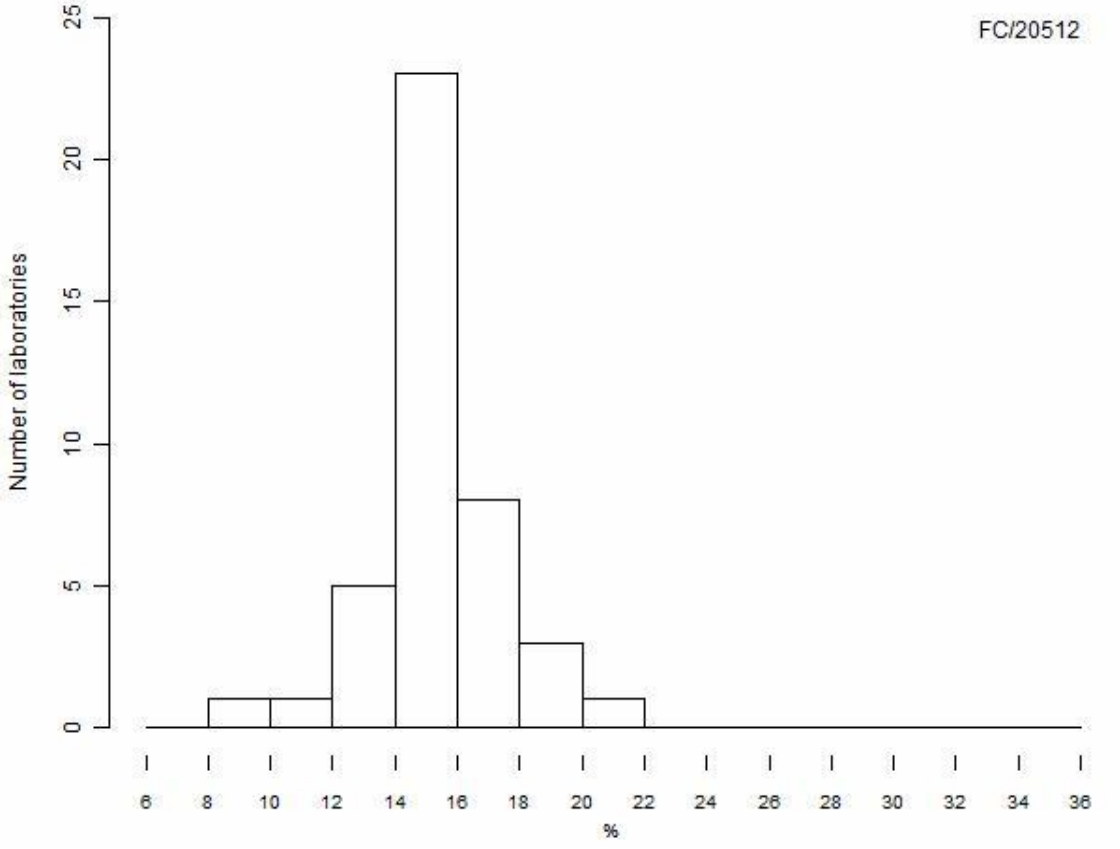
Lympho% haematology analyser



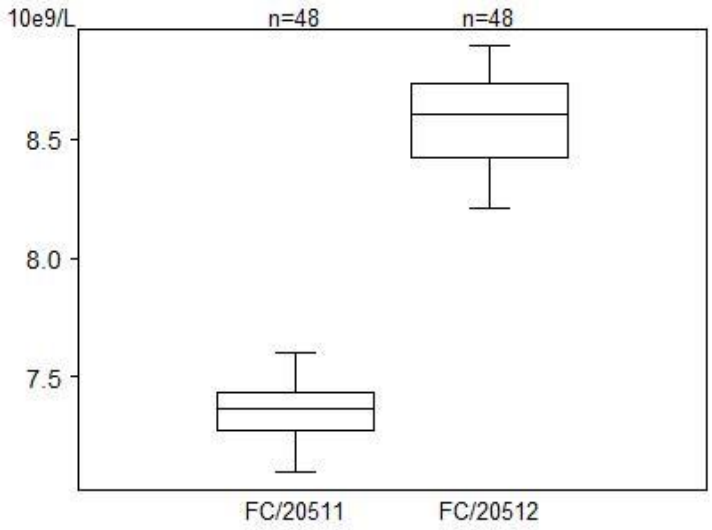


Lympho% flow cytometer





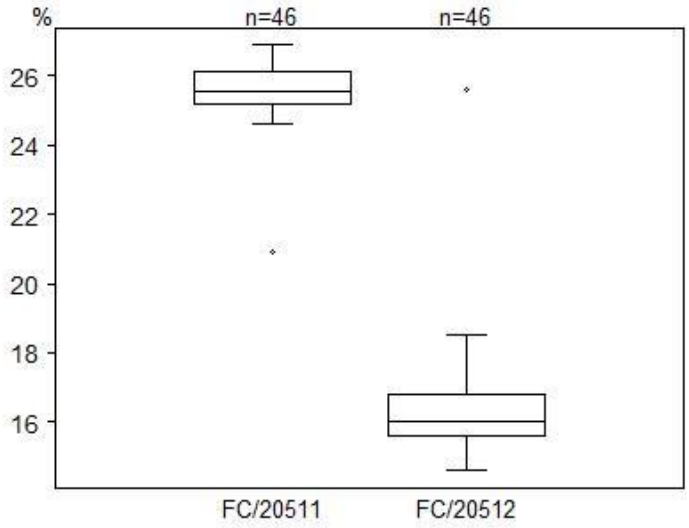
WBC 10E9/L



Results not represented on the graph

FC/20511 = 1.8 10e9/L
FC/20511 = 6019 10e9/
FC/20512 = 1.35 10e9/l
FC/20512 = 9.4 10e9/L
FC/20512 = 7302 10e9/

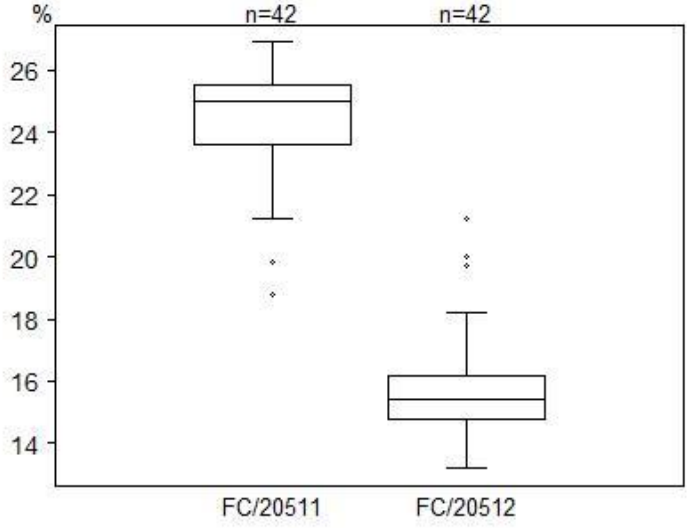
Lympho% haematology analyser



Results not represented on the graph

FC/20511 = 27.9 %
FC/20511 = 28.1 %
FC/20511 = 28.6 %
FC/20512 = 11.6 %

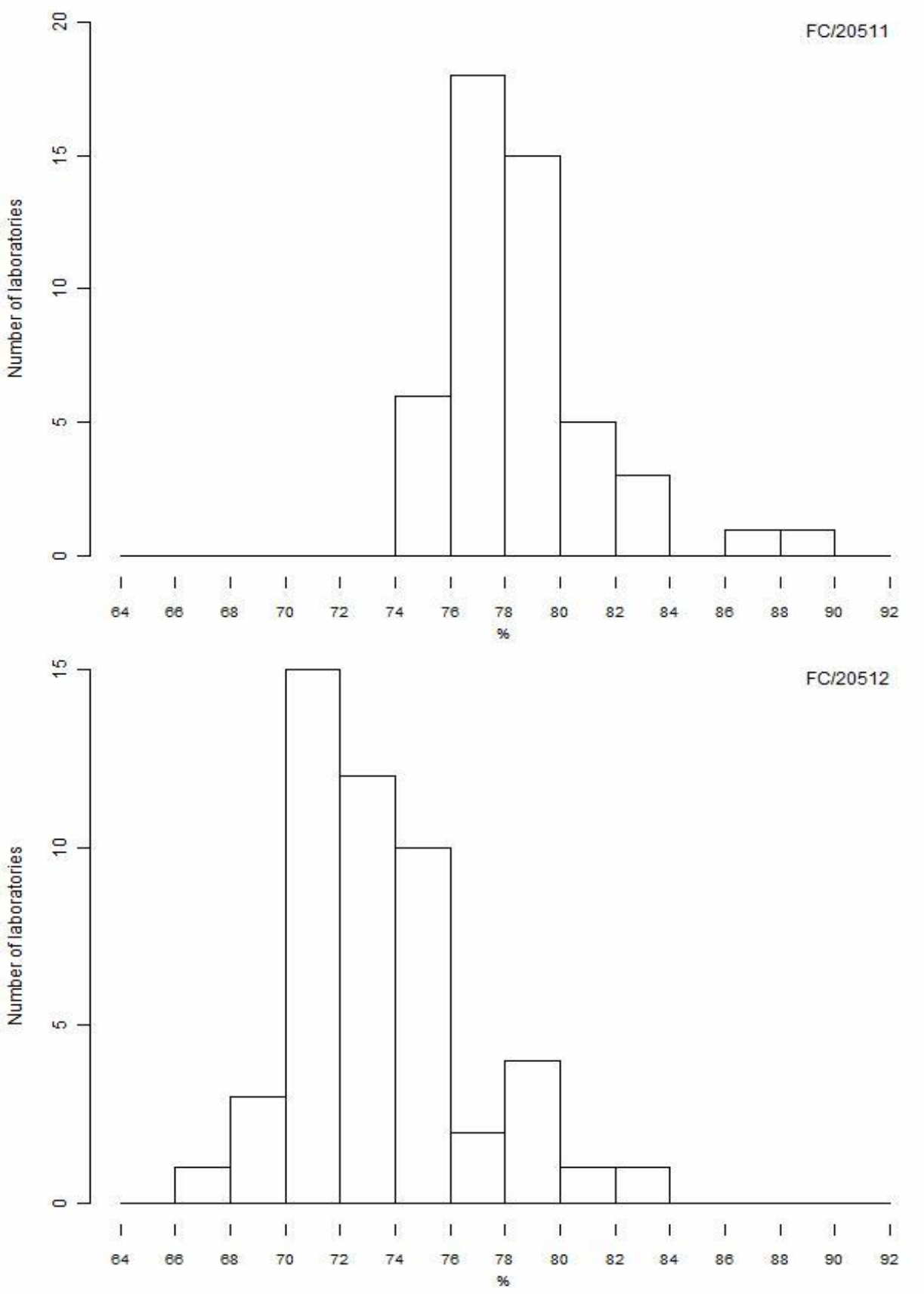
Lympho% flow cytometer



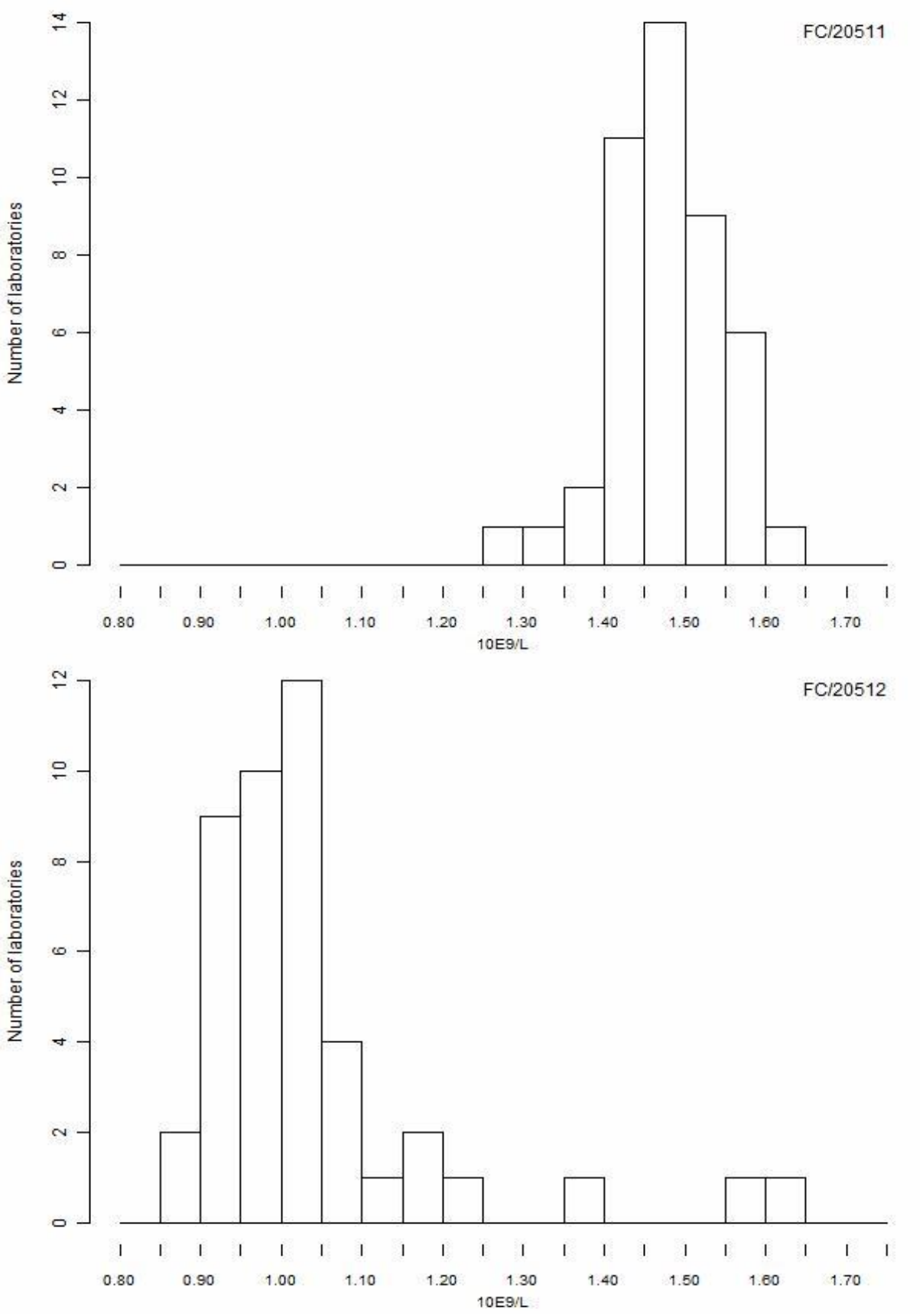
Results not represented on the graph

FC/20511 = 30.5 %
FC/20511 = 31 %
FC/20511 = 32.9 %
FC/20512 = 8.8 %
FC/20512 = 11.6 %

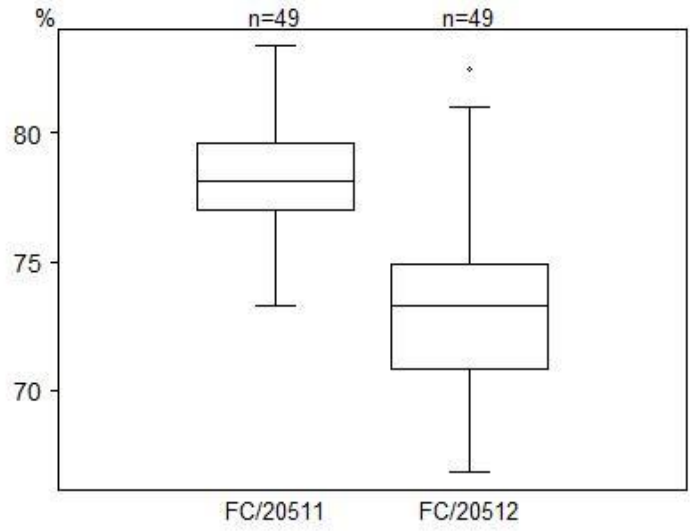
CD3 %


FC/20511
Number of laboratories
0
5
10
15
20
64
66
68
70
72
74
76
78
80
82
84
86
88
90
92
%
FC/20512
Number of laboratories
0
5
10
15
64
66
68
70
72
74
76
78
80
82
84
86
88
90
92
%

CD3 10E9/L


FC/20511
Number of laboratories
0
2
4
6
8
10
12
14
0.80
0.90
1.00
1.10
1.20
1.30
1.40
1.50
1.60
1.70
10E9/L
FC/20512
Number of laboratories
0
2
4
6
8
10
12
0.80
0.90
1.00
1.10
1.20
1.30
1.40
1.50
1.60
1.70
10E9/L

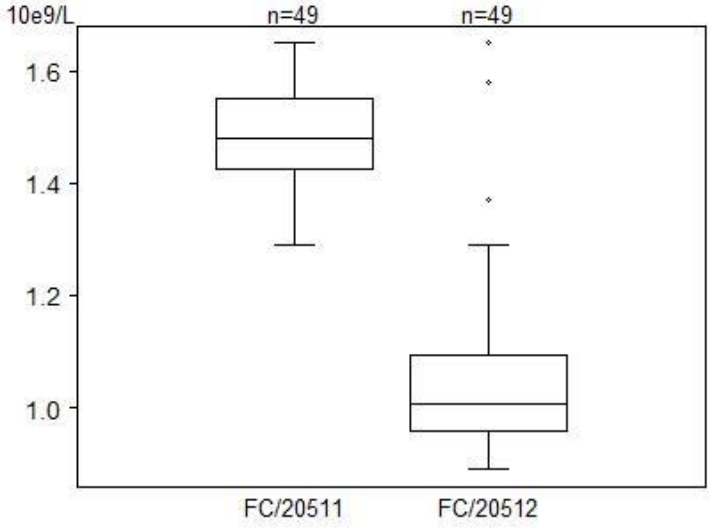
CD3 %



Results not represented on the graph

FC/20511 = 87.6 %
FC/20511 = 88.5 %

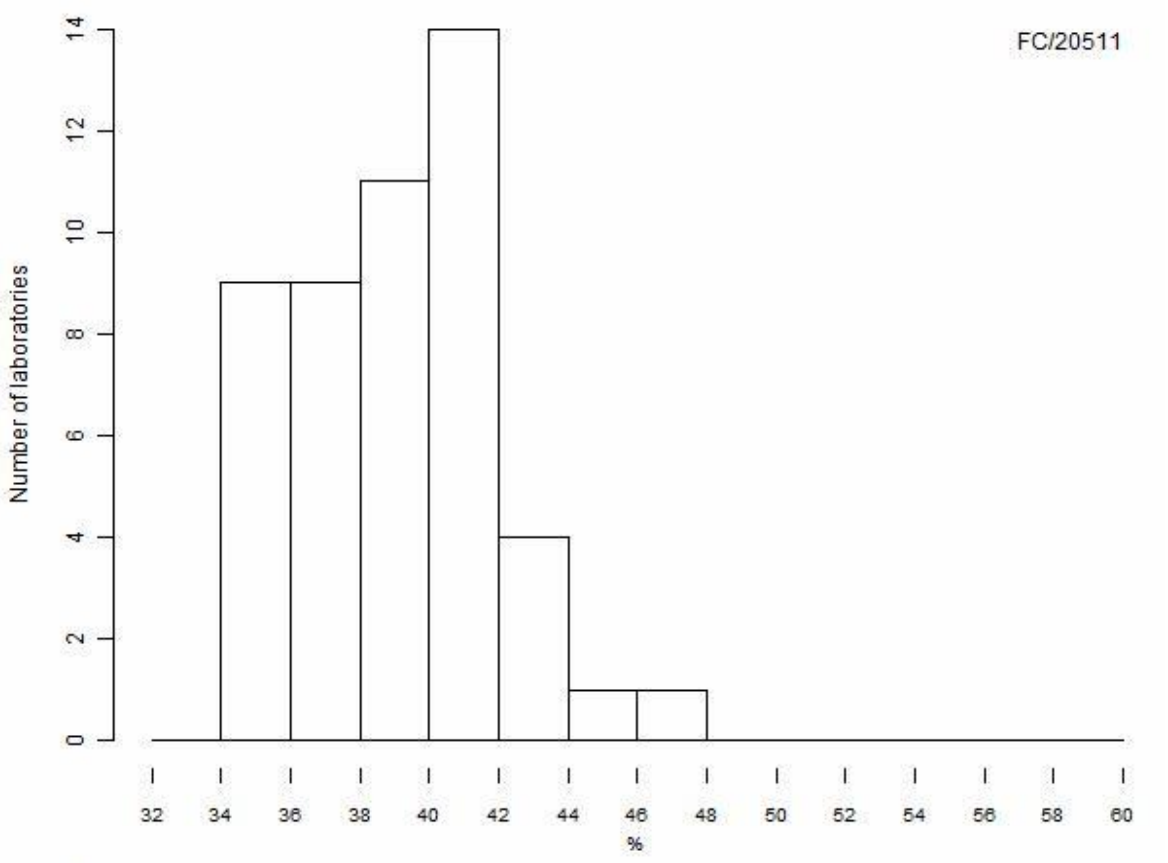
CD3 10E9/L

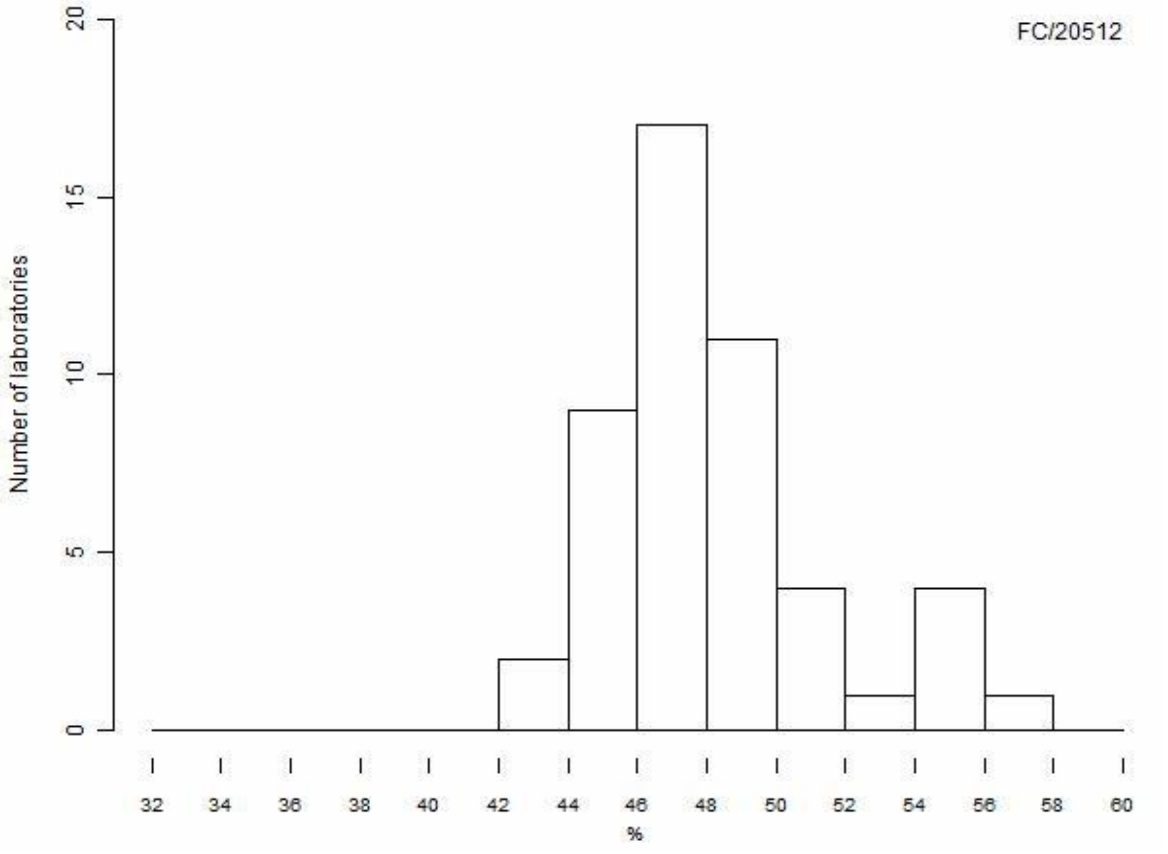


Results not represented on the graph

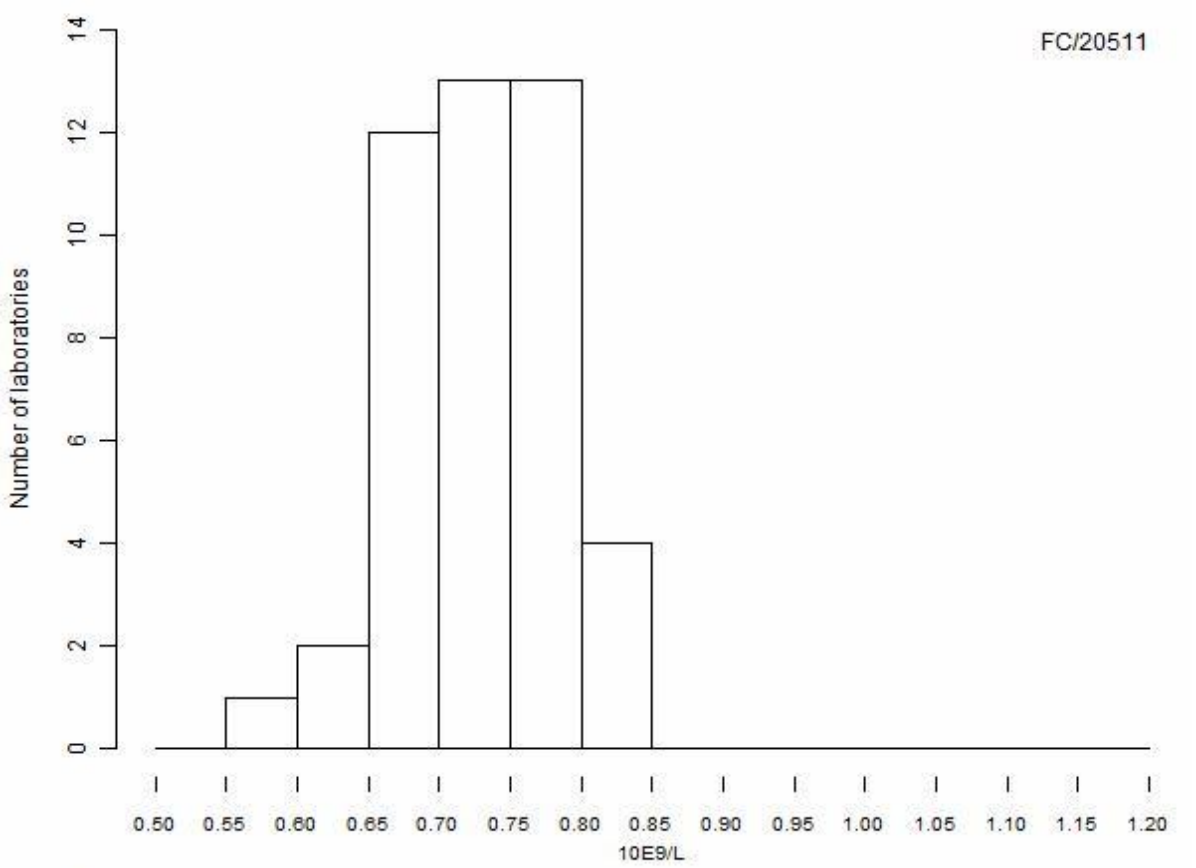
FC/20511 = 1229 10e9/
FC/20511 = 1349 10e9/
FC/20511 = 1460 10e9/
FC/20511 = 1491 10e9/
FC/20512 = 2.2 10e9/L
FC/20512 = 861 10e9/L
FC/20512 = 910 10e9/L
FC/20512 = 1068 10e9/
FC/20512 = 1098 10e9/

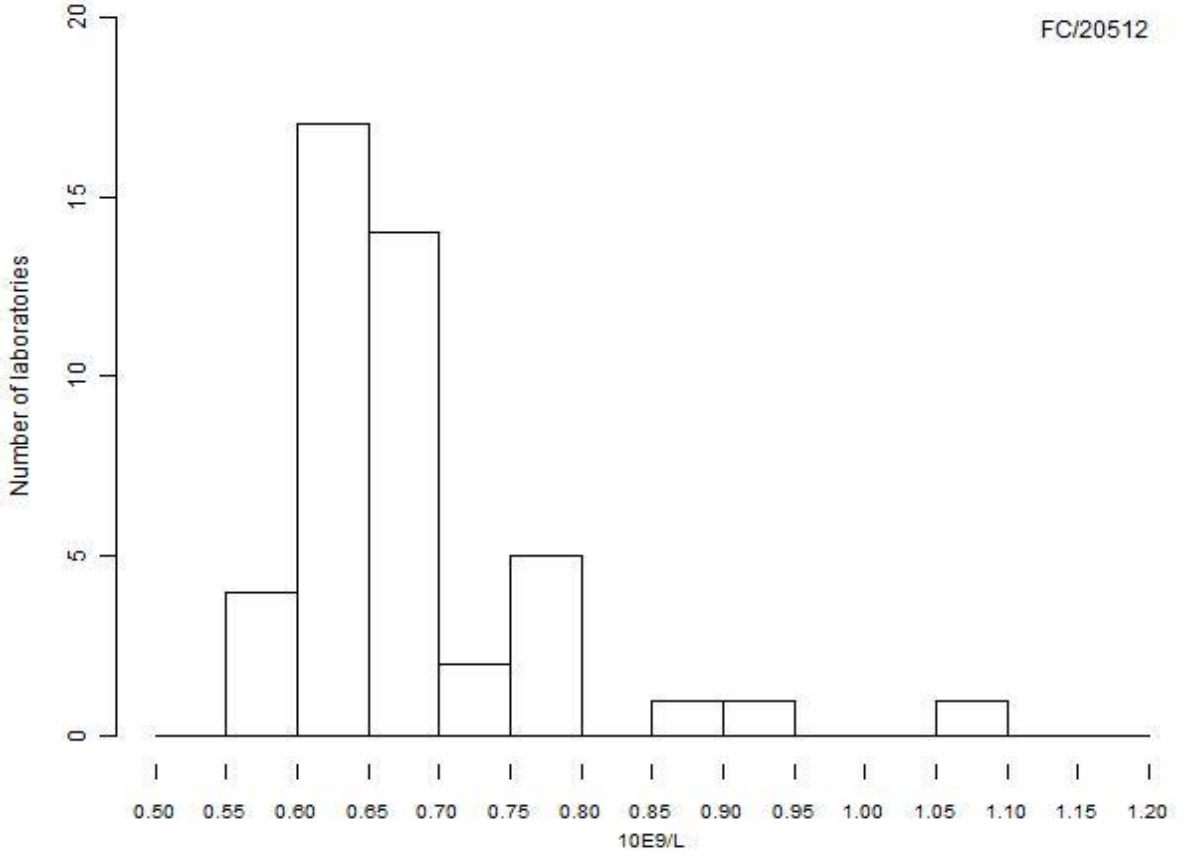
CD4 %


FC/20511
Number of laboratories
0
2
4
6
8
10
12
14
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
%

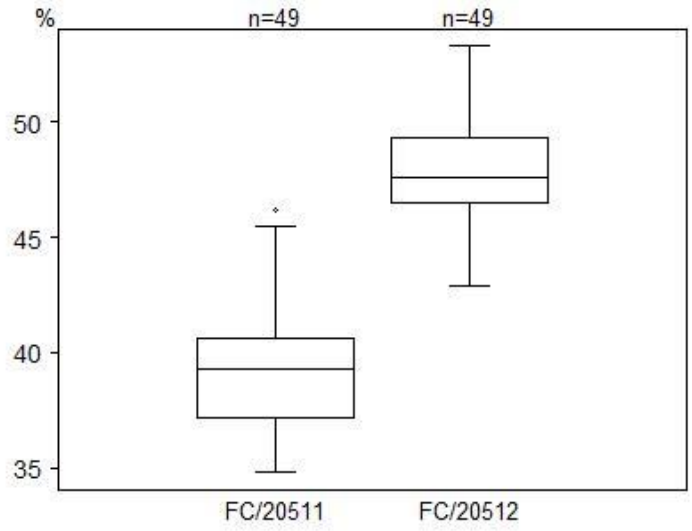

FC/20512
Number of laboratories
0
5
10
15
20
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
%

CD4 10E9/L


FC/20511
Number of laboratories
0
2
4
6
8
10
12
14
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
1.05
1.10
1.15
1.20
10E9/L


FC/20512
Number of laboratories
0
5
10
15
20
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
1.05
1.10
1.15
1.20
10E9/L

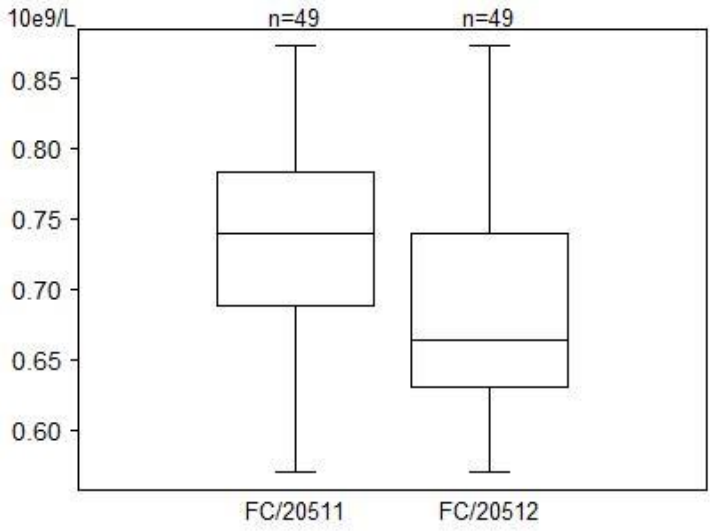
CD4 %



Results not represented on the graph

FC/20512 = 54.3 %
FC/20512 = 55.2 %
FC/20512 = 55.3 %
FC/20512 = 55.9 %
FC/20512 = 57 %

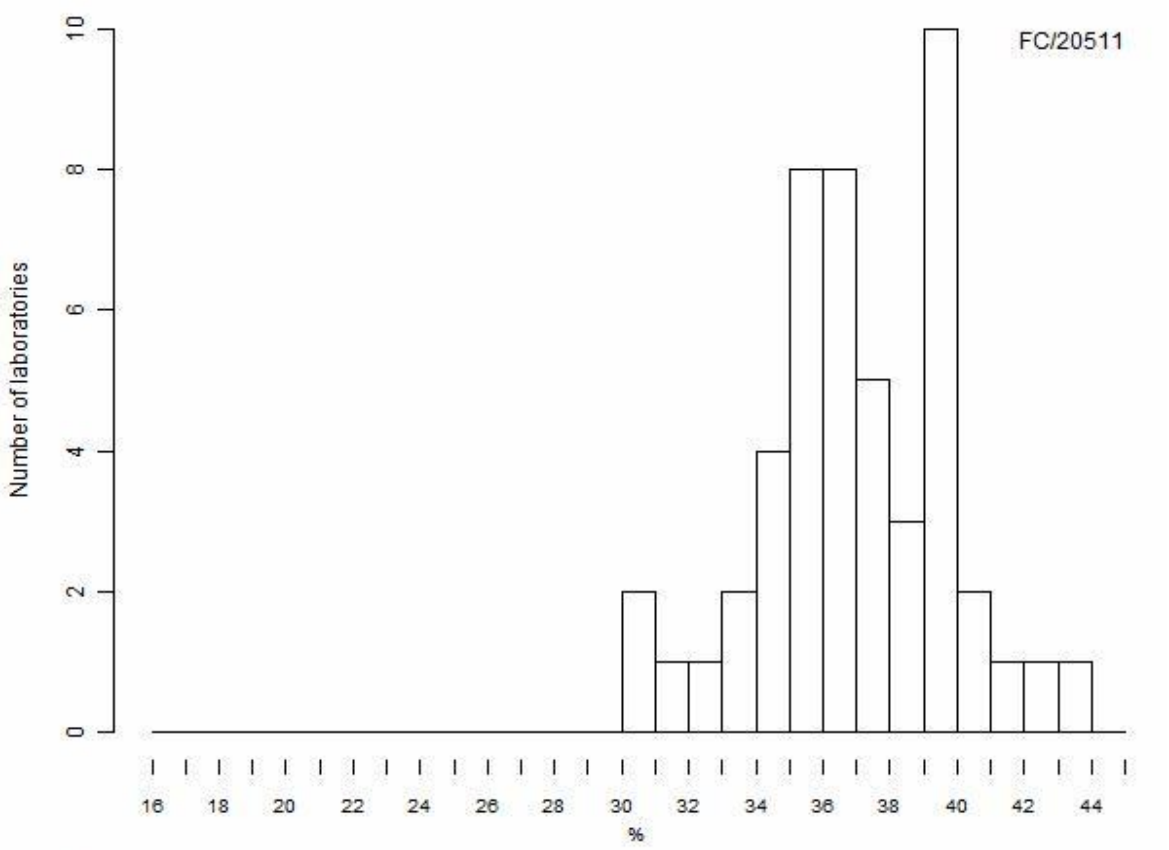
CD4 10E9/L

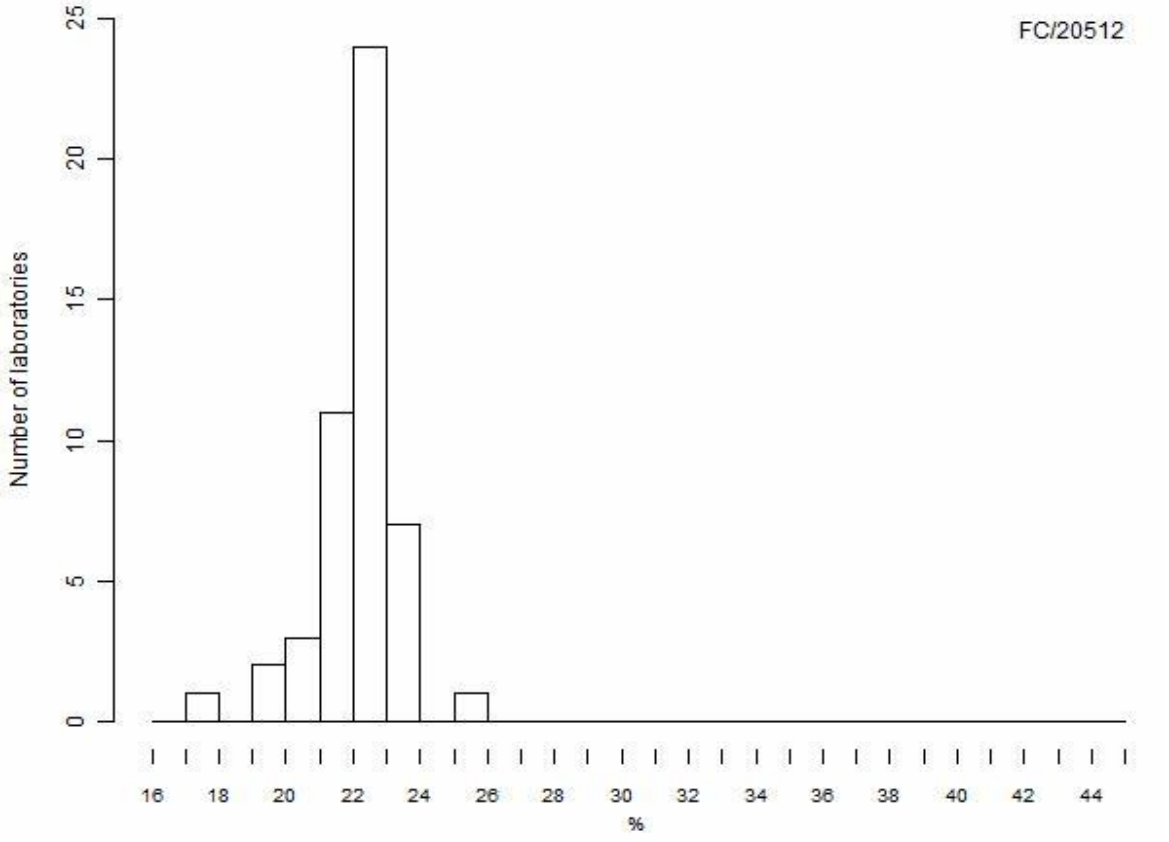


Results not represented on the graph

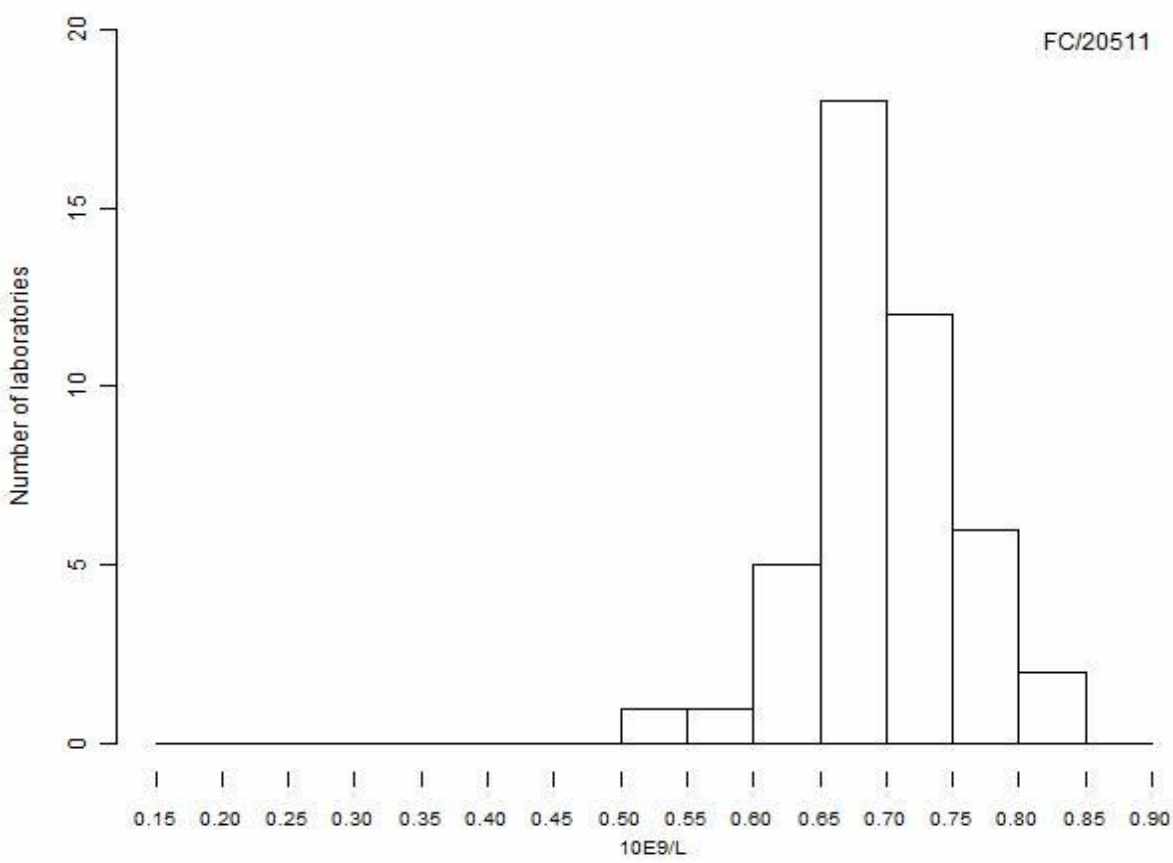
FC/20511 = 586 10e9/L
FC/20511 = 655 10e9/L
FC/20511 = 679 10e9/L
FC/20511 = 774 10e9/L
FC/20512 = 0.95 10e9/l
FC/20512 = 1.1 10e9/L
FC/20512 = 562 10e9/L
FC/20512 = 628 10e9/L
FC/20512 = 692 10e9/L
FC/20512 = 729 10e9/L

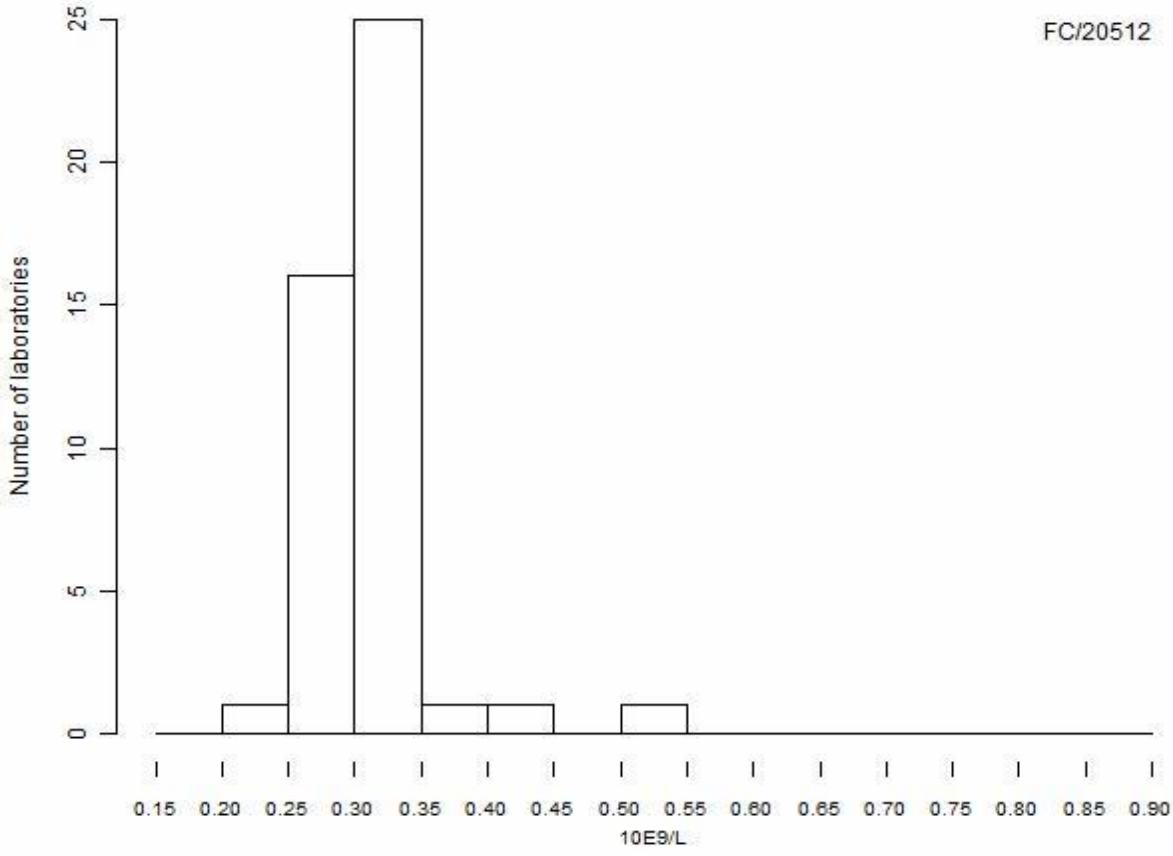
CD8 %


FC/20511
Number of laboratories
0
2
4
6
8
10
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
%

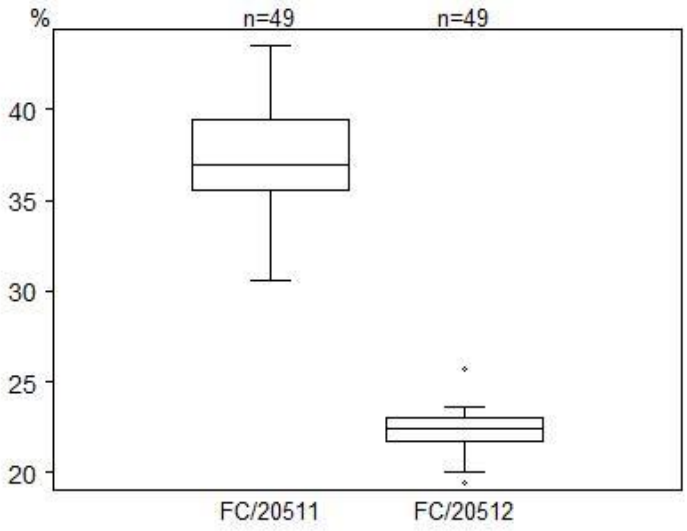

FC/20512
Number of laboratories
0
5
10
15
20
25
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
%

CD8 10E9/L


FC/20511
Number of laboratories
0
5
10
15
20
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
10E9/L


FC/20512
Number of laboratories
0
5
10
15
20
25
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
10E9/L

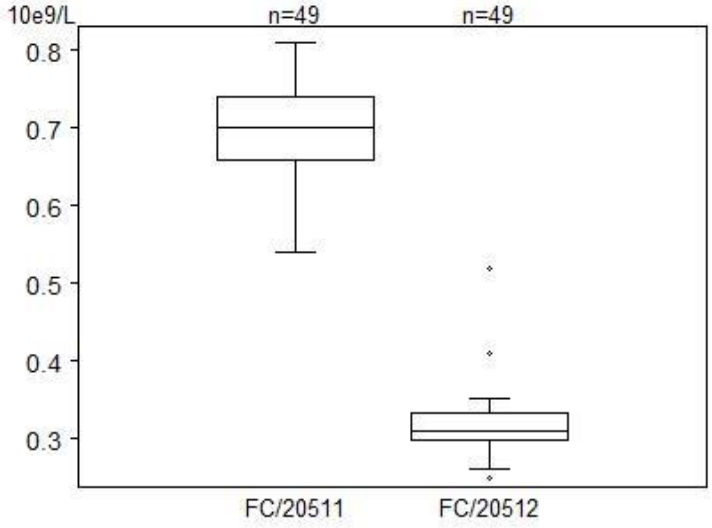
CD8 %



Results not represented on the graph

FC/20512 = 18 %

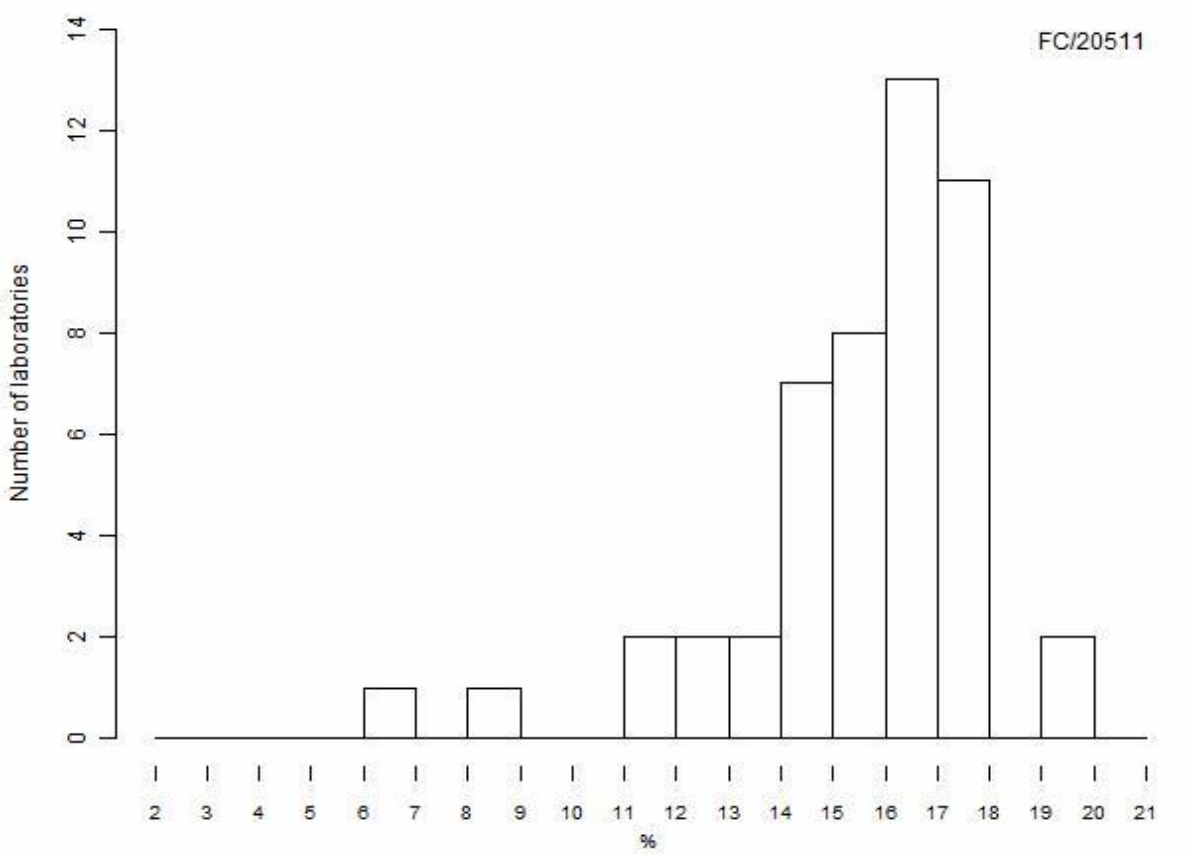
CD8 10E9/L

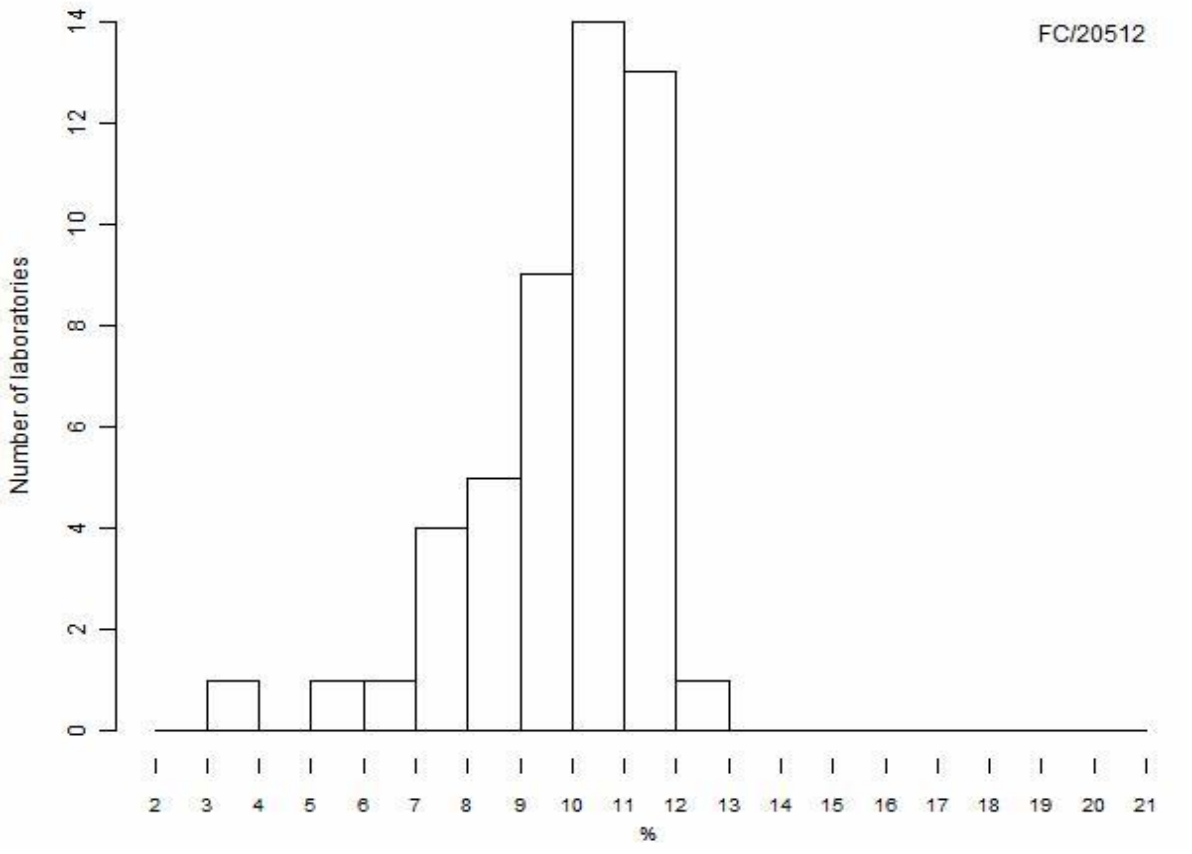


Results not represented on the graph

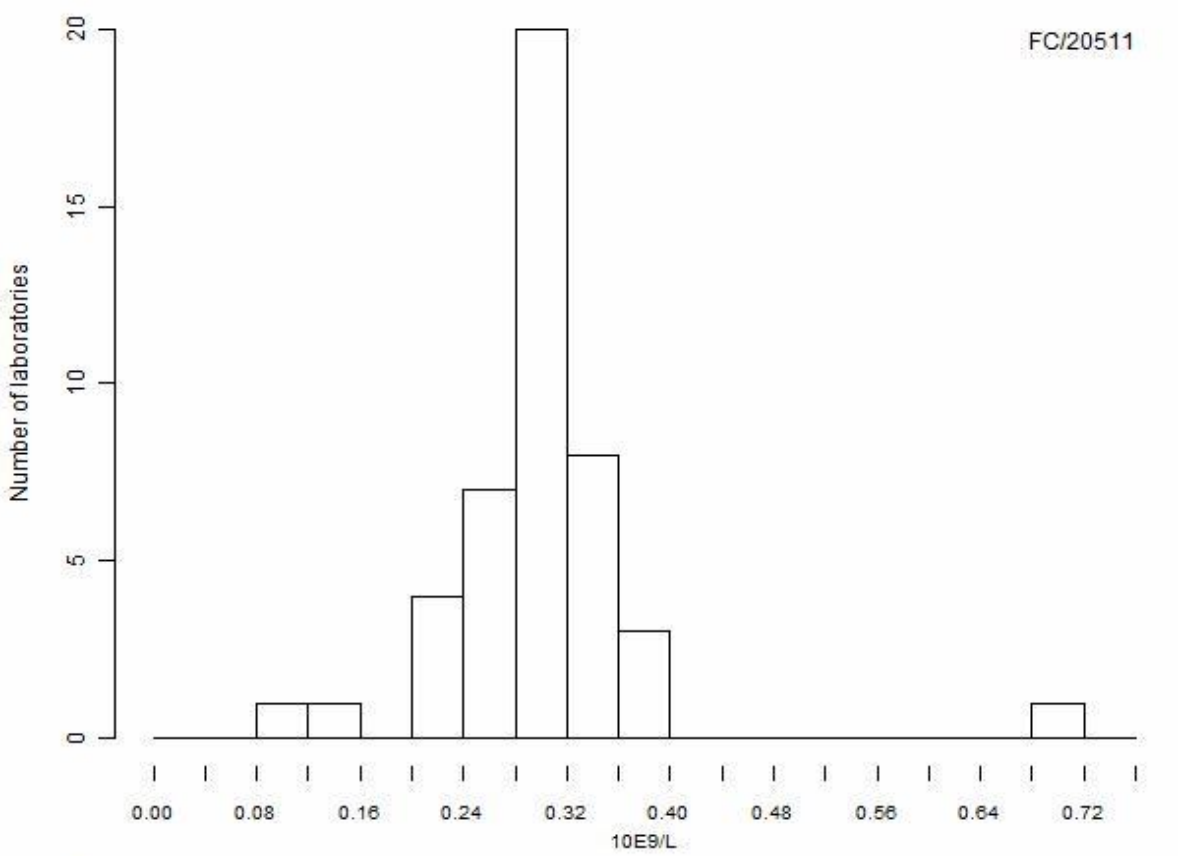
FC/20511 = 577 10e9/L
FC/20511 = 578 10e9/L
FC/20511 = 655 10e9/L
FC/20511 = 676 10e9/L
FC/20512 = 250 10e9/L
FC/20512 = 286 10e9/L
FC/20512 = 298 10e9/L
FC/20512 = 346 10e9/L

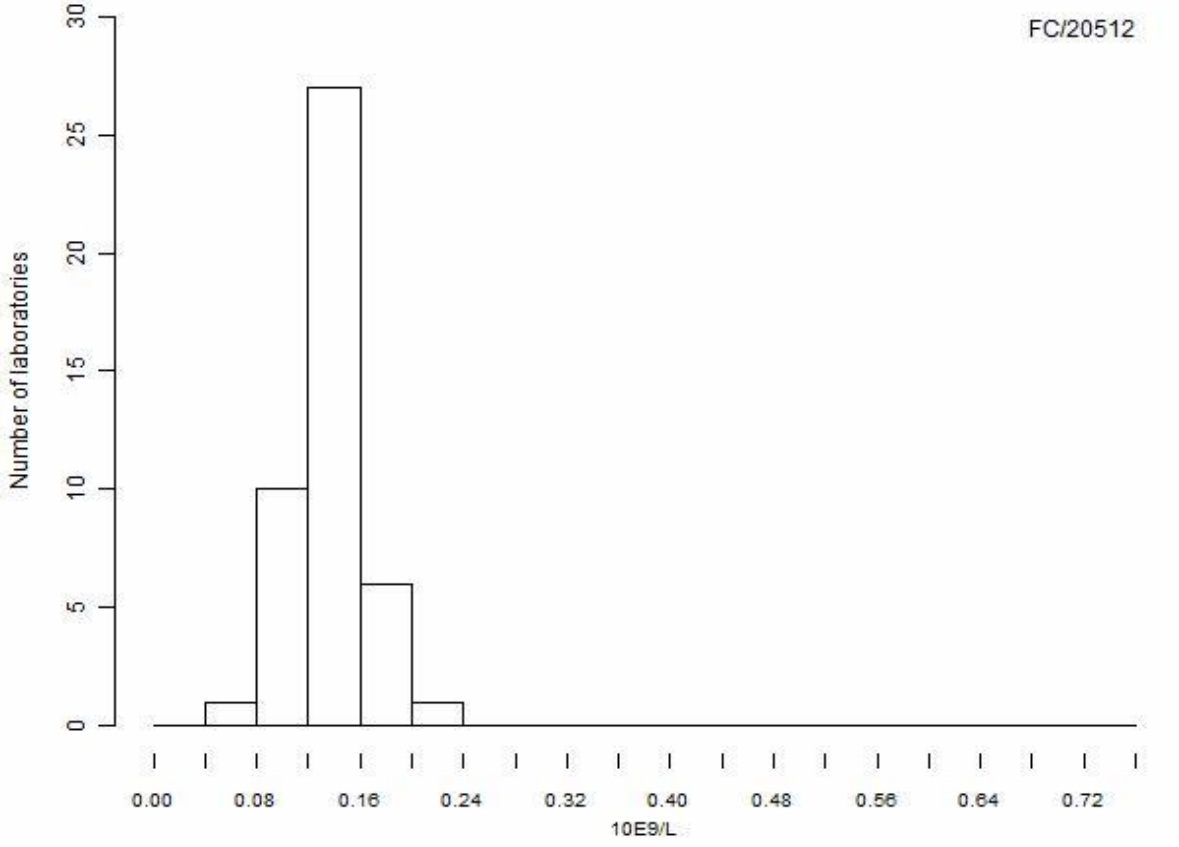
CD19 %


FC/20511
Number of laboratories
0
2
4
6
8
10
12
14
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
%

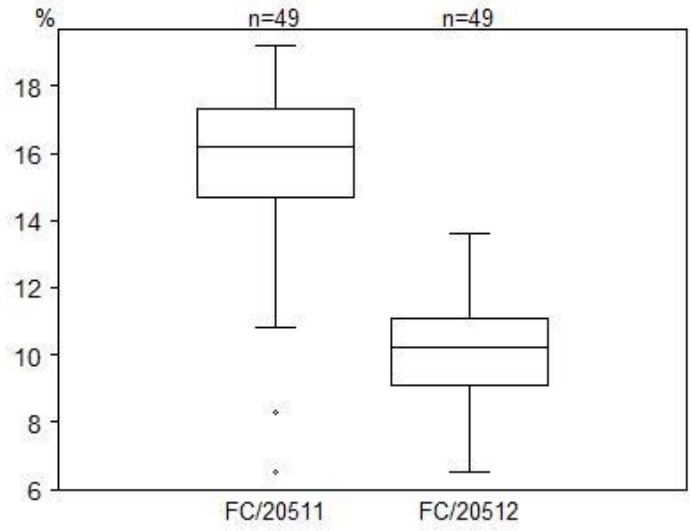

FC/20512
Number of laboratories
0
2
4
6
8
10
12
14
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
%

CD19 10E9/L


FC/20511
Number of laboratories
0
5
10
15
20
0.00
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
10E9/L


FC/20512
Number of laboratories
0
5
10
15
20
25
30
0.00
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
10E9/L

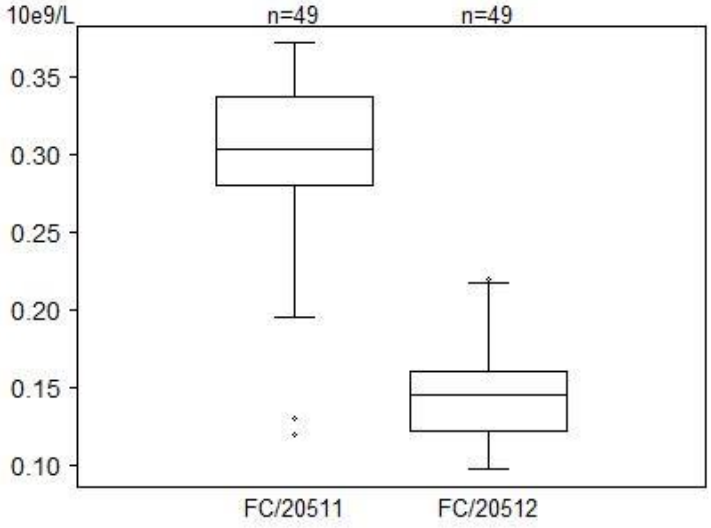
CD19 %



Results not represented on the graph

FC/20512 = 3.9 %

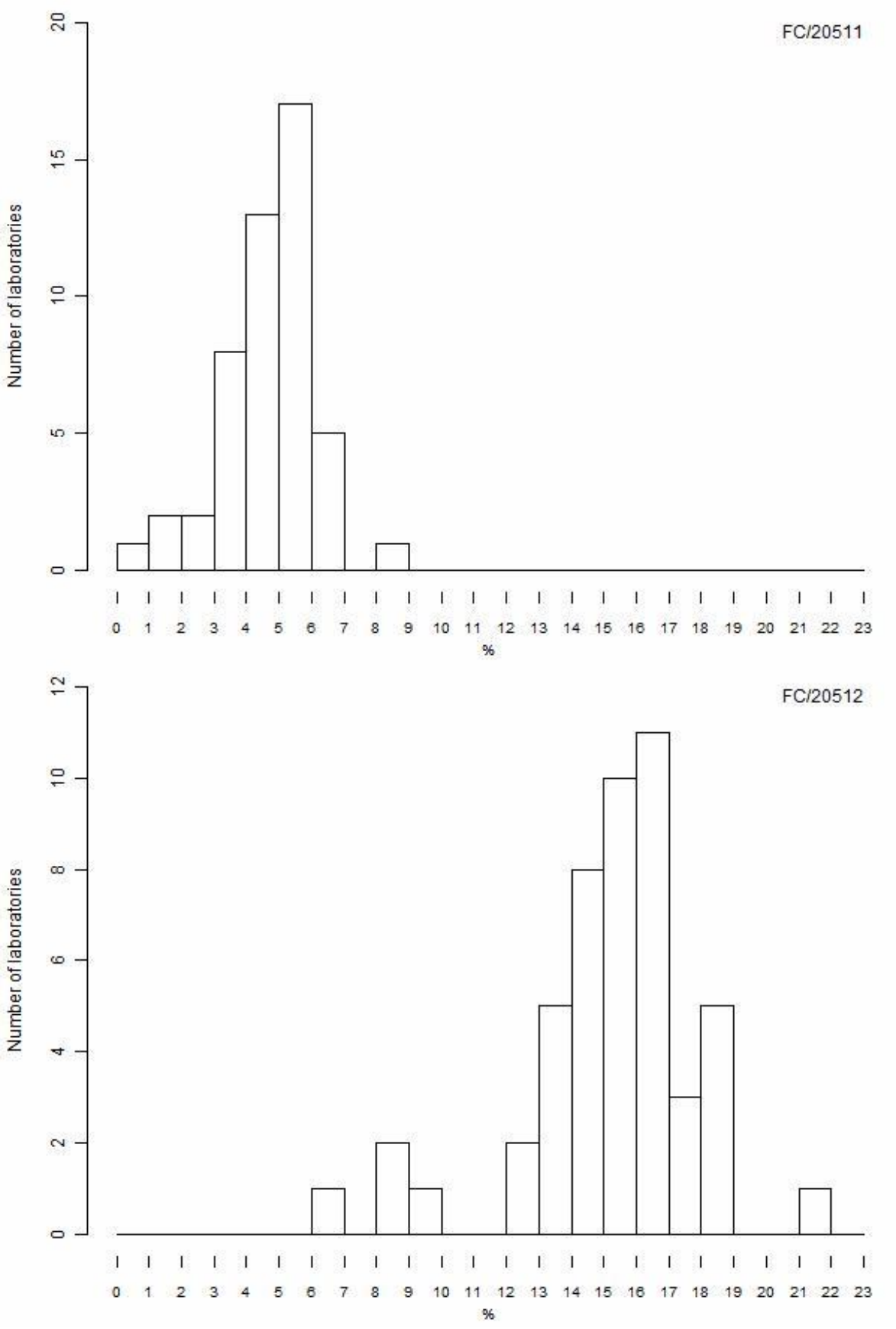
CD19 10E9/L



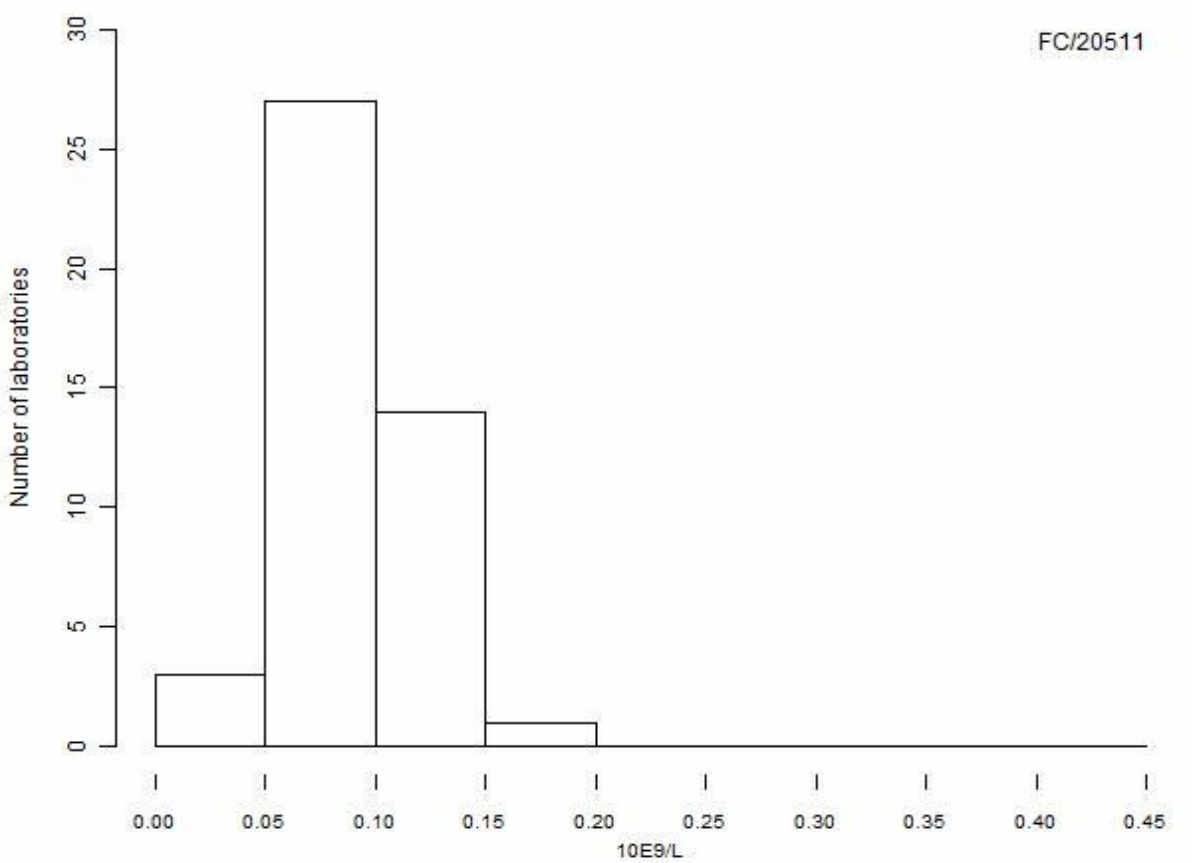
Results not represented on the graph

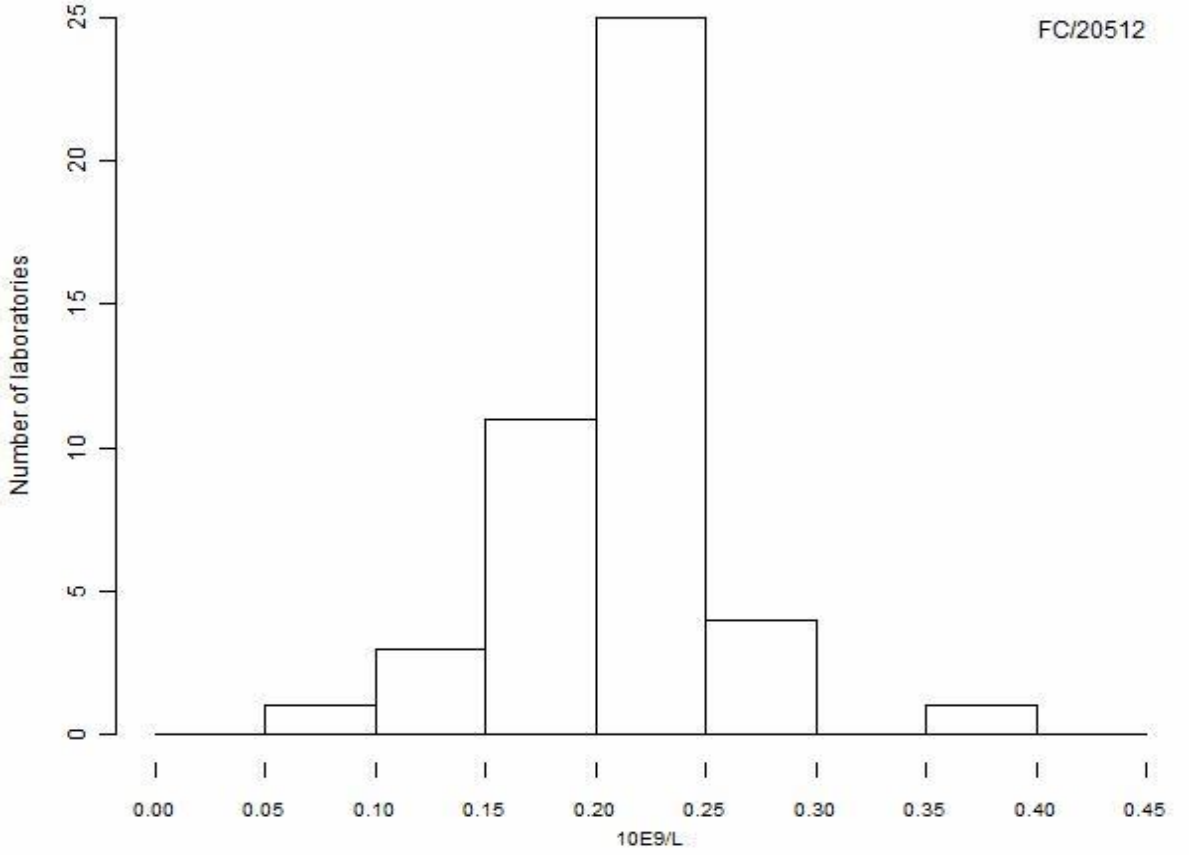
FC/20511 = 0.711 10e9
FC/20511 = 251 10e9/L
FC/20511 = 264 10e9/L
FC/20511 = 300 10e9/L
FC/20511 = 316 10e9/L
FC/20512 = 0.05 10e9/l
FC/20512 = 124 10e9/L
FC/20512 = 125 10e9/L
FC/20512 = 147 10e9/L
FC/20512 = 150 10e9/L

NKcells %

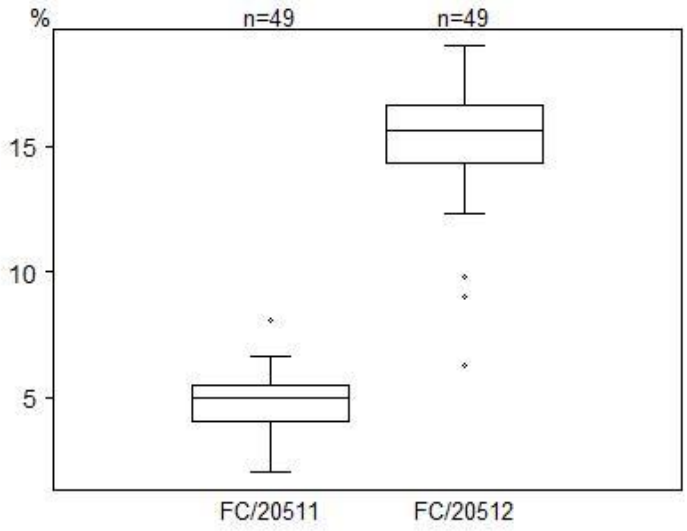

FC/20511
Number of laboratories
0
5
10
15
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
%
FC/20512
Number of laboratories
0
2
4
6
8
10
12
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
%

NKcells 10E9/L





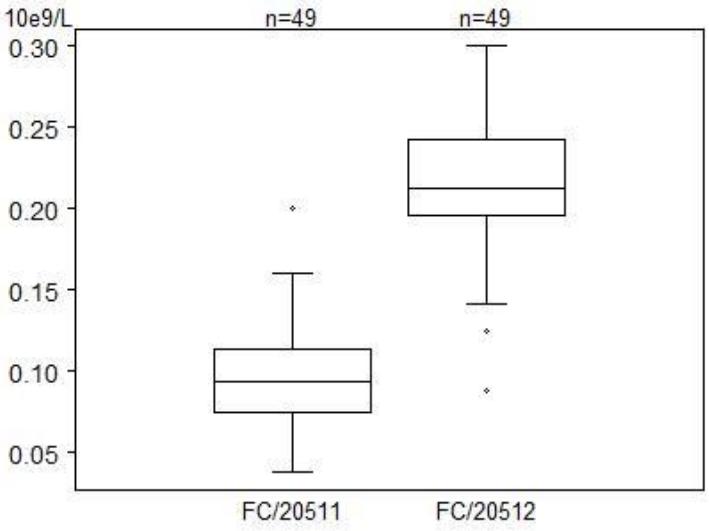
NKcells %



Results not represented on the graph

FC/20511 = 0.1 %
FC/20512 = 21.2 %

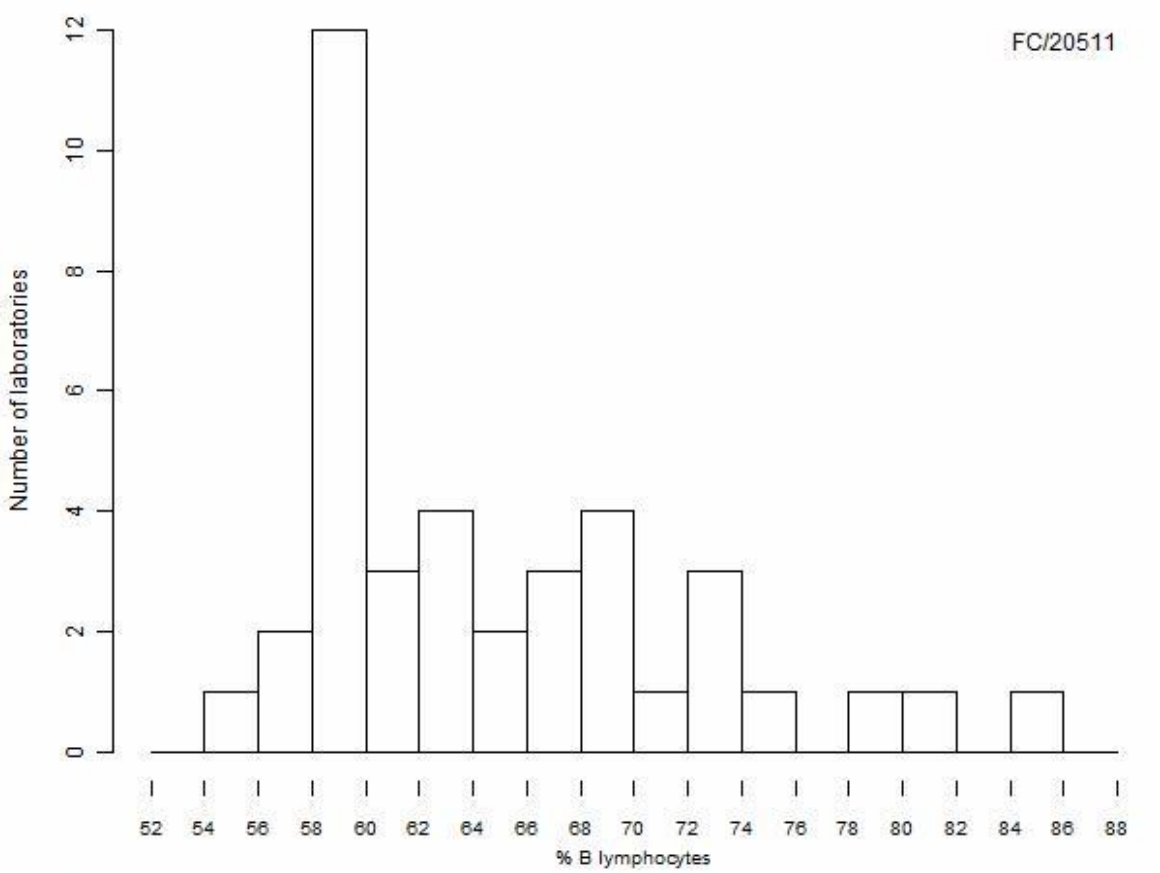
NKcells 10E9/L

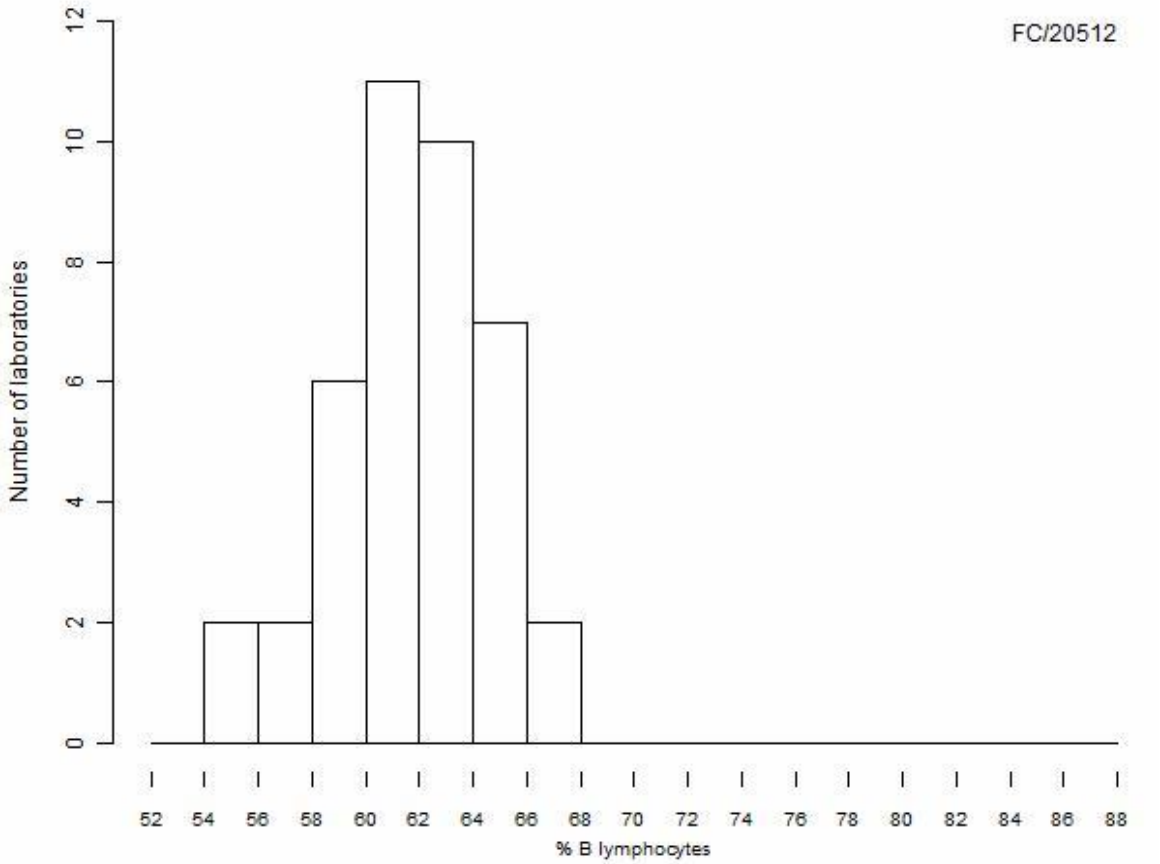


Results not represented on the graph

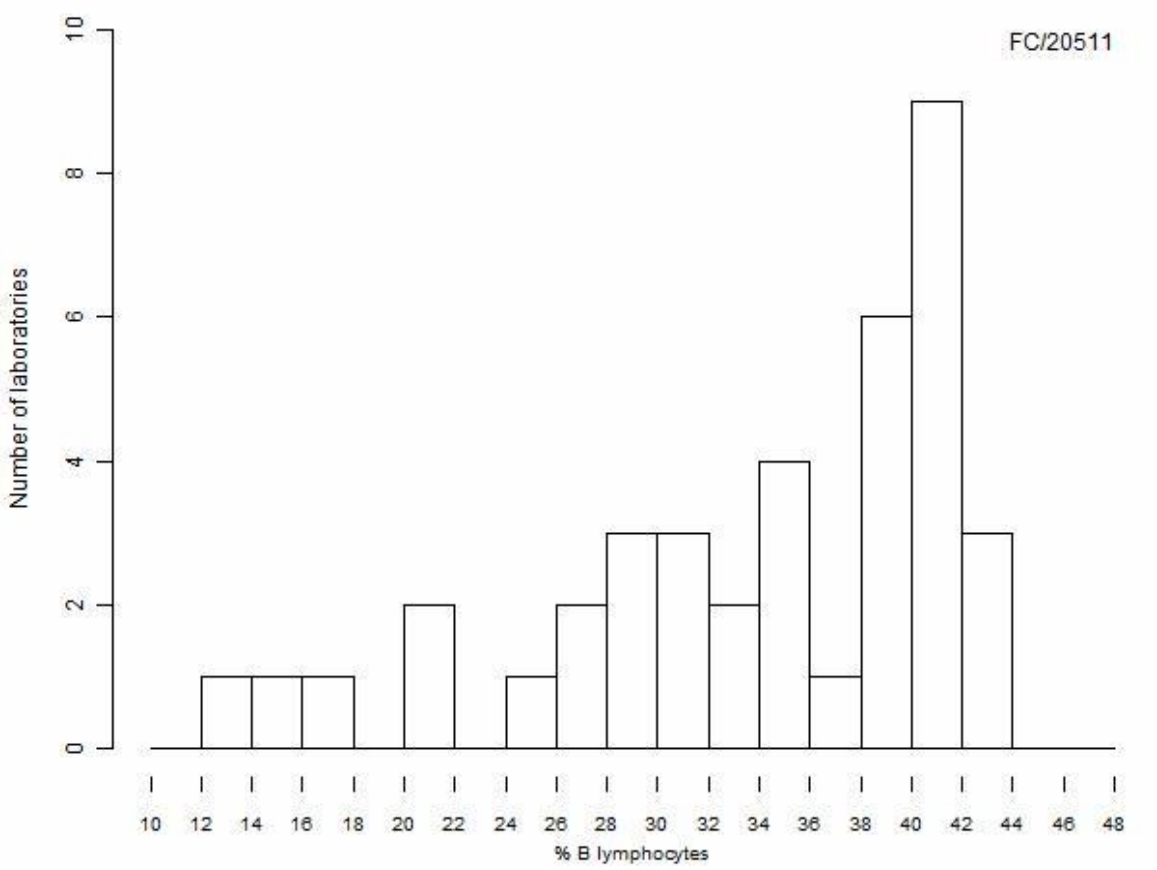
FC/20511 = 0 10e9/L
FC/20511 = 37 10e9/L
FC/20511 = 82 10e9/L
FC/20511 = 85 10e9/L
FC/20511 = 105 10e9/L
FC/20512 = 0.37 10e9/l
FC/20512 = 198 10e9/L
FC/20512 = 219 10e9/L
FC/20512 = 226 10e9/L
FC/20512 = 241 10e9/L

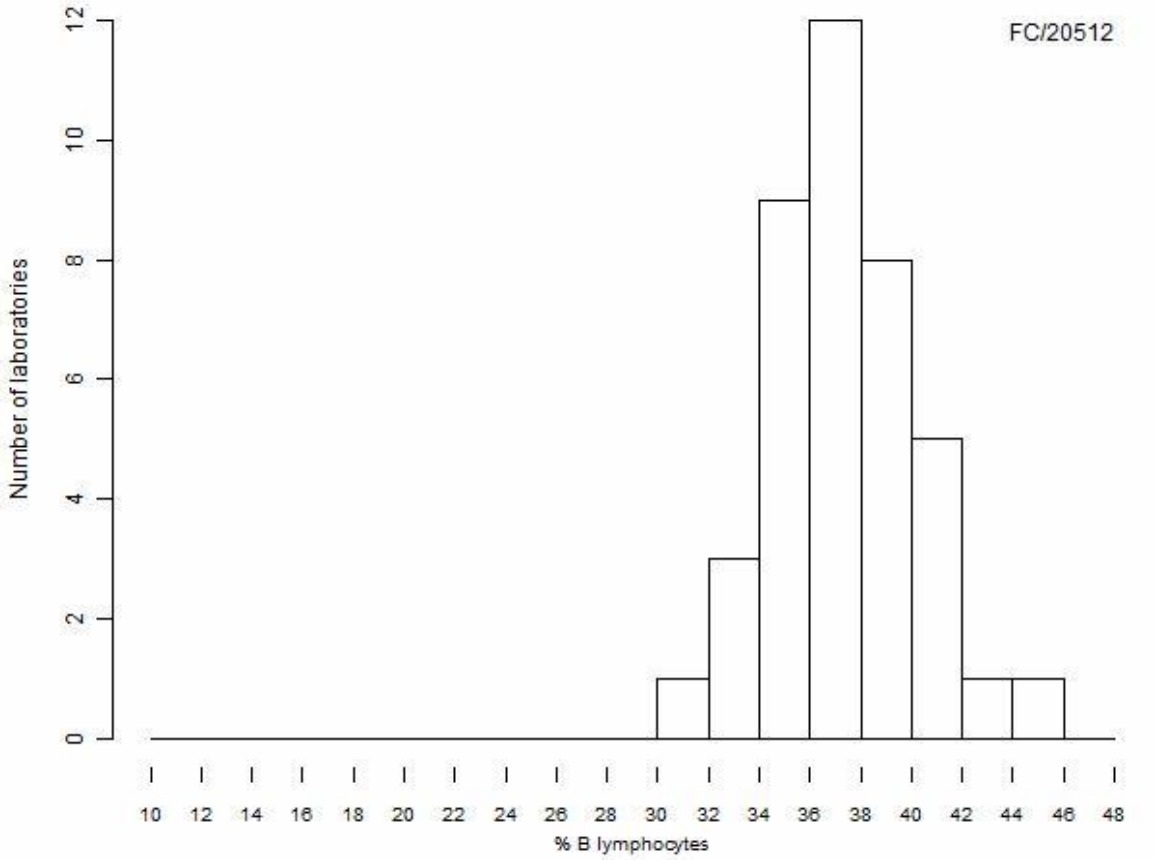
Kappa % B lymphocytes



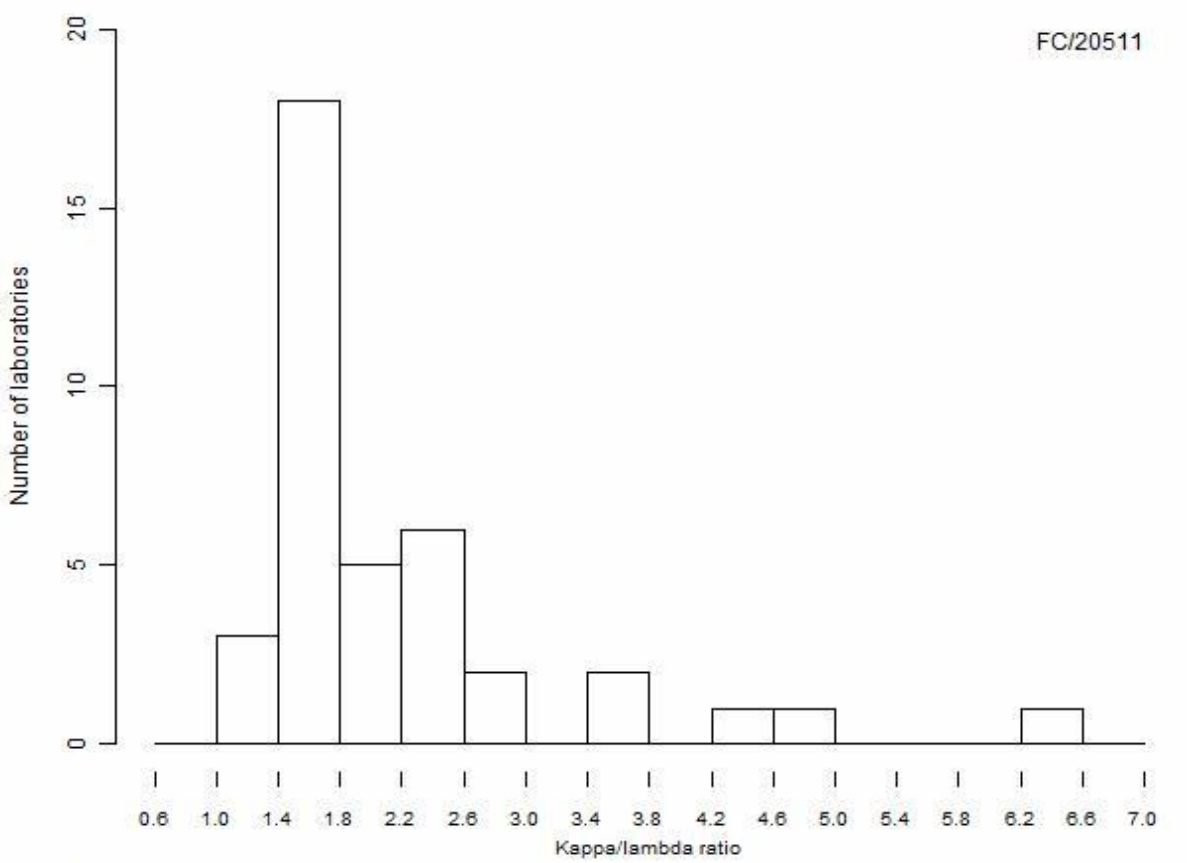


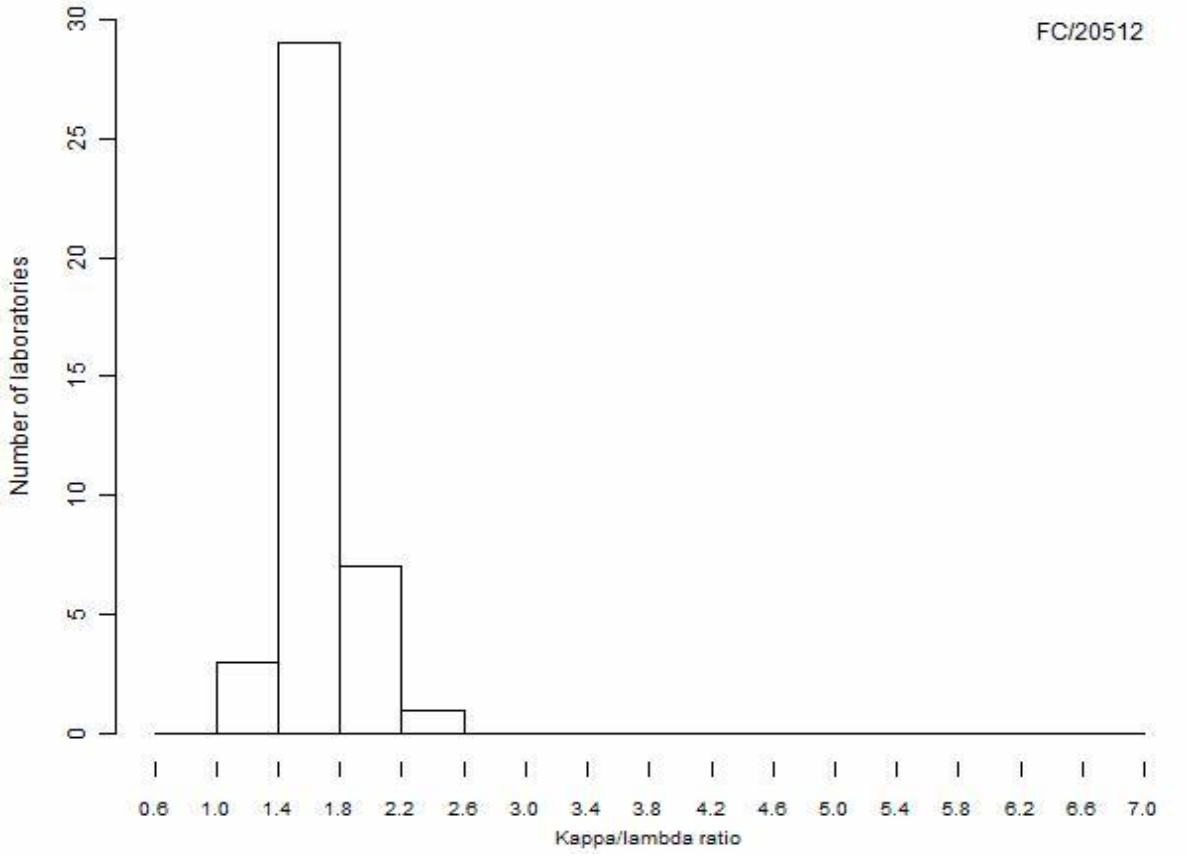
Lambda % B lymphocytes



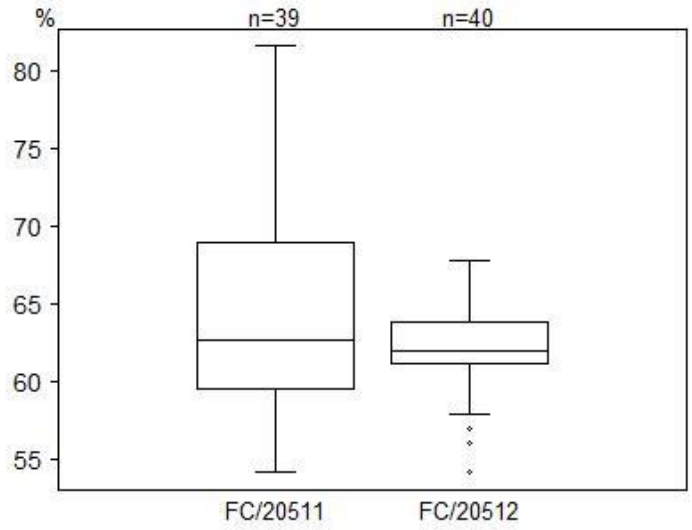


Kappa/lambda





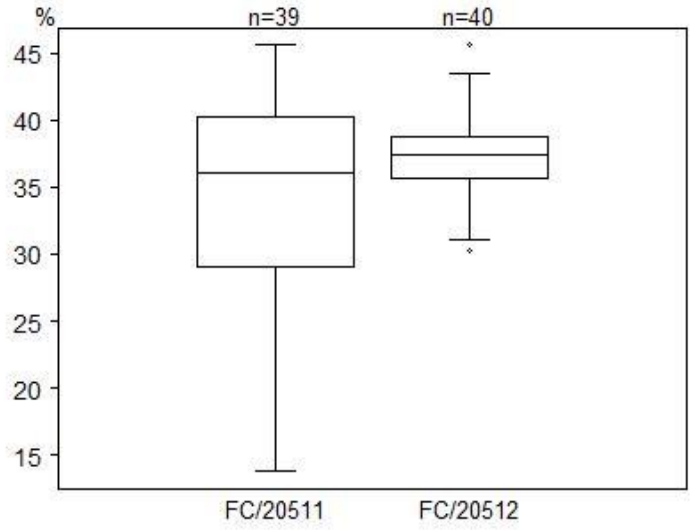
Kappa % B lymphocytes



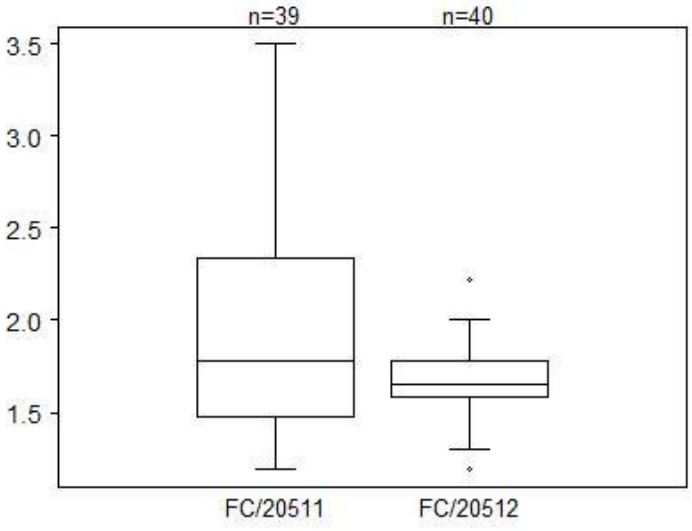
Results not represented on the graph

FC/20511 = 85.9 %

Lambda % B lymphocytes



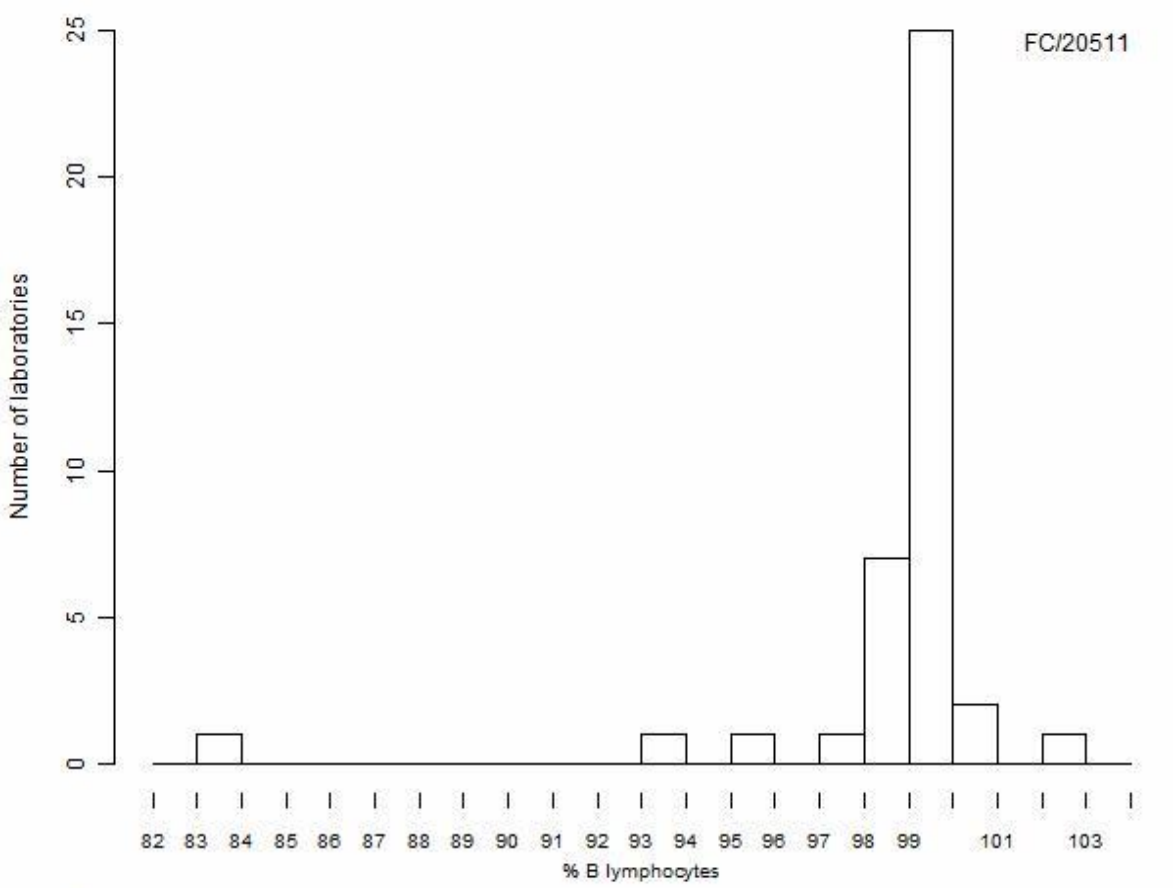
Kappa/lambda

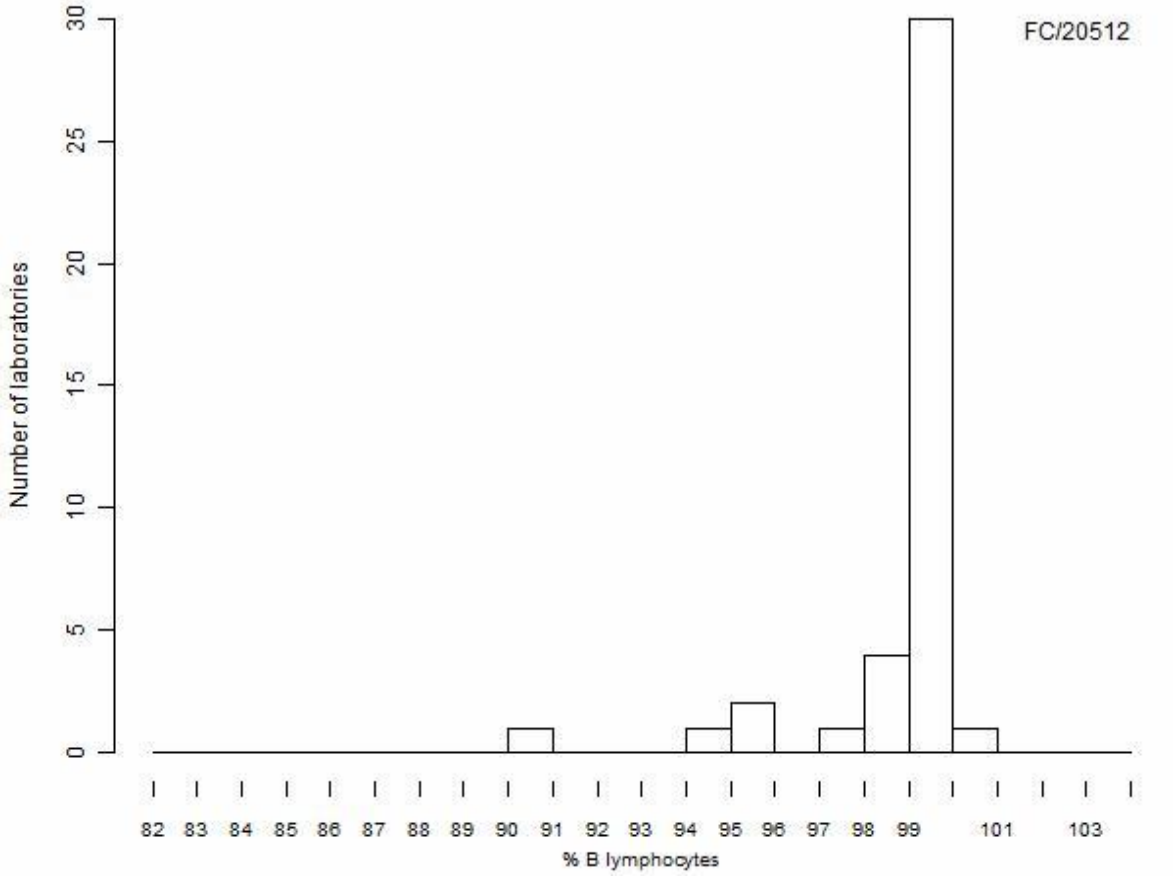


Results not represented on the graph

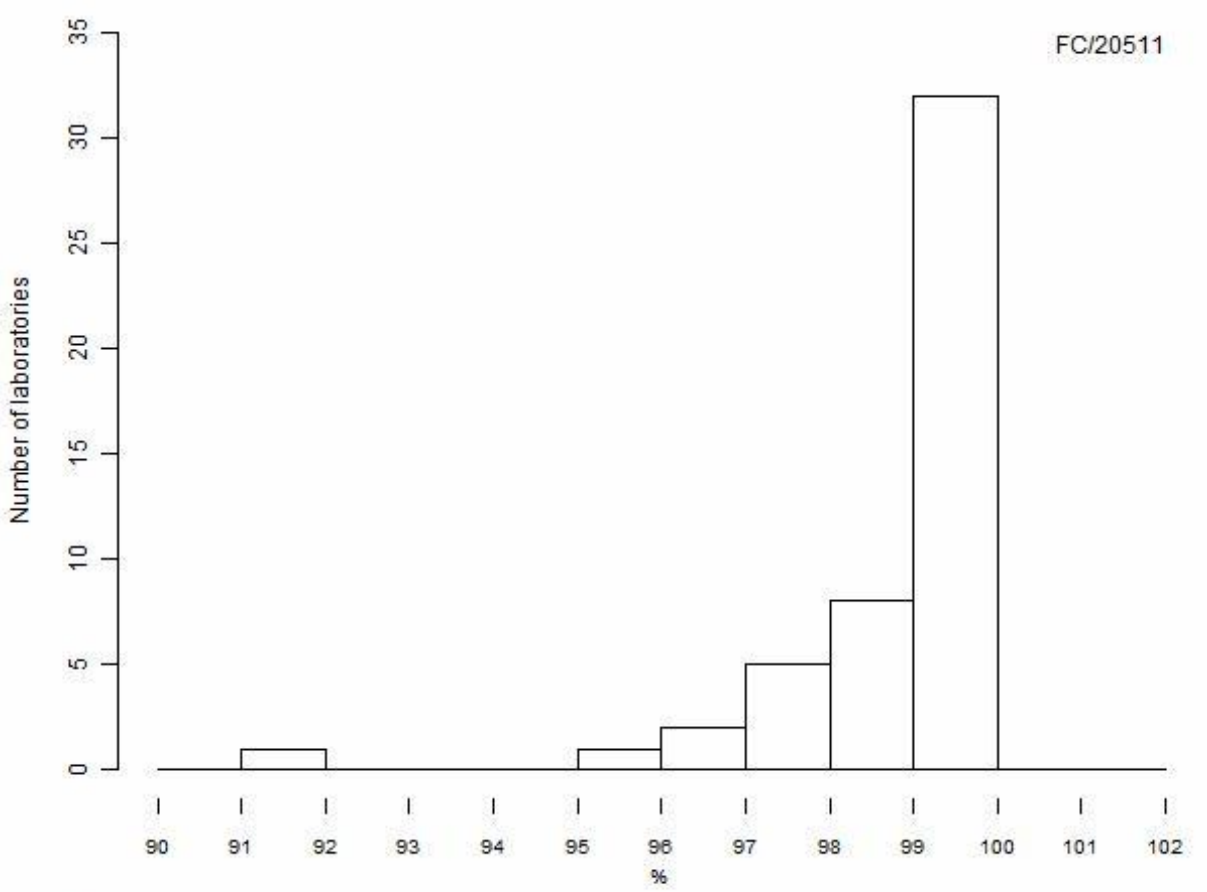
FC/20511 = 3.8
FC/20511 = 4.31
FC/20511 = 4.83
FC/20511 = 6.22

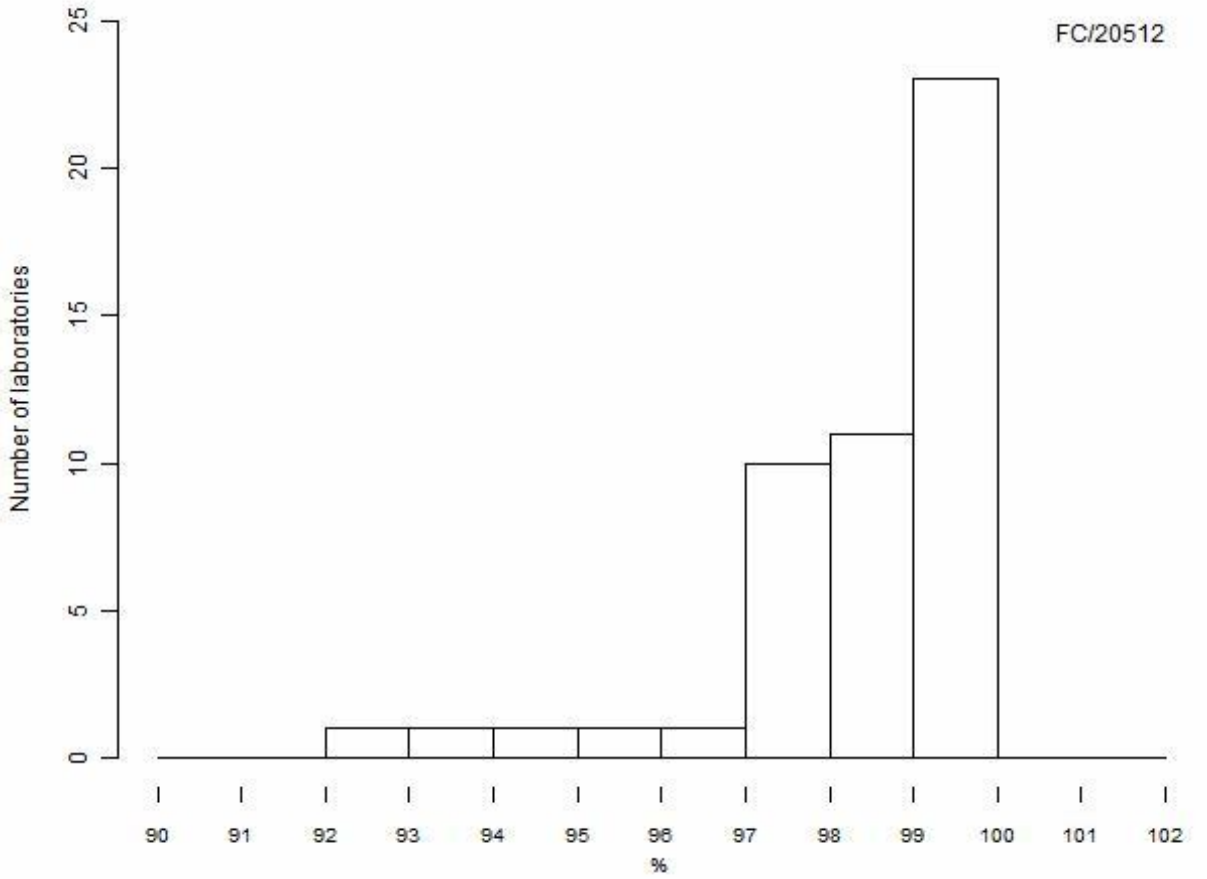
Sum K+L % B lymphocytes



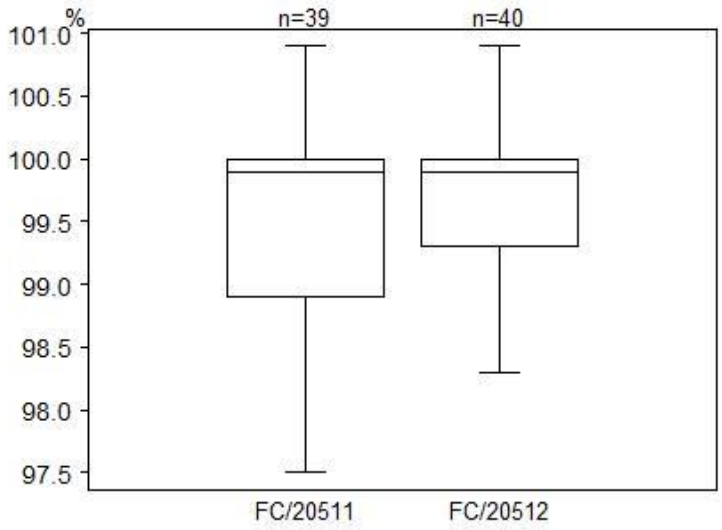


Lymphosum %


FC/20511
Number of laboratories
0
5
10
15
20
25
30
35
90
91
92
93
94
95
96
97
98
99
100
101
102
%


FC/20512
Number of laboratories
0
5
10
15
20
25
90
91
92
93
94
95
96
97
98
99
100
101
102
%

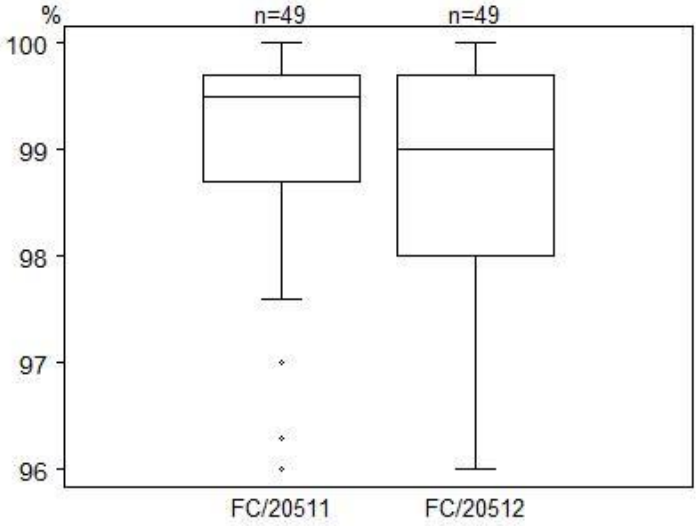
Sum K+L % B lymphocytes



Results not represented on the graph

FC/20511 = 83.4 %
FC/20511 = 93.9 %
FC/20511 = 95.3 %
FC/20511 = 102.1 %
FC/20512 = 90.7 %
FC/20512 = 94.6 %
FC/20512 = 95.7 %
FC/20512 = 95.8 %
FC/20512 = 97.1 %

Lymphosum %



Results not represented on the graph

FC/20511 = 91.6 %
FC/20512 = 92.6 %
FC/20512 = 93.9 %
FC/20512 = 94.9 %
FC/20512 = 95.4 %

For technical validation purposes it is worth noting that in non-pathological peripheral blood of adults the sum of kappa and lambda (expressed as a % of CD19+ B-cells) should be between 90 and 110. The lymphosum (sum of $CD3^{+}$% plus $CD19^{+}$% plus $CD3^{-}CD16^{+}$ and/or $CD56^{+}$%) should equal the purity of the lymphocytes in the gate ± 5%, with a maximum variability of ≤ 10%.

As mentioned above, the graphical representations do not include outliers. The following table presents an overview of outlier percentages for the different parameters during the 2024/1 survey, along with comparisons to previous surveys. Notably, absolute value results show a higher incidence of outliers compared to percentage results. This discrepancy primarily arises from unit errors during data encoding in the Toolkit interface.

	Percentage of outliers		
	2024/1	2023/3	2023/2
WBC 10E9/L	5%	3%	3%
Lympho% haematology analyser	4%	2%	10%
Lympho% flow cytometer	6%	7%	2%
CD3 %	2%	4%	4%
CD3 10E9/L	9%	4%	9%
CD4 %	5%	3%	8%
CD4 10E9/L	10%	5%	10%
CD8 %	1%	1%	2%
CD8 10E9/L	8%	3%	7%
CD19 %	1%	4%	7%
CD19 10E9/L	10%	3%	7%
NKcells %	2%	0%	1%
NKcells 10E9/L	10%	3%	5%
Kappa % B lymphocytes	1%	7%	1%
Lambda % B lymphocytes	0%	5%	1%
Kappa/lambda	5%	7%	2%
Sum K+L % B lymphocytes	11%	10%	4%
Lymphosum %	5%	7%	4%

END