EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
COMMITTEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

DEFINITIVE GLOBAL REPORT

FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS

SURVEY 2021/3

Sciensano/Flow cytometry/79-E

Expertise and service provision
Quality of laboratories
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1050 Brussels | Belgium

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Authorization to release the report: By Lobna Bouacida, scheme coordinator, on 14/02/2022.

Lobna Bouacida Digitally signed by Lobna Bouacida

All the reports are also available on our webpage:
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_nl/rapports_annee.htm
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_fr/rapports_annee.htm

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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 ($M_{M/G}$).

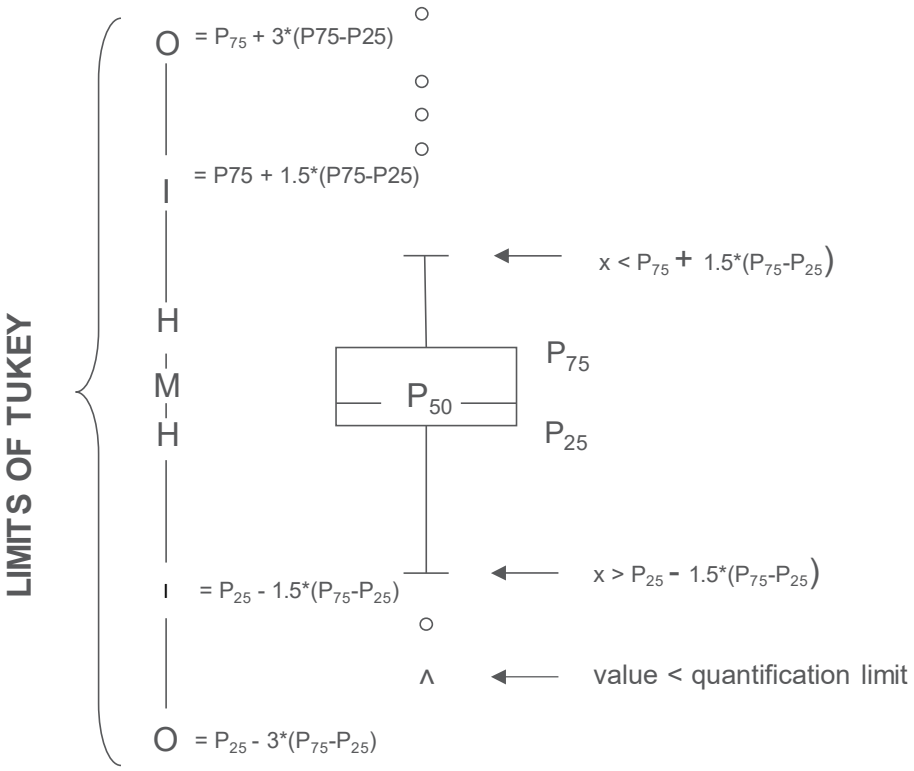
More information can be found in 3 brochures available on our website (only in Dutch and French):
https://www.wiv-isp.be/QML/index_nl.htm
https://www.wiv-isp.be/QML/index_fr.htm
(Choose “brochures” in the menu)
or directly on the following webpage (only in Dutch and French):
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_nl/brochures.htm
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_fr/brochures.htm

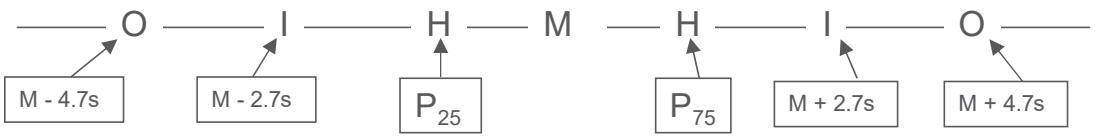
1. Informatiebrochure over de externe kwaliteitsevaluatieprogramma's voor klinische laboratoria (Algemene informatiebrochure over de externe evaluatie)/
   https://www.wiv-isp.be/QML/Informatiebrochure_EKE.pdf
   Brochure d’information sur les programmes d’évaluation externe de la qualité pour les laboratoires cliniques (Brochure d’information générale sur l’évaluation externe).
   https://www.wiv-isp.be/QML/Brochure_information_EEQ.pdf
2. Statistische brochure (Algemene statistische berekeningsprocedure opgesteld door Professor Albert)/ Brochure statistique (Procédure générale de calcul statistique mis au point par le professeur Albert).
3. Verwerking van gecensureerde waarden (Statistische berekeningsprocedure toegepast op de gecensureerde waarden opgesteld door Professor Albert)/
   Traitement des valeurs censurées (Procédure de calcul statistique appliquée aux valeurs censurées rédigée par le Professeur Albert).

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/18731 and FC/18732) collected on K2EDTA were sent to the laboratories.

Sample FC/18731 was collected from a healthy and voluntary blood donor, whereas sample FC/18732 was collected from a patient and was kindly provided by Dr Christian Chatelain (CHR Val de Sambre).

The two samples were distributed into aliquots at Sciensano and sent on the day of blood collection by Taxipost 24h. The laboratories were informed by e-mail of the send-out of the control material (day 0).

The samples tested negative for HIV 1 and 2, hepatitis B surface antigen and hepatitis C.

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

FC18731

	%	10^9/L
Leukocytes		8.8
Lymphocytes	16.3	
$CD3^+$ cells	63.6	0.91
$CD4^+CD3^+$ cells	40.7	0.58
$CD8^+CD3^+$ cells	21.4	0.31
$CD19^+$ cells	11.4	0.16
NK cells	22.6	0.32
κ % B lymphocytes	64.3	
λ % B lymphocytes	35.7	
κ/λ ratio	1.80	

FC18732

	%	10^9/L
Leukocytes		6.7
Lymphocytes	42.3	
$CD3^+$ cells	75.5	2.14
$CD4^+CD3^+$ cells	65.6	1.86
$CD8^+CD3^+$ cells	10.1	0.29
$CD19^+$ cells	17.0	0.48
NK cells	3.9	0.11
κ % B lymphocytes	24.6	
λ % B lymphocytes	75.2	
κ/λ ratio	0.33	

PARTICIPATION

Fifty-three laboratories (1 Canadian and 52 Belgian clinical laboratories) participated in the survey 2021/3 (send-out of blood samples on November 22, 2021 (day 0)).

RESULTS

100% of the Belgian laboratories received the samples on day 1 or 2. 46 laboratories (88%) received the samples on day 1 and six (12%) received them on day 2.

75% (n=39) of the Belgian laboratories performed the analyses on day 1, 15% (n=8) on day 2 and 10% (n=5) on day 3.

Since the samples are fresh and not stabilised, it is extremely important to perform sample testing as soon as possible upon receipt.

Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=52). 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 are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=47).

The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/18731 and FC/18732:

FC/18731	Median	SD	CV,%	N
WBC 10E9/L	10.70	0.40	3.7	45
Lympho% haematology analyser	15.7	0.7	4.7	46
Lympho% flow cytometer	15.4	1.4	9.1	45
CD3 %	62.2	2.5	4.0	52
CD3 10E9/L	1.046	0.097	9.2	47
CD4 %	39.4	1.9	4.9	52
CD4 10E9/L	0.660	0.070	10.5	47
CD8 %	20.1	1.0	5.2	52
CD8 10E9/L	0.340	0.038	11.3	47
CD19 %	11.1	1.0	**8.7**	52
CD19 10E9/L	0.183	0.021	**11.3**	47
NKcells %	24.8	2.4	9.5	52
NKcells 10E9/L	0.410	0.048	11.6	47
Kappa % B lymphocytes	63.8	2.8	**4.3**	44
Lambda % B lymphocytes	36.0	2.8	**7.8**	44
Kappa/lambda	1.78	0.20	11.2	44
Sum K+L % B lymphocytes	99.8	0.6	0.6	44
Lymphosum %	98.2	1.9	2.0	52

FC/18732	Median	SD	CV,%	N
WBC 10E9/L	7.36	0.23	3.1	46
Lympho% haematology analyser	41.7	1.0	2.3	46
Lympho% flow cytometer	40.3	2.8	6.9	45
CD3 %	78.8	2.2	2.8	52
CD3 10E9/L	2.402	0.134	5.6	47
CD4 %	68.0	2.1	3.2	52
CD4 10E9/L	2.055	0.121	5.9	47
CD8 %	10.3	0.9	8.6	52
CD8 10E9/L	0.318	0.026	8.1	47
CD19 %	15.5	1.5	**9.5**	52
CD19 10E9/L	0.470	0.067	**14.2**	47
NKcells %	4.4	0.7	16.8	52
NKcells 10E9/L	0.132	0.024	18.2	47
Kappa % B lymphocytes	28.0	4.4	**15.9**	43
Lambda % B lymphocytes	69.8	9.3	**13.3**	43
Kappa/lambda	0.41	0.18	43.3	43
Sum K+L % B lymphocytes	99.7	1.3	1.3	43
Lymphosum %	99.3	1.0	1.0	52

The inter-laboratory variability of the B-cells in the patient (FC/18732) was roughly similar to that of the healthy donor (FC/18731). This is particularly true for the percentage of CD19+ cells (CV = 9.5% in FC/18732 vs 8.7% in FC/18731). The distribution of the results follows a usual dispersion with only one peak. It can be deduced that the results were reported in a homogeneous way. The results for the light chains kappa and lambda, on the other hand, show a higher variability than those of the donor, as well as in comparison with previous surveys.

For sample FC/18732, 21 laboratories (40%) reported the detection of a monoclonal B-cell population. This population was estimated at 251 cells/µL (M=251 cells/µL, CV= 12.4%, n=7) or 56.6% (M=56.6%, CV=7.2%, n=7) of the B-cells.

Among the laboratories, 11 referred to a chronic lymphocytic leukaemia (CLL) type monoclonal B-cell lymphocytosis (MBL).

Below are the detailed comments of all the laboratories.

	Comment
1	Monoclonal B-cells expressing Lambda light chains: **251/µL**. This population shows weaker expression compared to the normal Lambda B-cells.
2	Small monoclonal B-cell population with the phenotype CD19+/ CD20 weak+/CD5+ /Lambda weak. This population is approximately **3.3%** of the WBC.
3	Very weak Ig expression in the pathological population in the sample FC/18732.
4	Clonal lymphocytes: very weak expression of the light chains (appear negative) CD5+ CD20 weak: probable ***CLL***
5	Within the B population, 26% of the cells are polyclonal and **72%** are Ig negative. Intracytoplasmic labeling is recommended.
6	Monoclonal population: Lambda weak CD19+ CD20dim CD5+ CD23+ CD10- CD200+ Fmc7- CD79b- (Catovsky-score 5/5) accounting for **55%** of the B-cells (**250 cells/µL**). Consistent with a ***CLL type MBL***.
7	***Low-count MBL of CLL phenotype*** accounting for **52%** of B-cells (**272 cells/µL**).
8	Monoclonal population: CD19+ CD5+ CD10- CD20+dim CD22+dim CD38- and monoclonal Lambda accounting for **61%** of the B-cells.
9	***MBL*** CD5+ CD20 weak Lambda weak (**502/µL**)
10	We found 2 B-cell populations, one CD5+ and the other CD5-
11	B-cell clone with the phenotype CD45+weak CD19+ CD20 weak+ CD5+ CD38- Lambda weak. ***MBL*** at **228/µl**. Consistent with a ***CLL*** phenotype. Matutes score to be considered.
12	Slight increase in the CD4/CD8 ratio. 2 distinct B-cell populations: a) **55%** of B-cells with the phenotype CD19+ CD20 weak+ CD5+ CD10- monoclonal Kappa (Kappa: 98%, Lambda: 2%, decreased fluorescence) consistent with a ***low-count MBL type B-CLL***. To be confronted with the clinical presentation. b) 45% of B-cells without evidence of monoclonality (Kappa: 64.5%, Lambda: 35.5%).
13	B-cell population with the phenotype CD20+, CD5+ and not expressing surface immunoglobulins. ***MBL type CLL.***
14	***MBL type CLL.***
15	Monoclonal B-cell population with the immunophenotype of a ***CLL*** (Matutes score 5/5) accounting for **56.6%** of total B-cells. Consistent with a ***MBL***.
16	Monoclonal B-cell population with the phenotype CD19+ CD5+ Lambda weak.
17	Malignant B-cell population expressing the Lambda light chain at low density. It also expresses strongly CD5 and CD20 at low density. This malignant cell population represents **60%** of total B cells or **290 cells/µl**. These results are highly suggestive of a "***Low-count CLL-type MBL***". Evaluation of the Matutes score on this population is recommended to confirm the diagnosis with a higher certainty. These results must be interpreted in regard to the clinical presentation of the patient (absence of "B symptoms"?).
18	Lymphoma population CD19+ CD5+ with negative expression of Kappa and Lambda. We can't interpret the Kappa/Lambda ratio.
19	B-cell clone at **215 cells/µL** (**8.1%** of the lymphocytes) with the phenotype CD19+ CD5+ CD10- CD20 weak+ Lambda weak.
20	Clonal weak lambda B-cell population with the phenotype: CD20 dim/CD5+/CD38-. Immunophenotype suggestive for ***low-count MBL***.
21	Presence of a CD19+ CD5+ population (+/- **9.2%**). Increase in the CD4/CD8 ratio. This increase is often observed in autoimmune disorders.

Comment

Among the 31 laboratories that gave no indication of the presence of an abnormal population, 77% (n=24) analysed the light chain expression, but only two assessed CD5 expression.

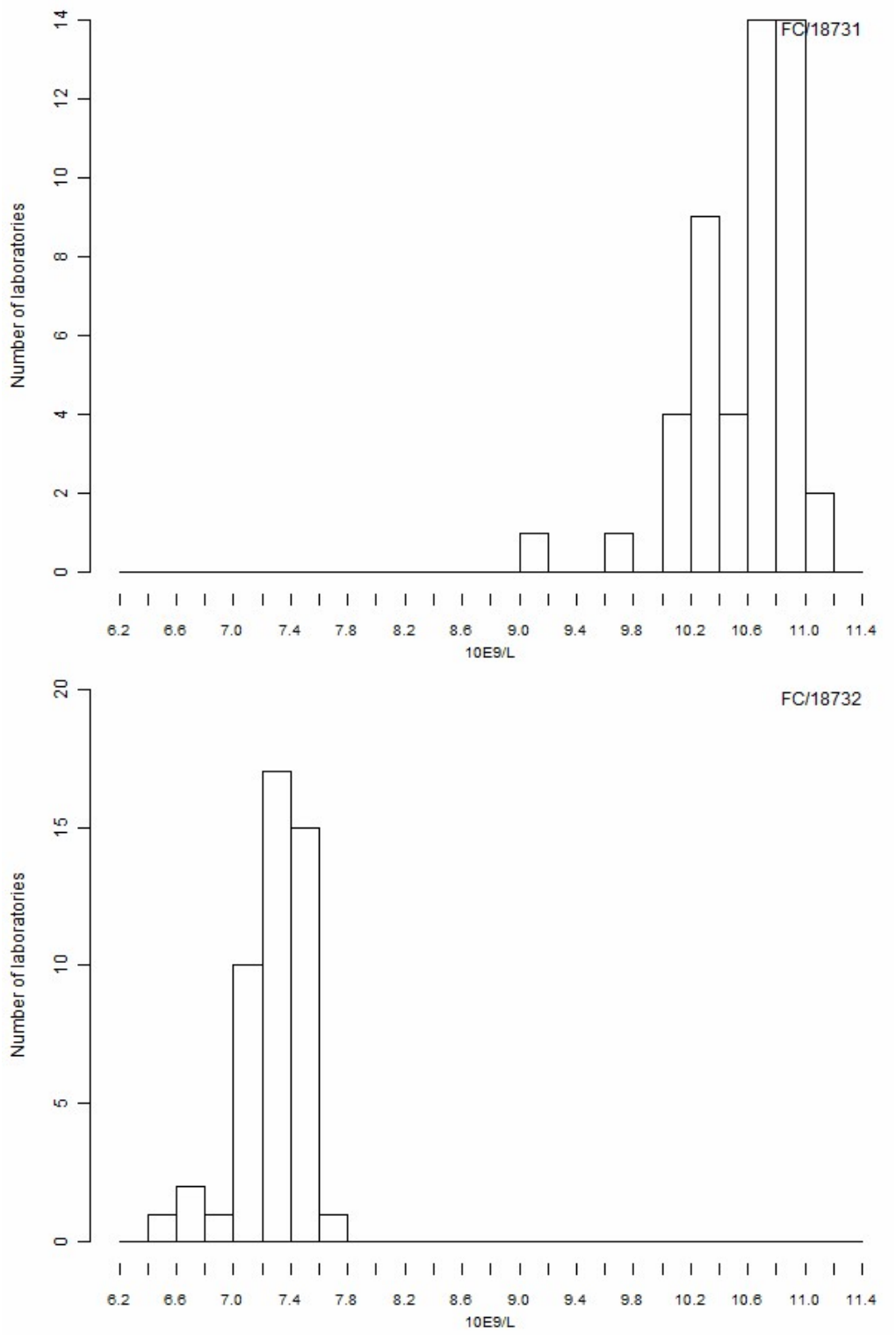
Among the 24 laboratories that analysed the light chain expression, 20 (83%) used polyclonal antibodies.

Only 40% of the laboratories reported the detection of a monoclonal B-cell population. The presence of a population with the phenotype CD19+ CD45 weak should however always be reported to the clinician and additional markers should be proposed, in this case CD5.

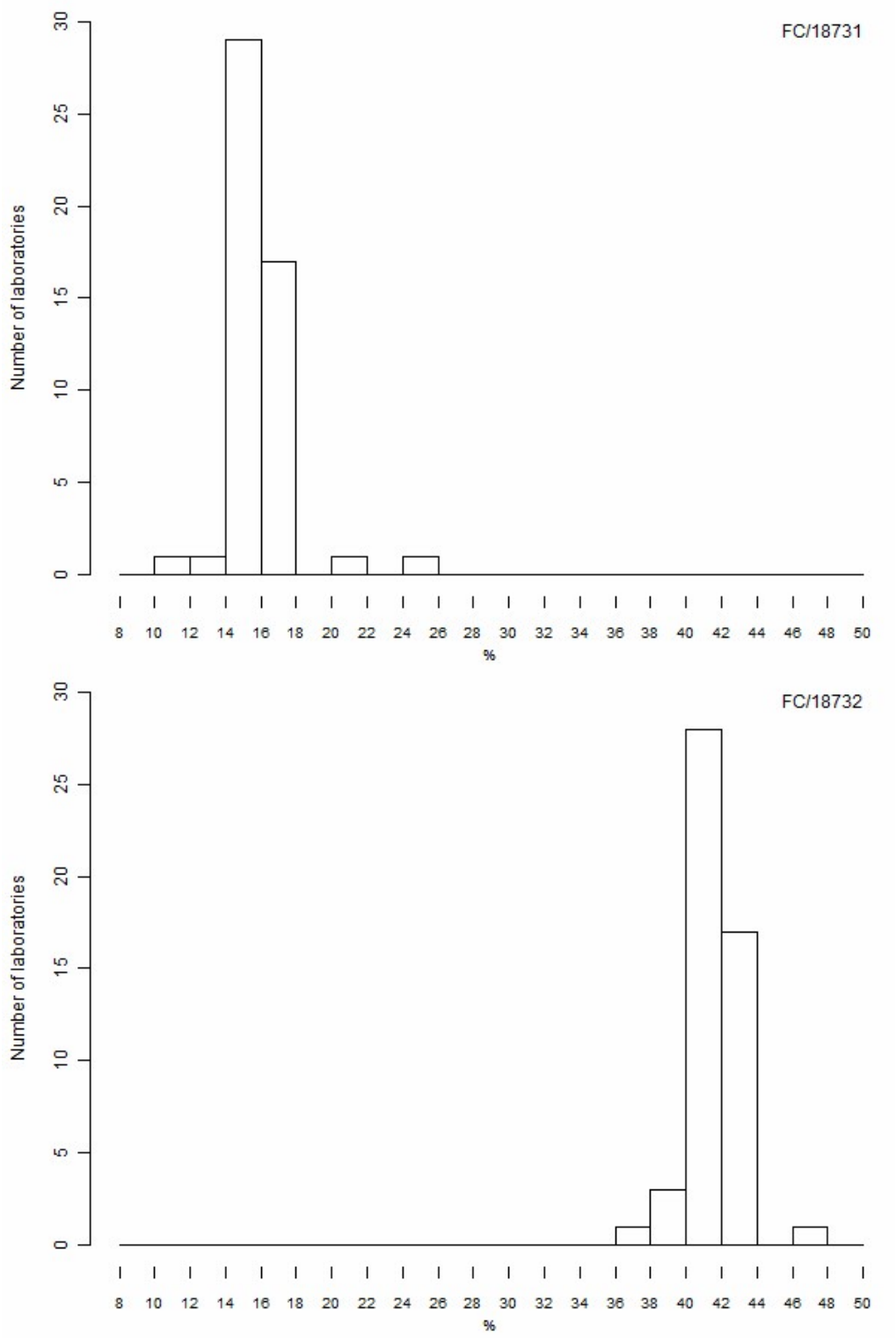
This sample contains a monoclonal B-cell population with the phenotype CD19+ CD5+ CD20 weak CD45 weak. This educational case serves to remind that any abnormal population should be mentioned to the clinician.

Eight laboratories found a kappa/lambda ratio > 1, but the median of the results was 0.41. These laboratories used different methods and kits from different manufacturers. Two of these laboratories explained this result by the fact that this ratio is of the polyclonal B-cells. The monoclonal B-population was found to be double negative (kappa-lambda-) by these two laboratories. Two other participants redid the gating and found a ratio of around 0.5. For two other laboratories, it was an inversion of the results when encoding the results in the Toolkit.

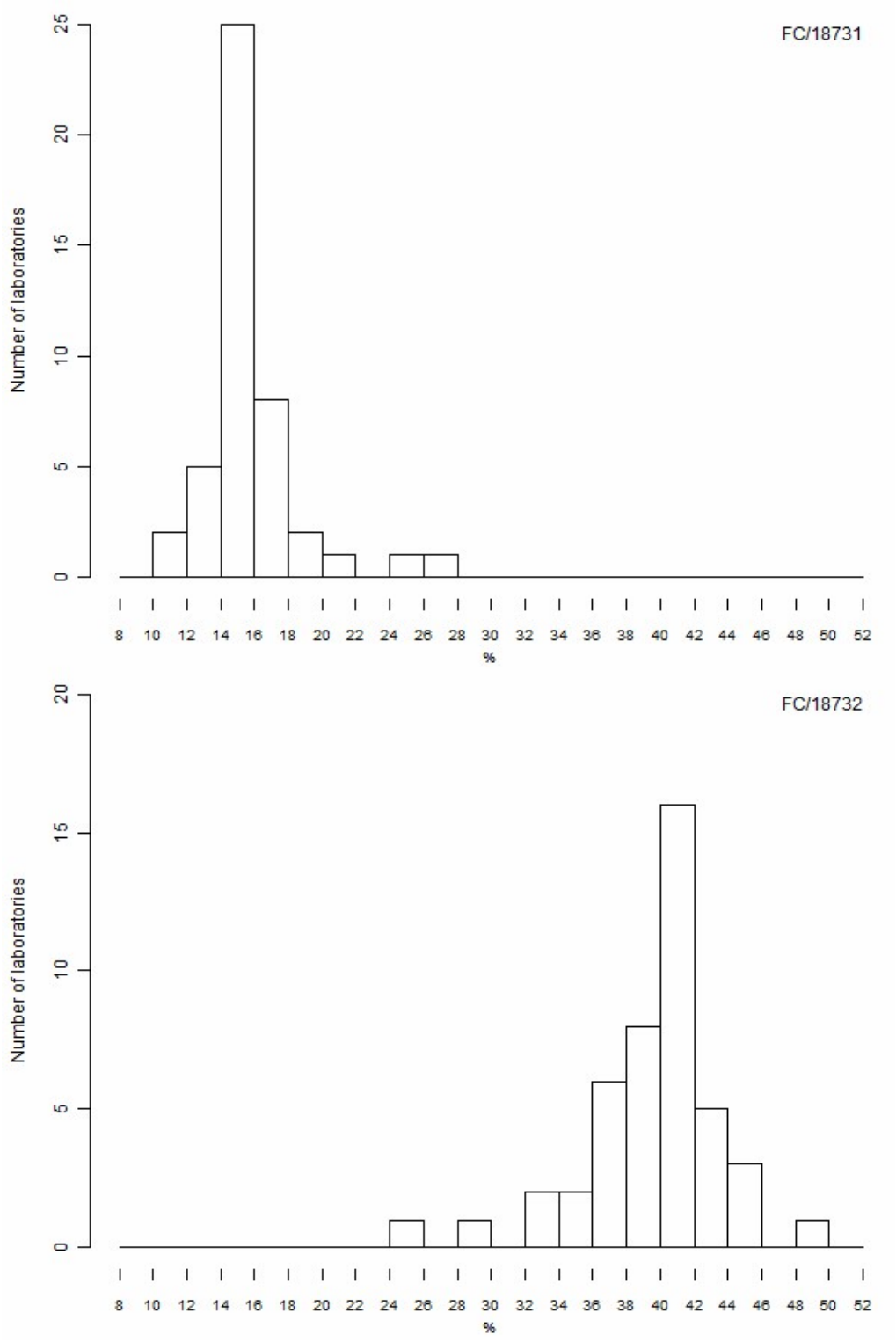
WBC 10E9/L


FC/18731
Number of laboratories
0
2
4
6
8
10
12
14
6.2
6.6
7.0
7.4
7.8
8.2
8.6
9.0
9.4
9.8
10.2
10.6
11.0
11.4
10E9/L
FC/18732
Number of laboratories
0
5
10
15
20
6.2
6.6
7.0
7.4
7.8
8.2
8.6
9.0
9.4
9.8
10.2
10.6
11.0
11.4
10E9/L

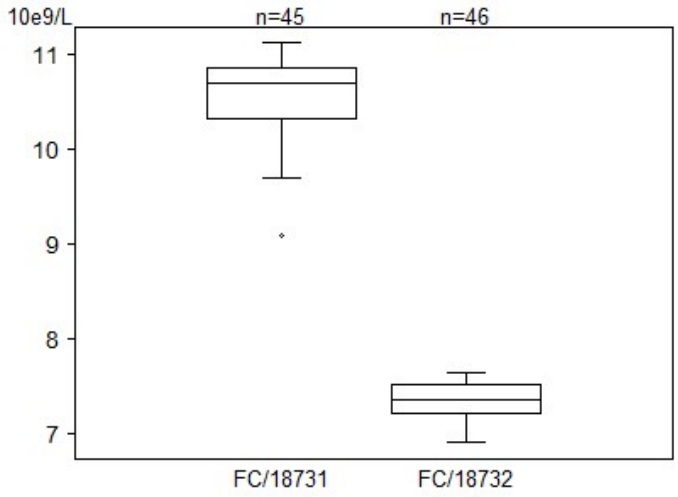
Lympho% haematology analyser



Lympho% flow cytometer



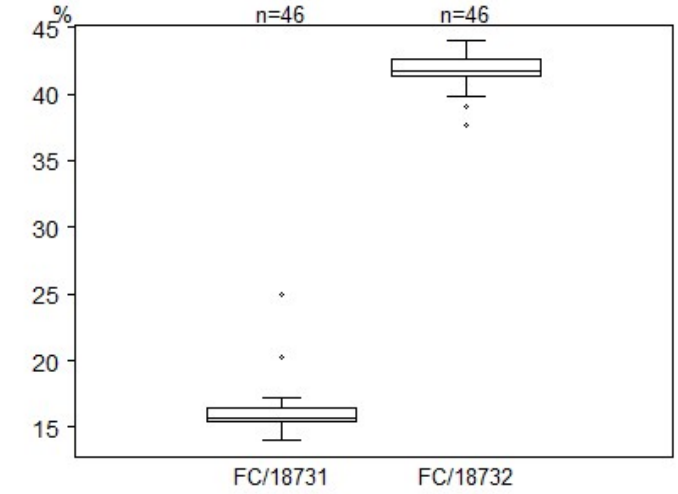
WBC 10E9/L



Results not represented on the graph

FC/18731 = 1.04 10e9/l
FC/18732 = 6.5 10e9/L
FC/18732 = 6.62 10e9/l
FC/18732 = 6.65 10e9/l
FC/18732 = 70 10e9/L
FC/18732 = 70.56 10e9
FC/18732 = 73 10e9/L
FC/18732 = 73.1 10e9/l

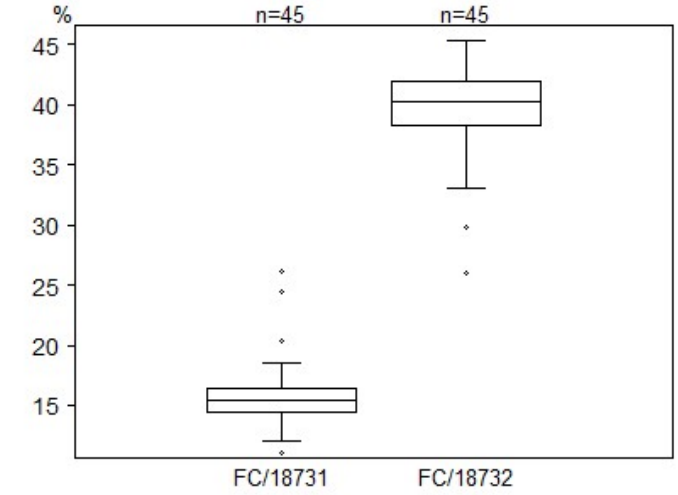
Lympho% haematology analyser



Results not represented on the graph

FC/18731 = 11.4 %
FC/18732 = 46.3 %

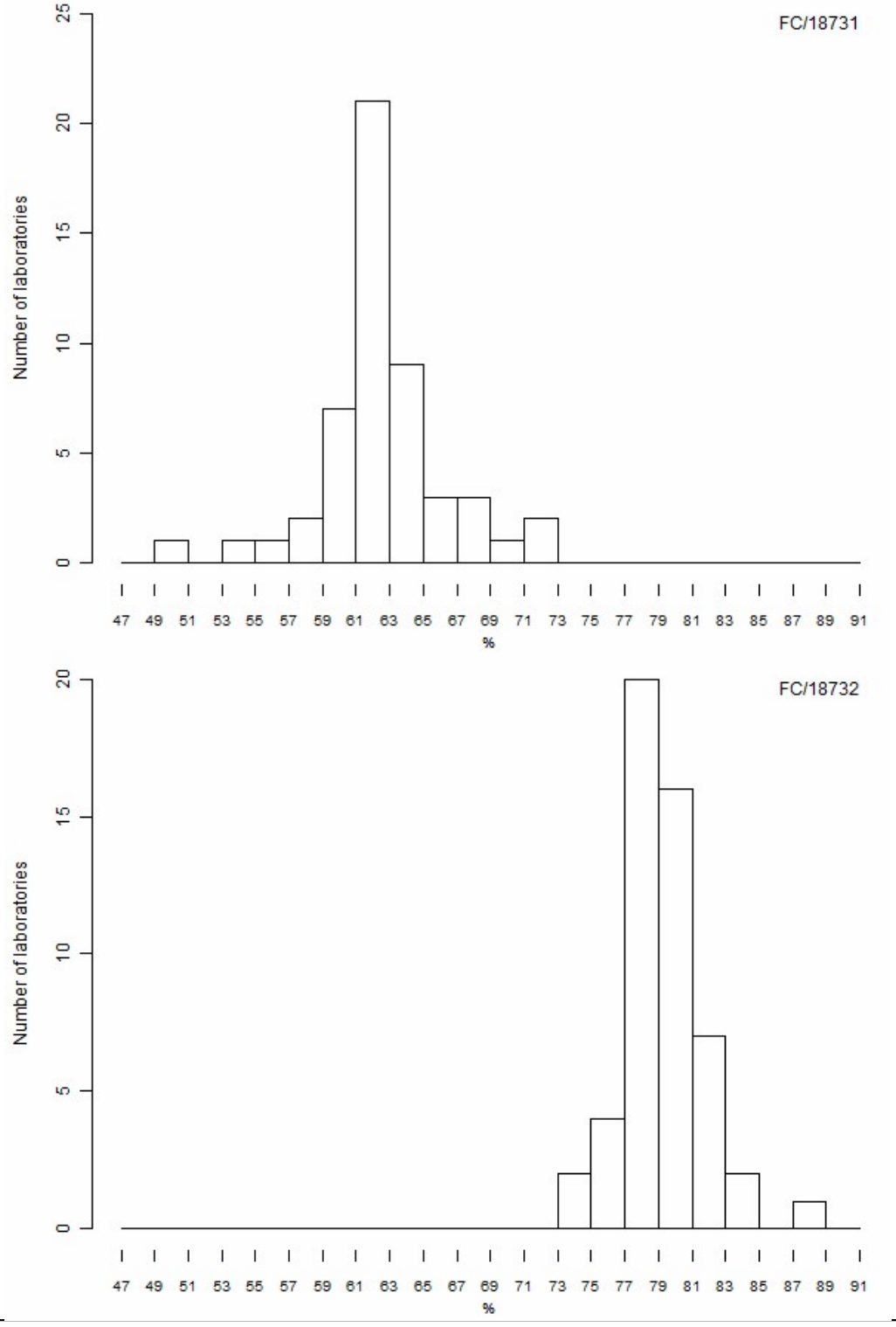
Lympho% flow cytometer



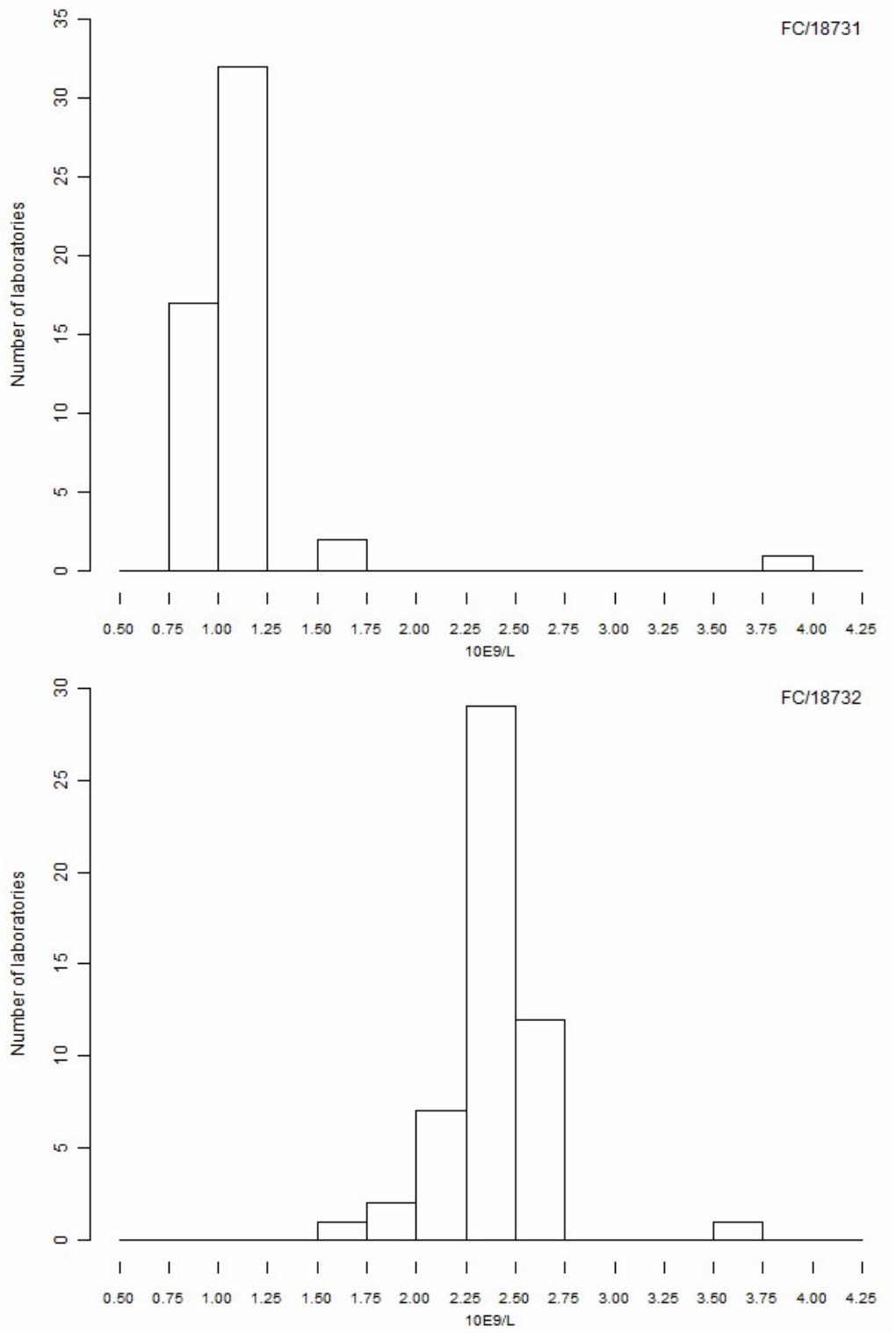
Results not represented on the graph

FC/18732 = 48.5 %

CD3 %


FC/18731
Number of laboratories
0
5
10
15
20
25
47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91
%
FC/18732
Number of laboratories
0
5
10
15
20
47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91
%

CD3 10E9/L


FC/18731
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10E9/L
FC/18732
Number of laboratories
0
5
10
15
20
25
30
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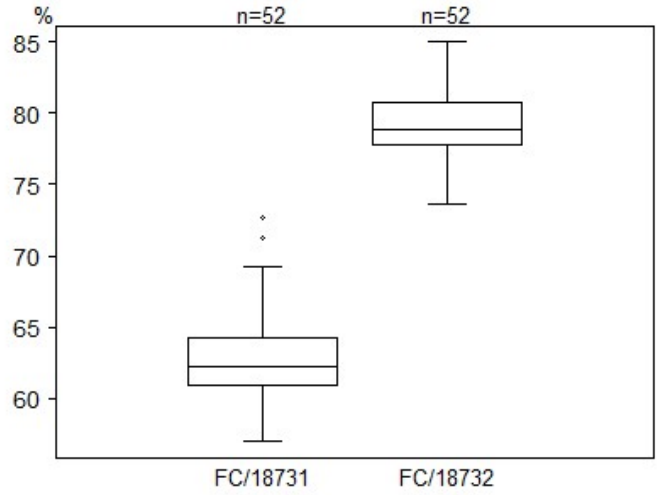
10E9/L

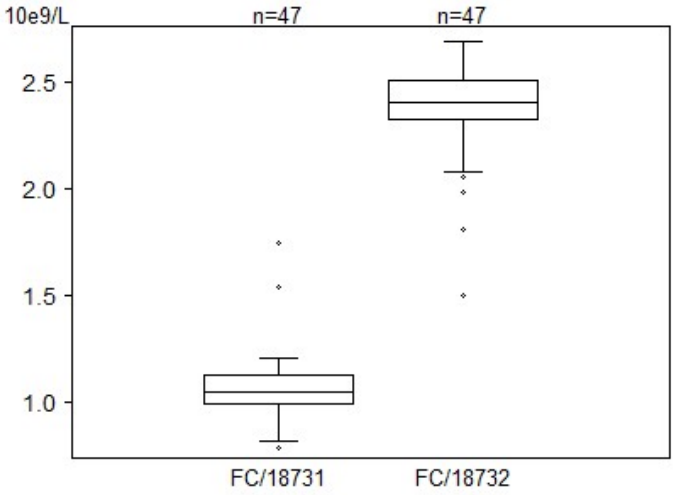
CD3 %



Results not represented on the graph

FC/18731 = 0.6 %
FC/18731 = 49.7 %
FC/18731 = 54.8 %
FC/18732 = 87.2 %

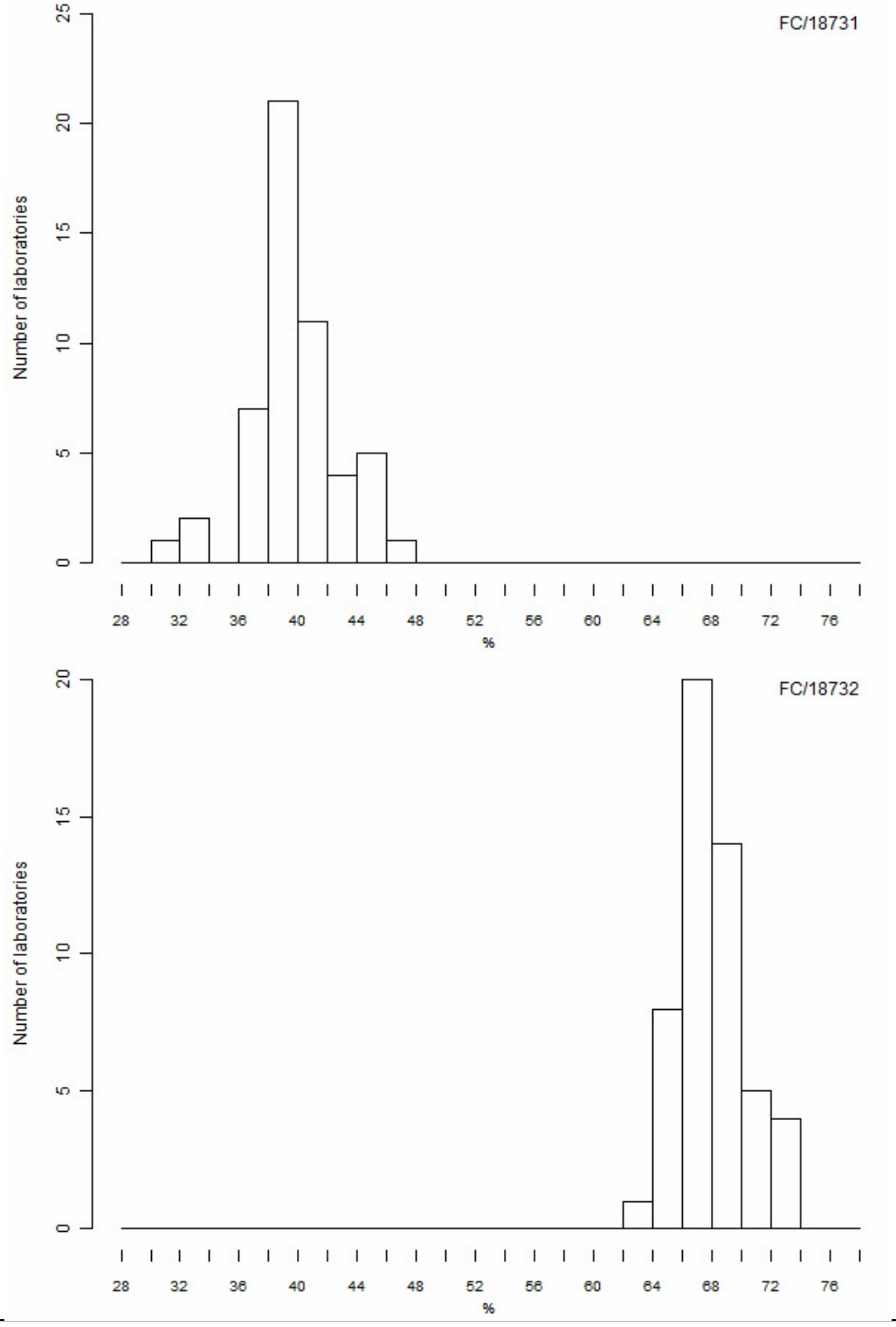
CD3 10E9/L



Results not represented on the graph

FC/18731 = 3.945 10e9
FC/18732 = 3.746 10e9

CD4 %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%
FC/18732
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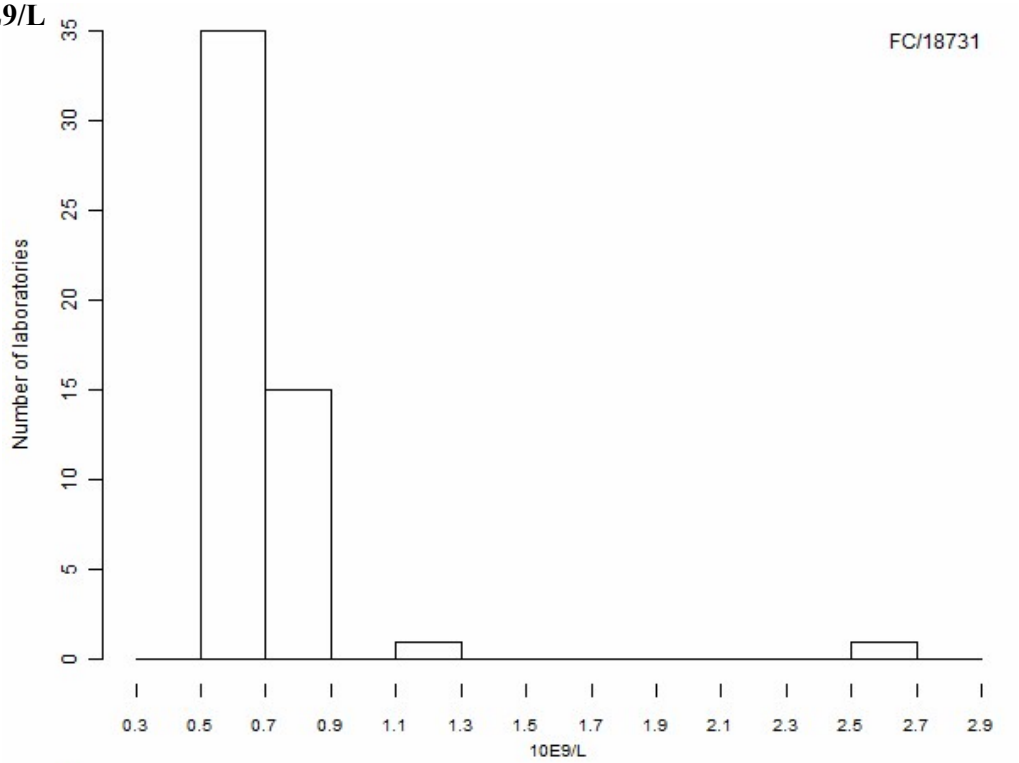
64
68
72
76
%

CD4 10E9/L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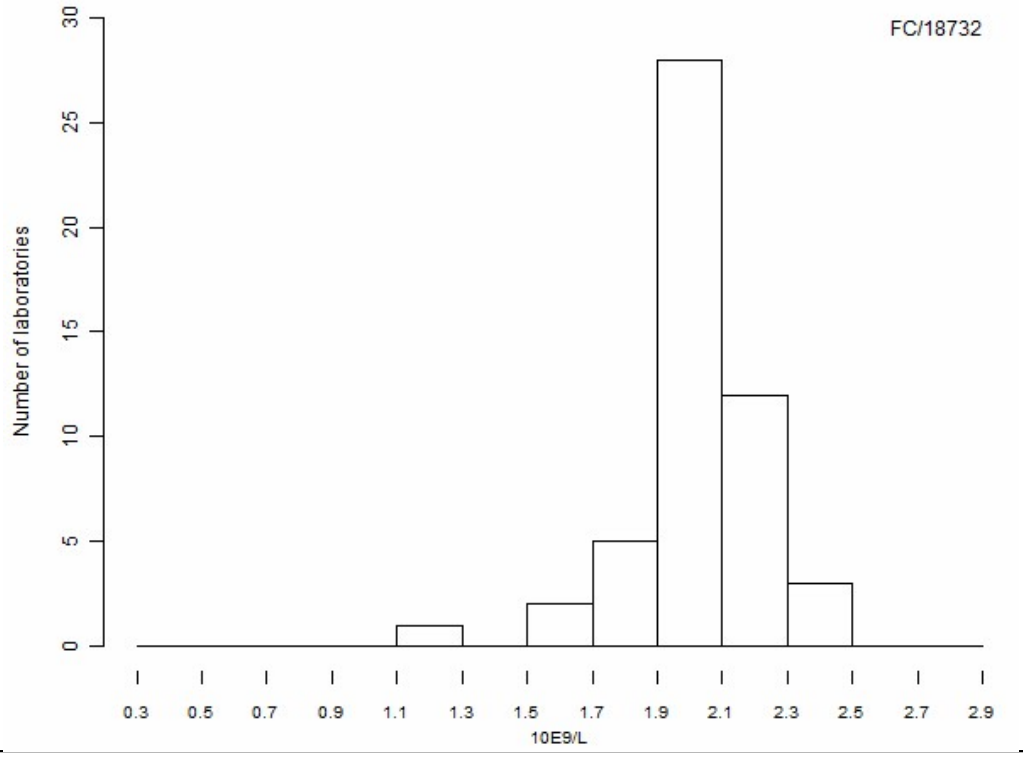
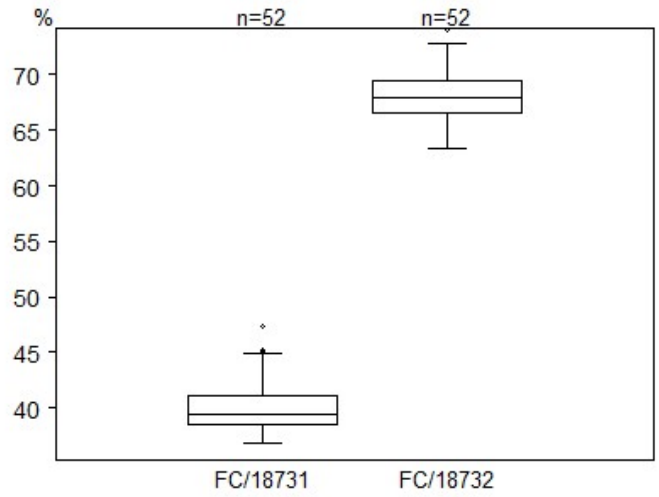
2.9
10E9/L


FC/18732
Number of laboratories
0
5
10
15
20
25
30
0.3
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
10E9/L

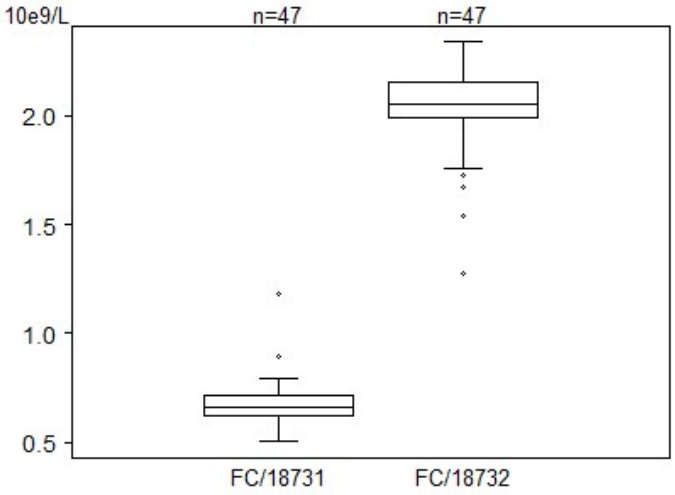
CD4 %



Results not represented on the graph

FC/18731 = 30.3 %
FC/18731 = 33 %
FC/18731 = 33.9 %
FC/18732 = 74 %
FC/18732 = 74 %

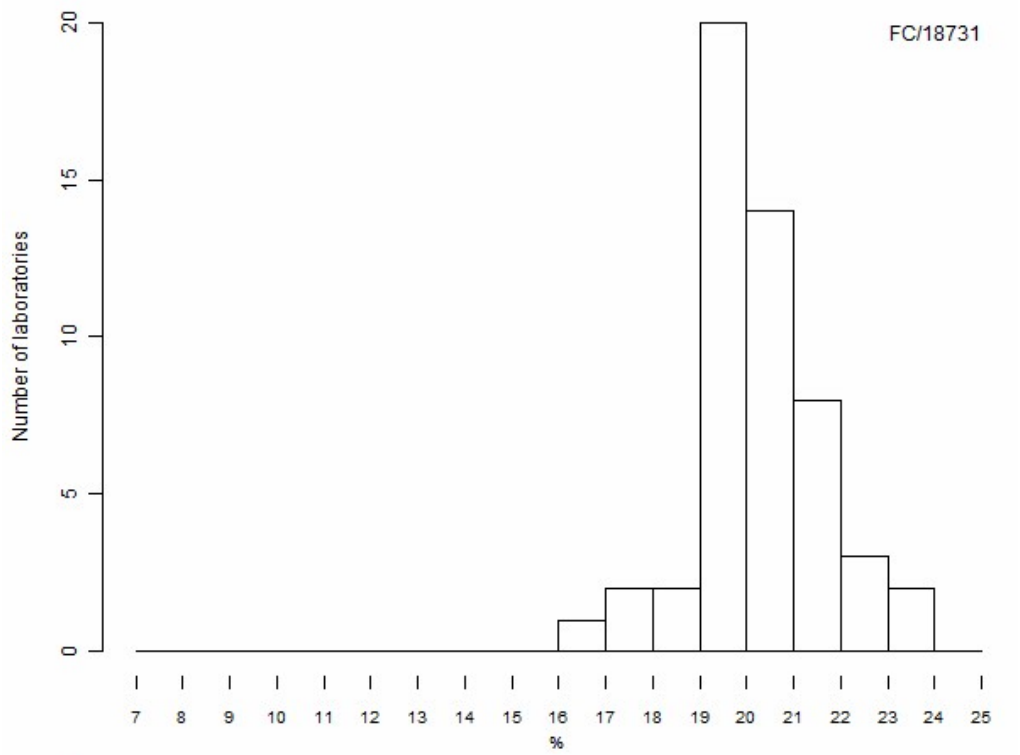
CD4 10E9/L

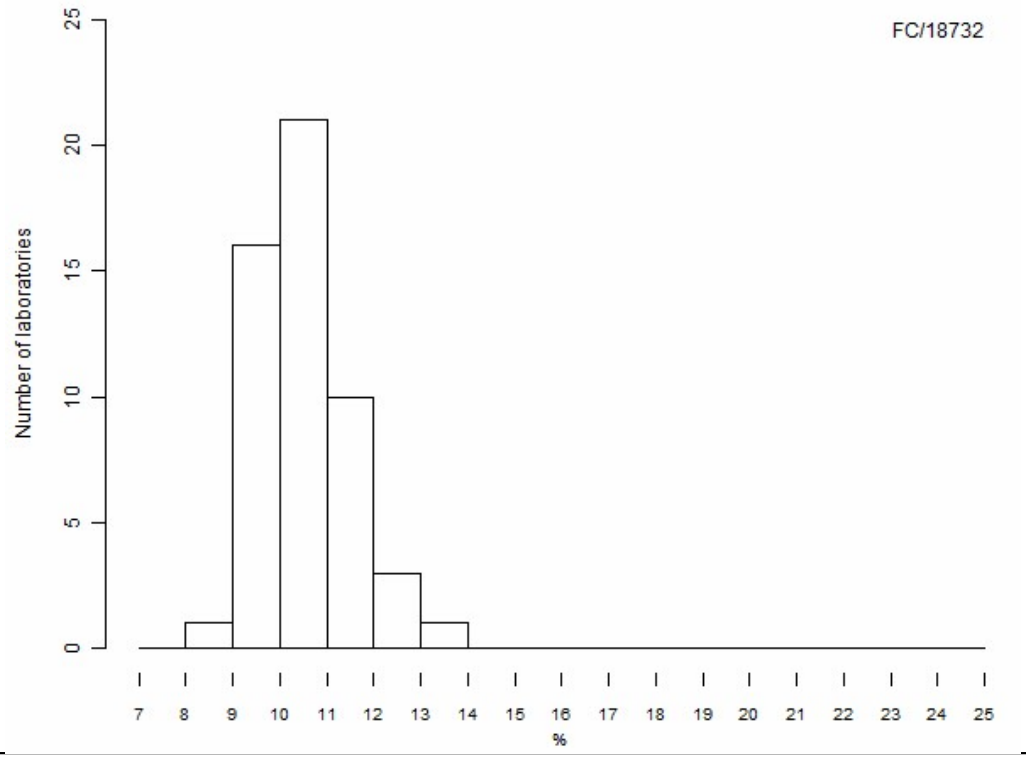


Results not represented on the graph

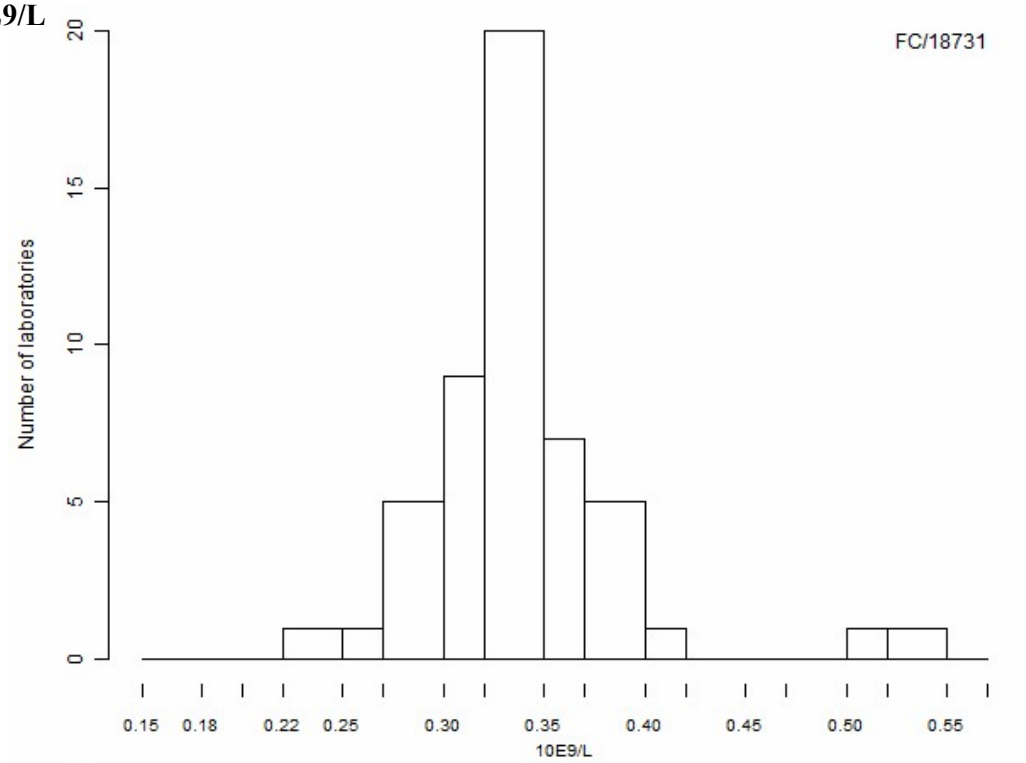
FC/18731 = 2.571 10e9
FC/18732 = 3.265 10e9

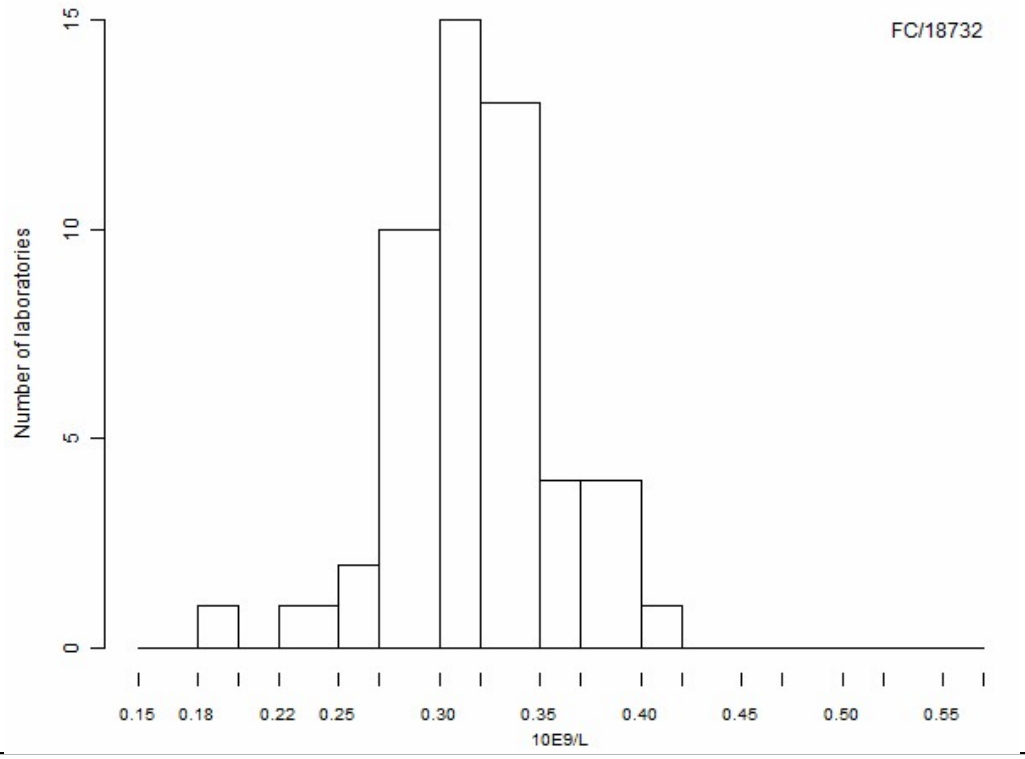
CD8 %


FC/18731
Number of laboratories
0
5
10
15
20
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
%

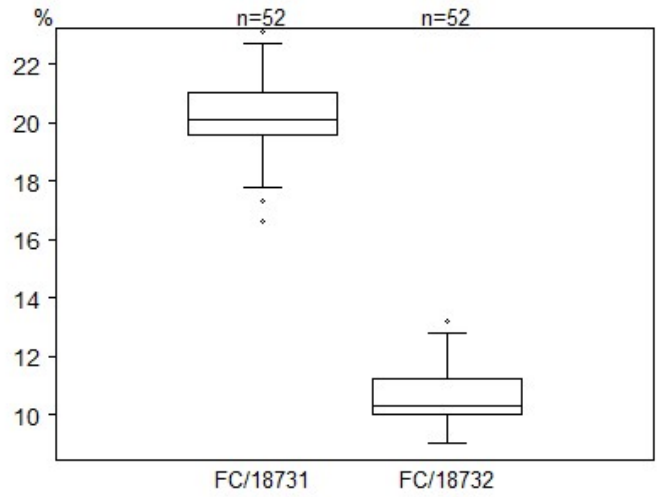

FC/18732
Number of laboratories
0
5
10
15
20
25
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
%

CD8 10E9/L

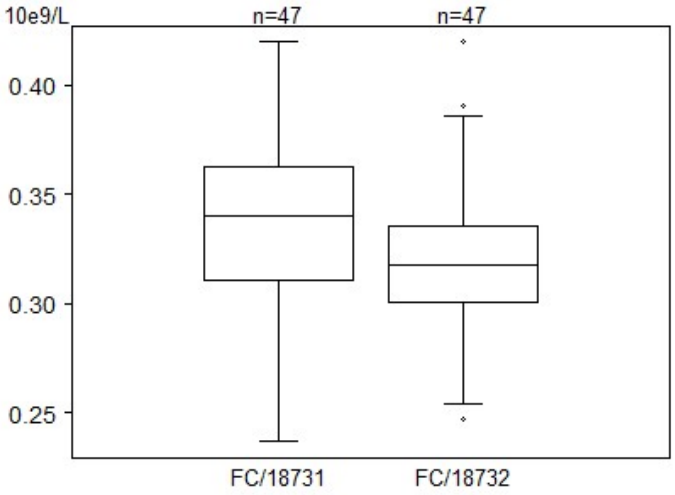

FC/18731
Number of laboratories
0
5
10
15
20
0.15
0.18
0.22
0.25
0.30
0.35
0.40
0.45
0.50
0.55
10E9/L


FC/18732
Number of laboratories
0
5
10
15
0.15
0.18
0.22
0.25
0.30
0.35
0.40
0.45
0.50
0.55
10E9/L

CD8 %



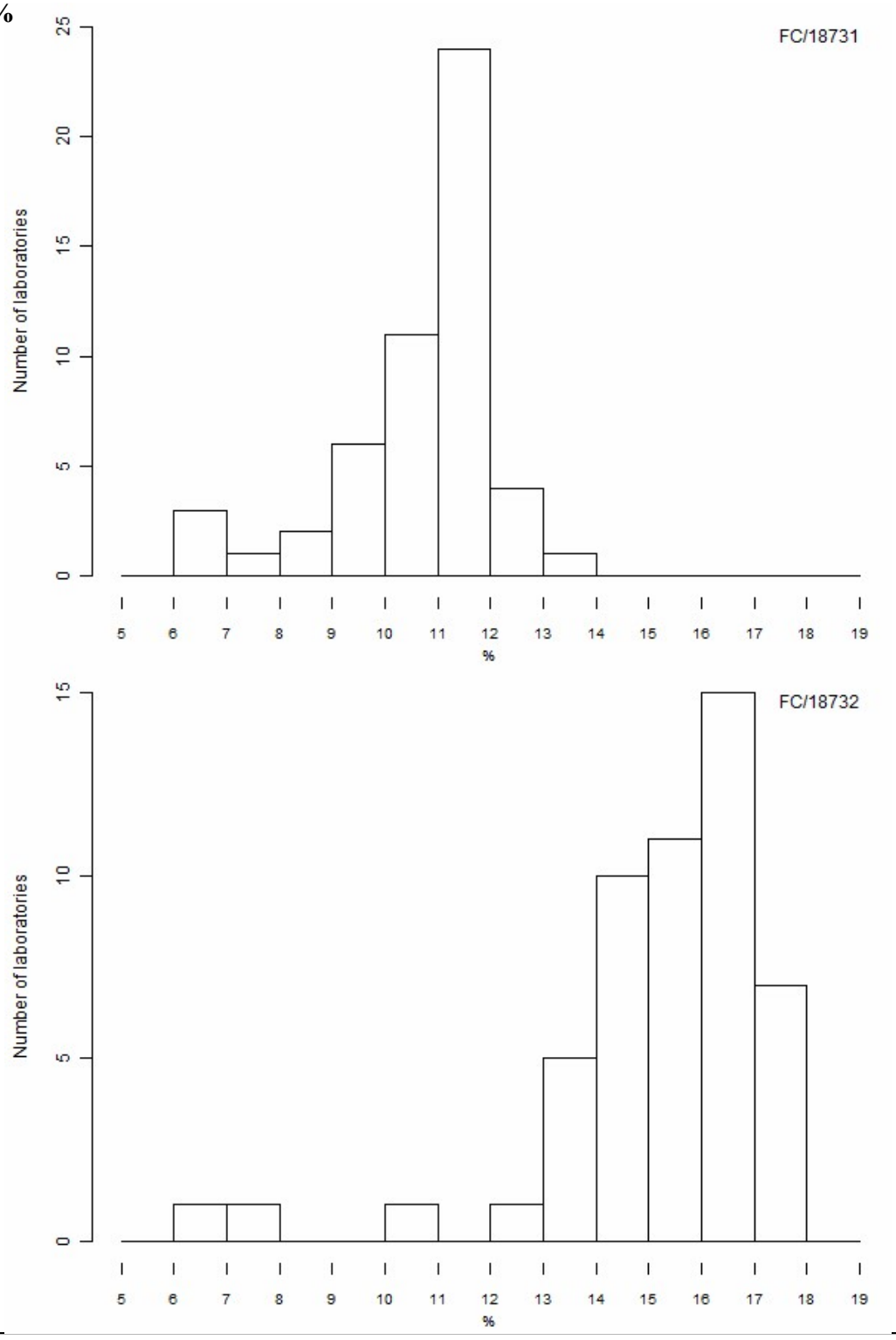
CD8 10E9/L



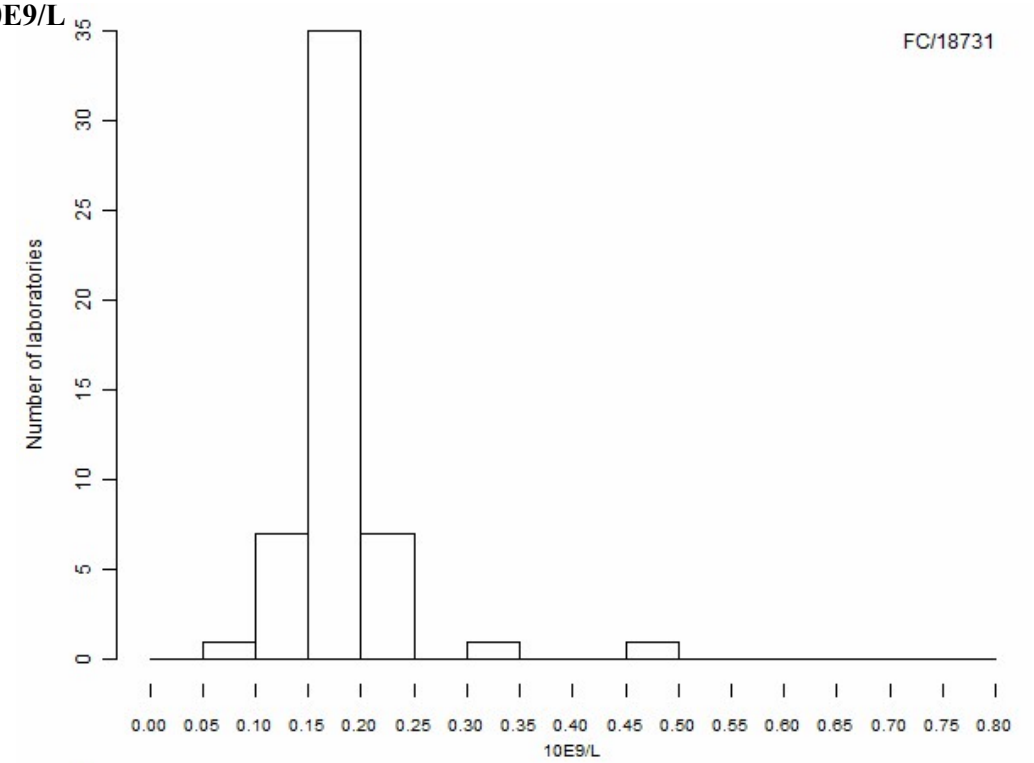
Results not represented on the graph

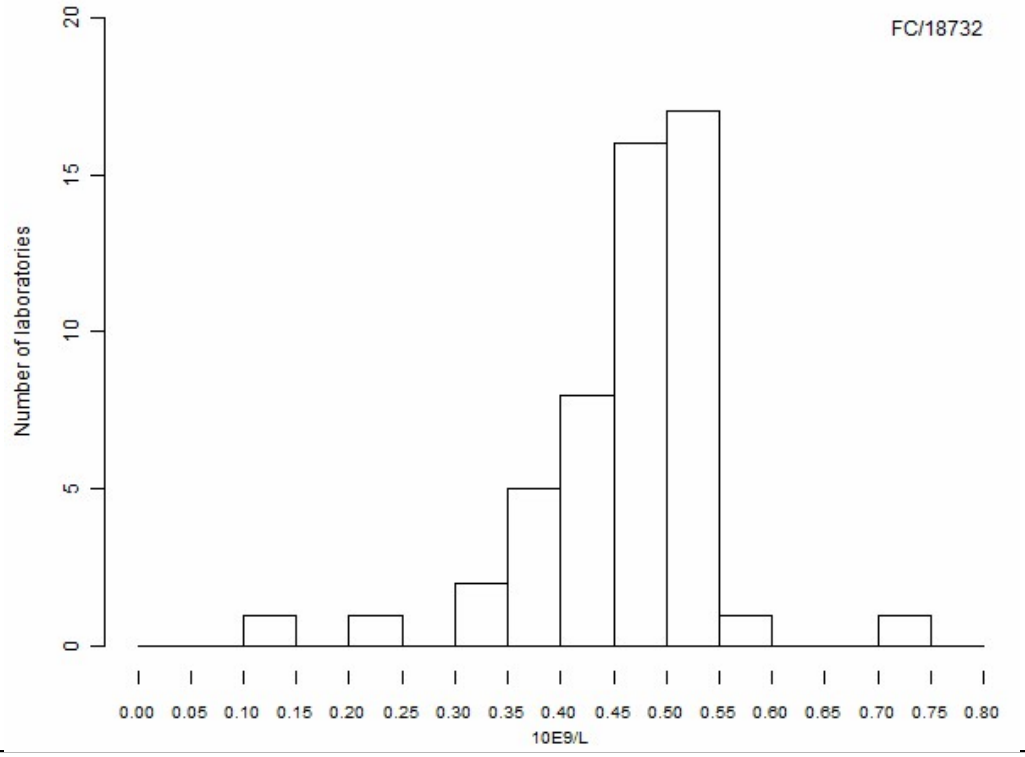
FC/18731 = 0.52 10e9/l
FC/18731 = 0.54 10e9/l
FC/18731 = 1.229 10e9
FC/18732 = 0.184 10e9
FC/18732 = 3.28 10e9/l

CD19 %

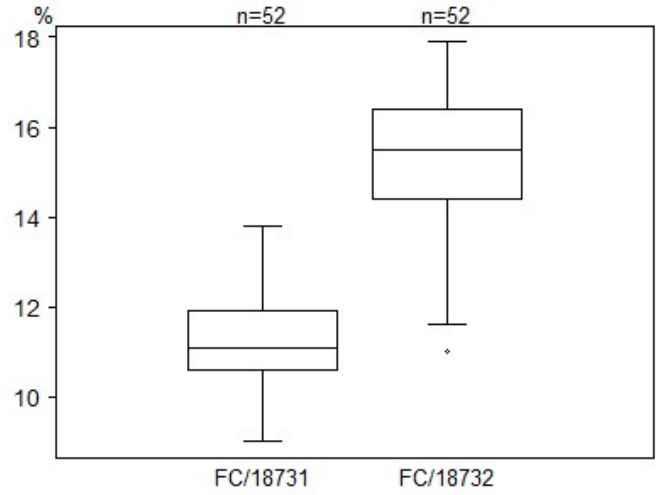

FC/18731
Number of laboratories
0
5
10
15
20
25
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
%
FC/18732
Number of laboratories
0
5
10
15
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
%

CD19 10E9/L


FC/18731
Number of laboratories
0
5
10
15
20
25
30
35
0.00 0.05 0.10 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
10E9/L


FC/18732
Number of laboratories
0
5
10
15
20
0.00 0.05 0.10 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
10E9/L

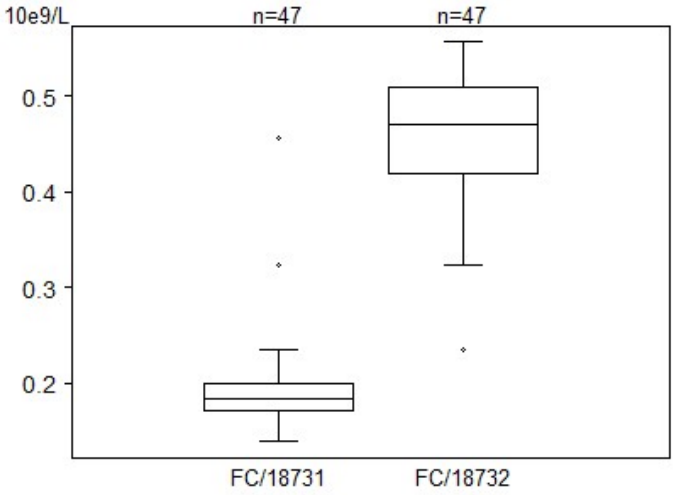
CD19 %



Results not represented on the graph

FC/18731 = 6.3 %
FC/18731 = 6.5 %
FC/18731 = 6.9 %
FC/18731 = 7.9 %
FC/18731 = 8.4 %
FC/18732 = 6.7 %
FC/18732 = 7.4 %

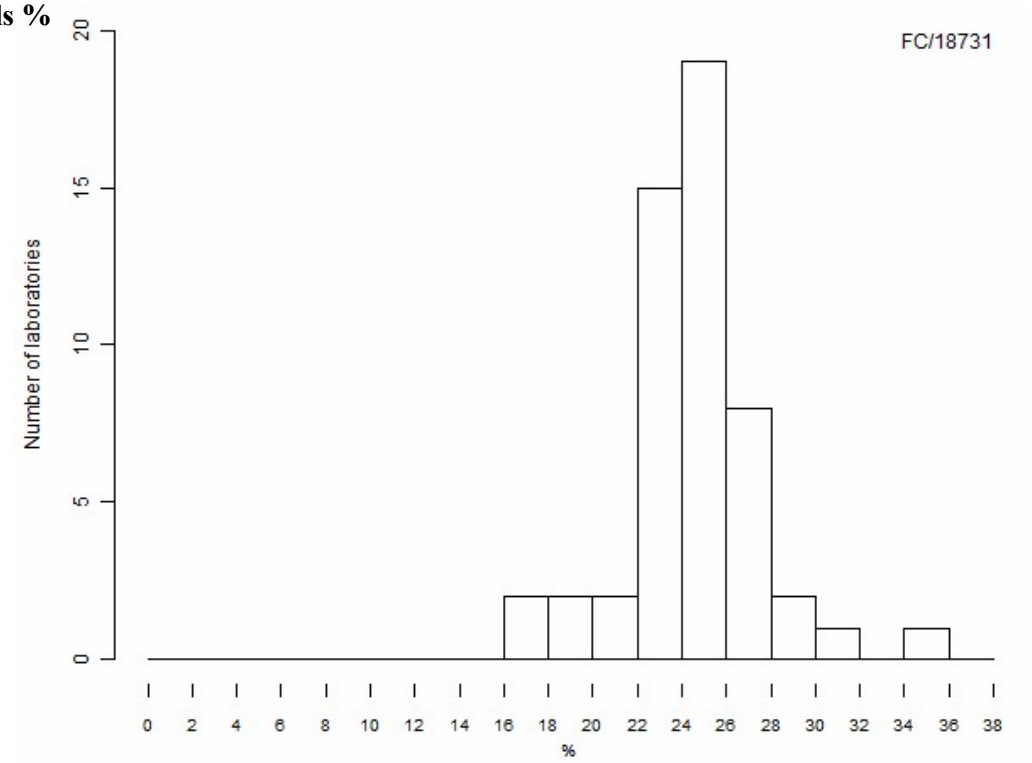
CD19 10E9/L

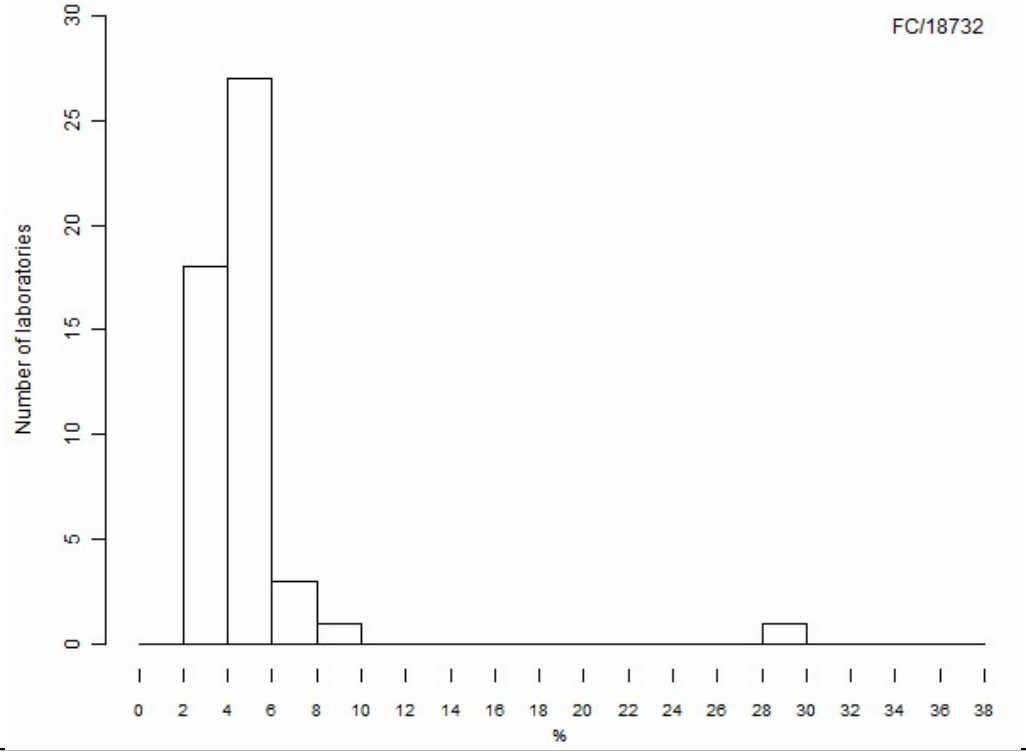


Results not represented on the graph

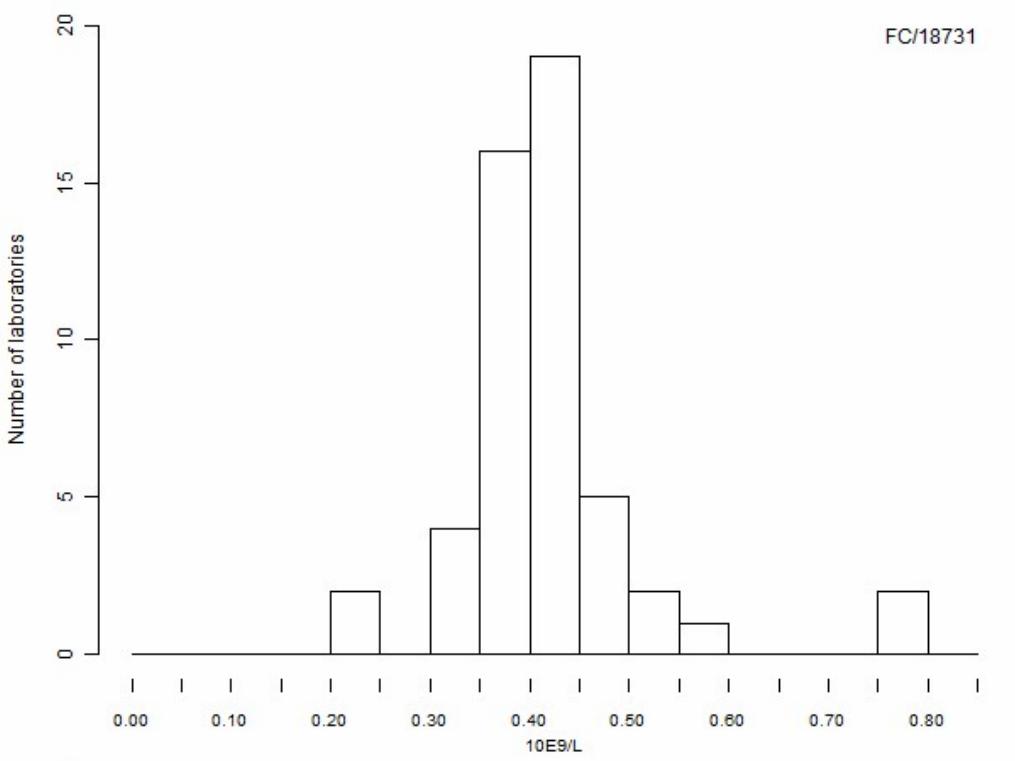
FC/18731 = 0.08 10e9/l
FC/18732 = 0.115 10e9
FC/18732 = 0.719 10e9

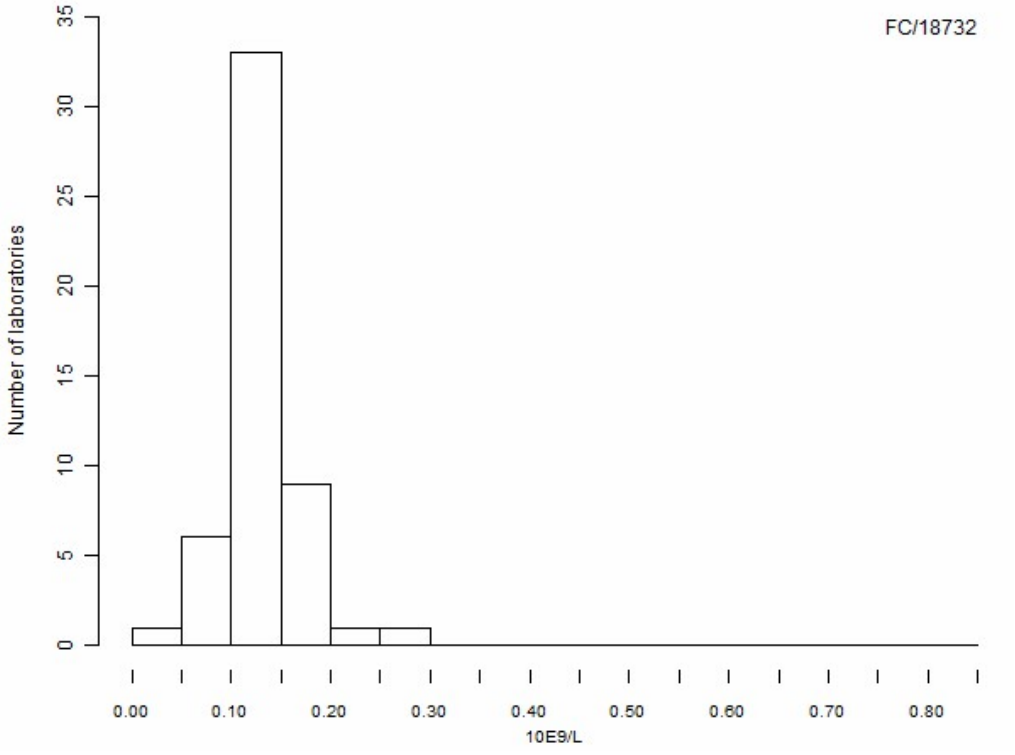
NKcells %


FC/18731
Number of laboratories
0
5
10
15
20
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
%

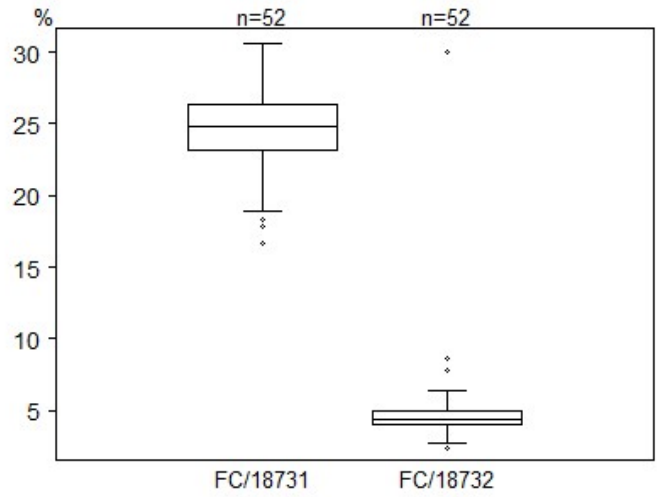

FC/18732
Number of laboratories
0
5
10
15
20
25
30
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
%

NKcells 10E9/L





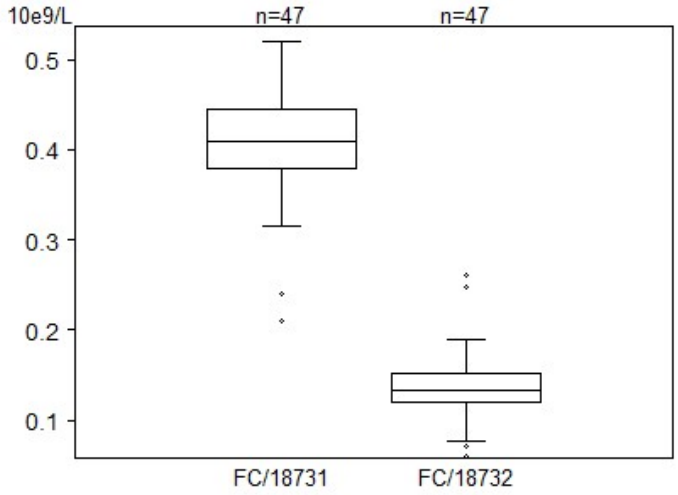
NKcells %



Results not represented on the graph

FC/18731 = 34.2 %
FC/18732 = 43 %
FC/18732 = 52 %

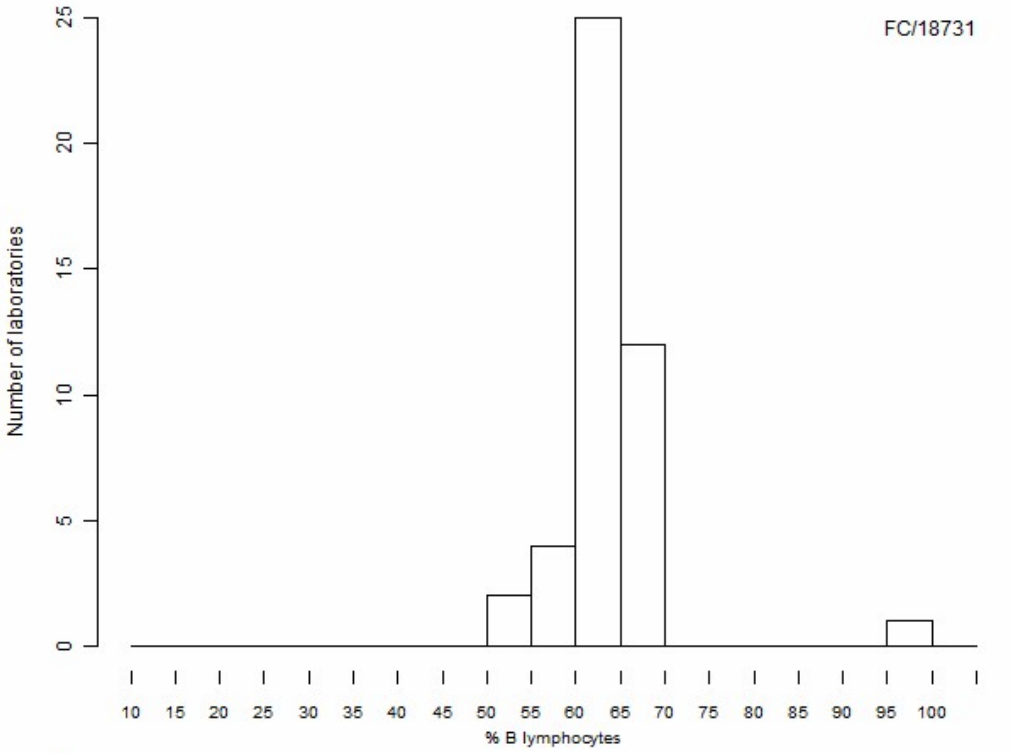
NKcells 10E9/L

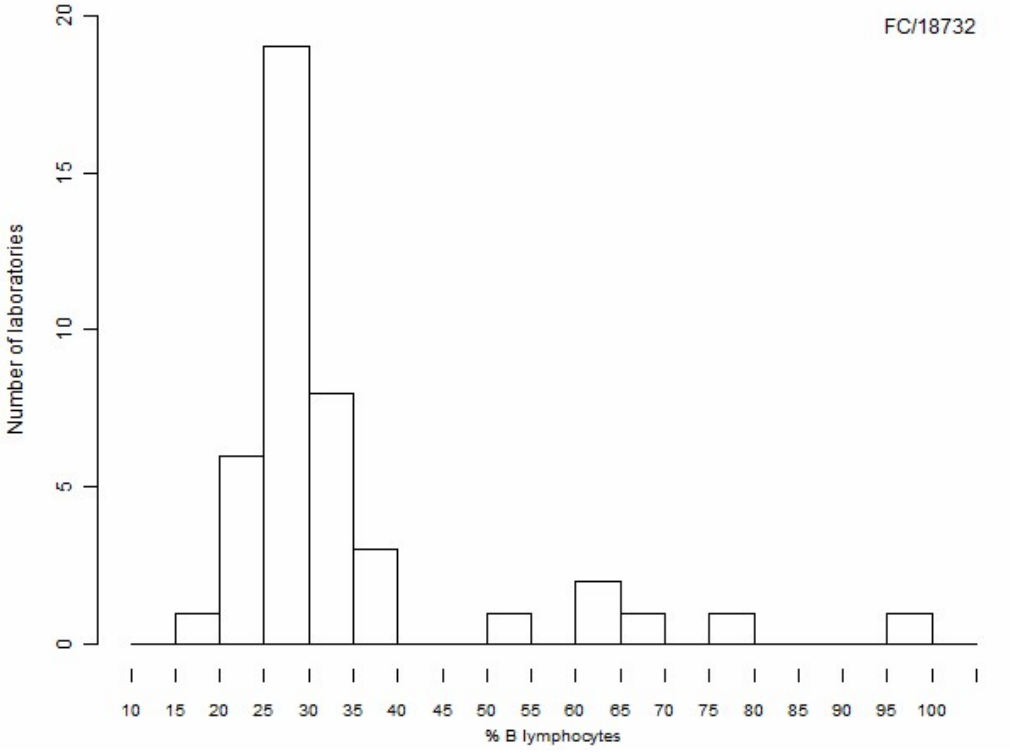


Results not represented on the graph

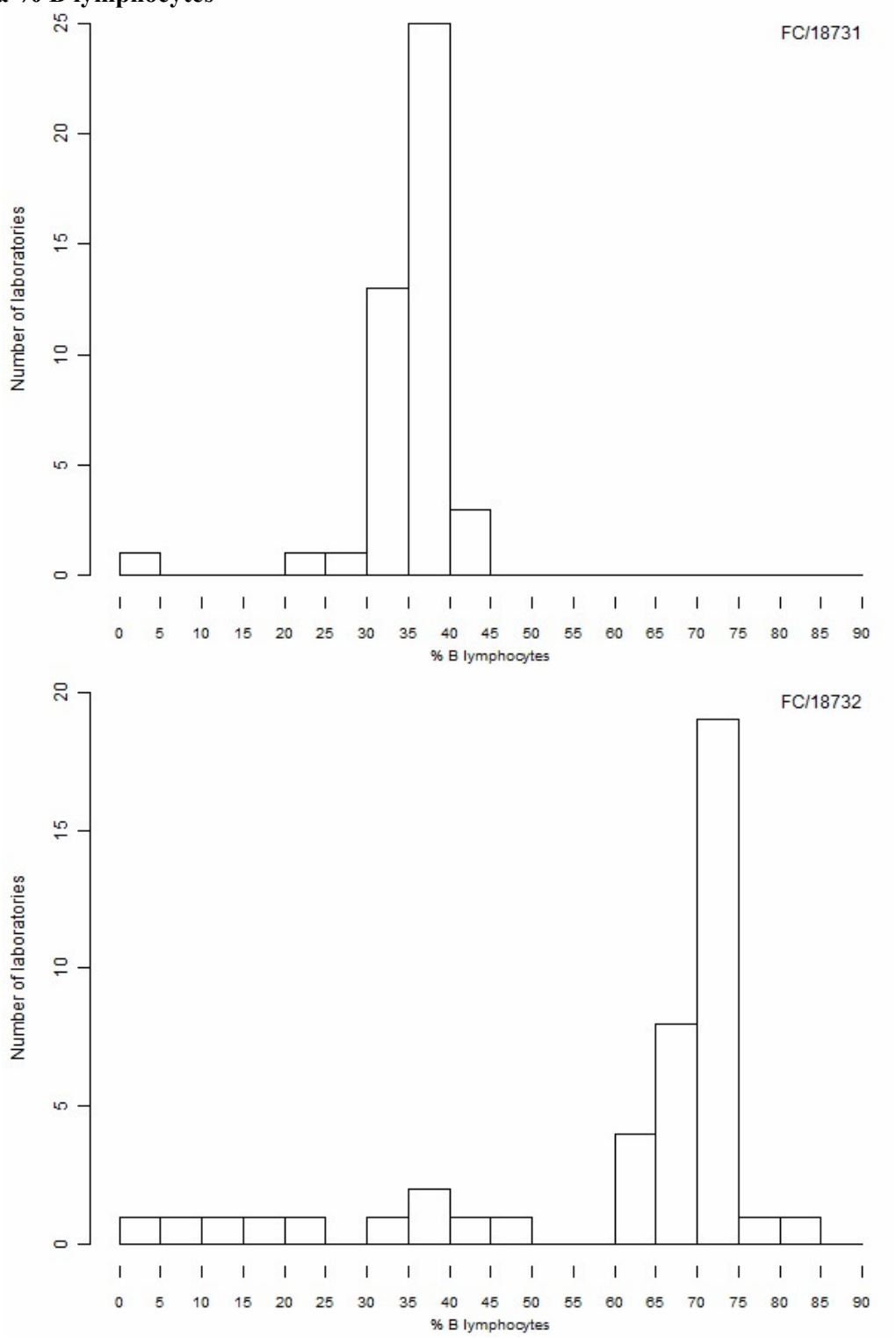
FC/18731 = 0.58 10e9/l
FC/18731 = 0.756 10e9
FC/18731 = 0.76 10e9/l
FC/18731 = 0.906 10e9
FC/18732 = 0.05 10e9/l
FC/18732 = 0.92 10e9/l

Kappa % B lymphocytes

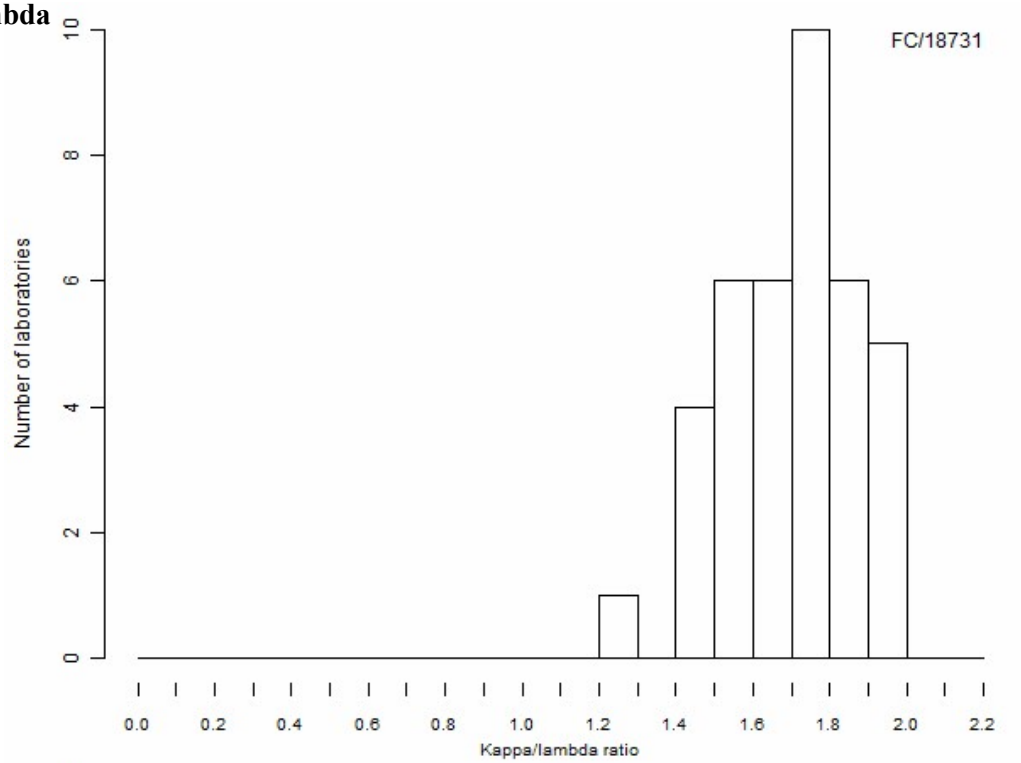


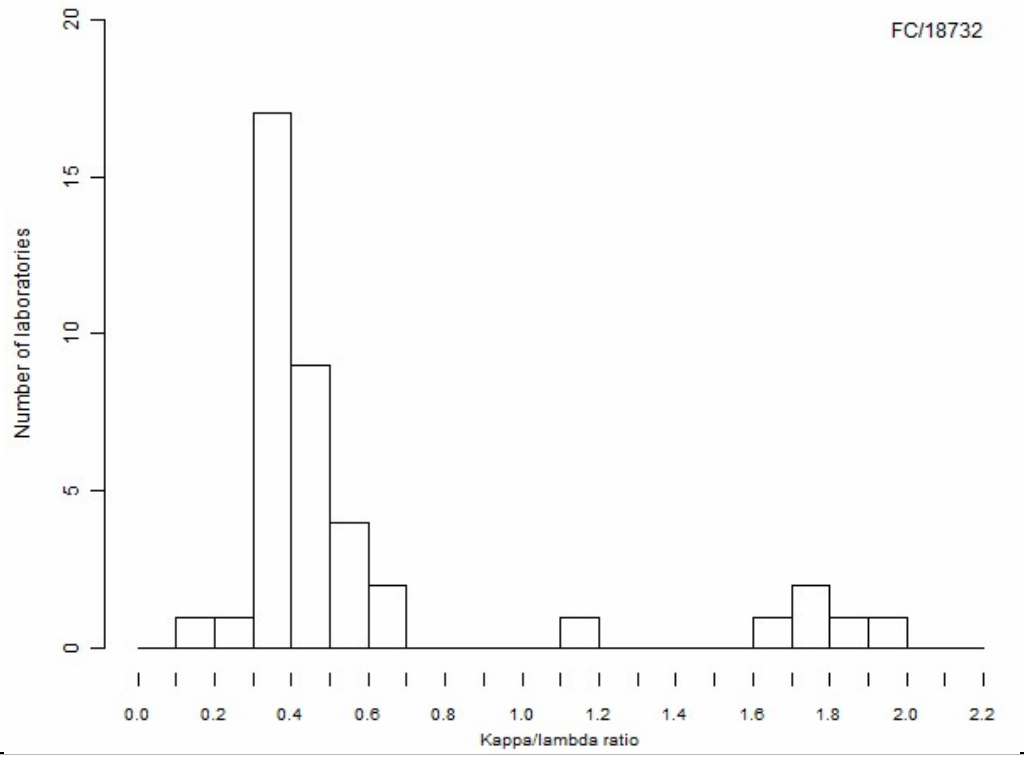


Lambda % B lymphocytes

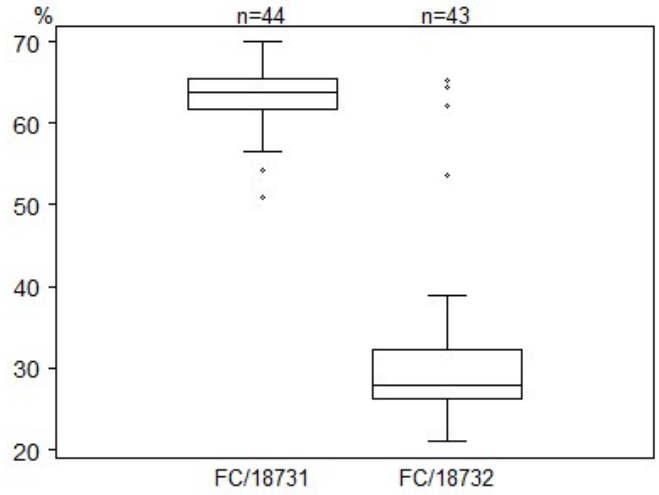


Kappa/lambda





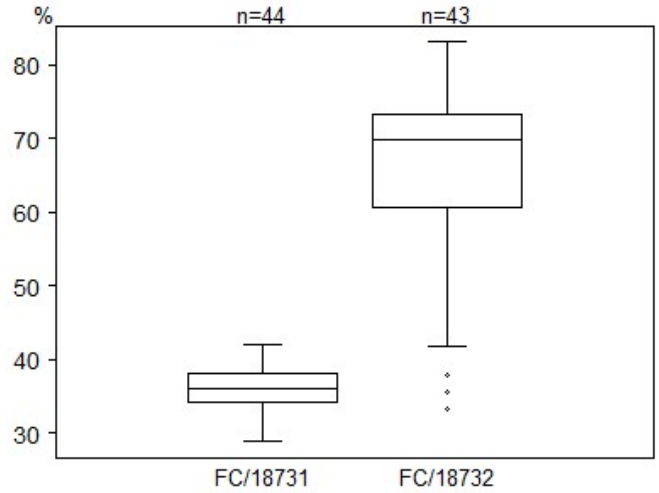
Kappa % B lymphocytes



Results not represented on the graph

FC/18731 = 95.1 %
FC/18732 = 15.6 %
FC/18732 = 75.9 %
FC/18732 = 99.8 %

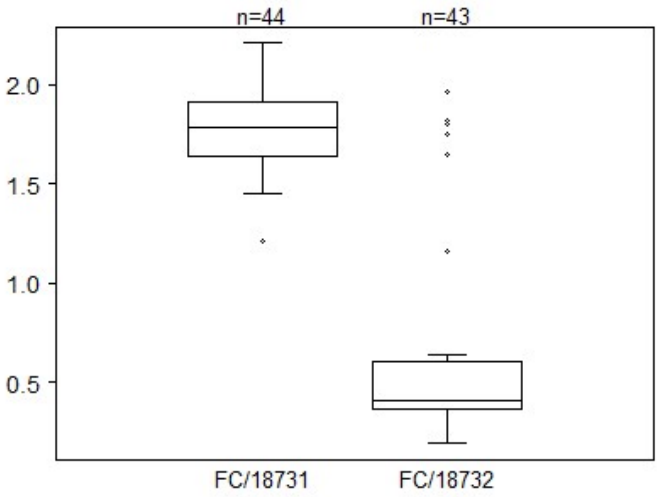
Lambda % B lymphocytes



Results not represented on the graph

FC/18731 = 3.2 %
FC/18731 = 22 %
FC/18732 = 0.2 %
FC/18732 = 8.9 %
FC/18732 = 12 %
FC/18732 = 15.6 %
FC/18732 = 23.8 %

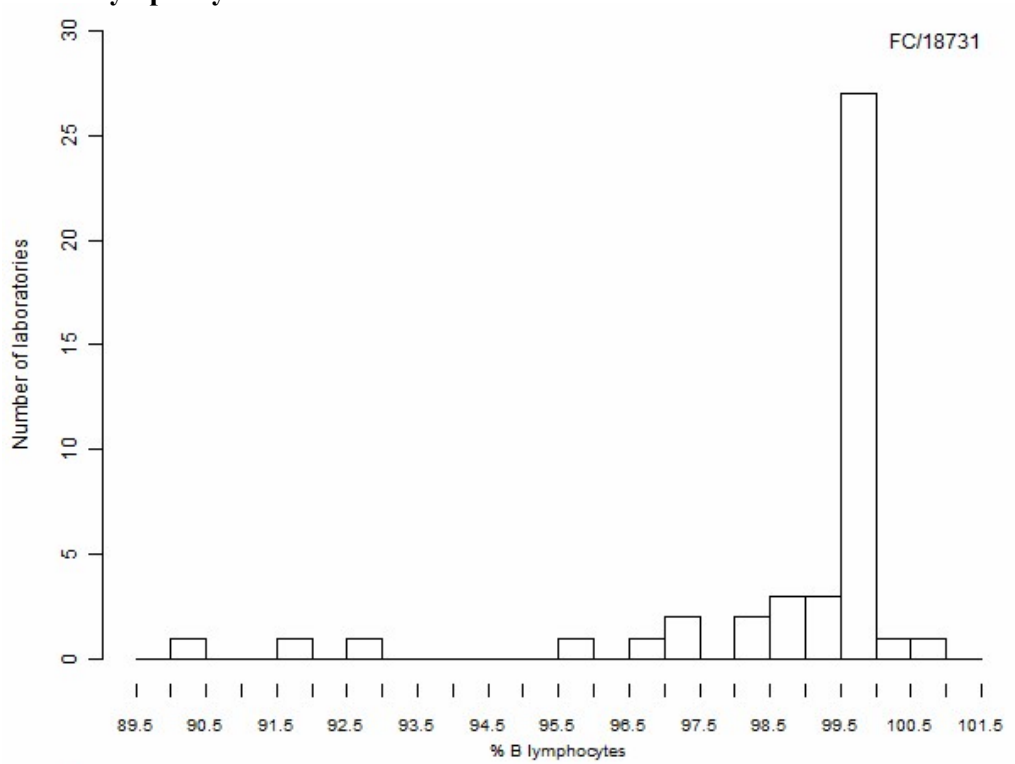
Kappa/lambda

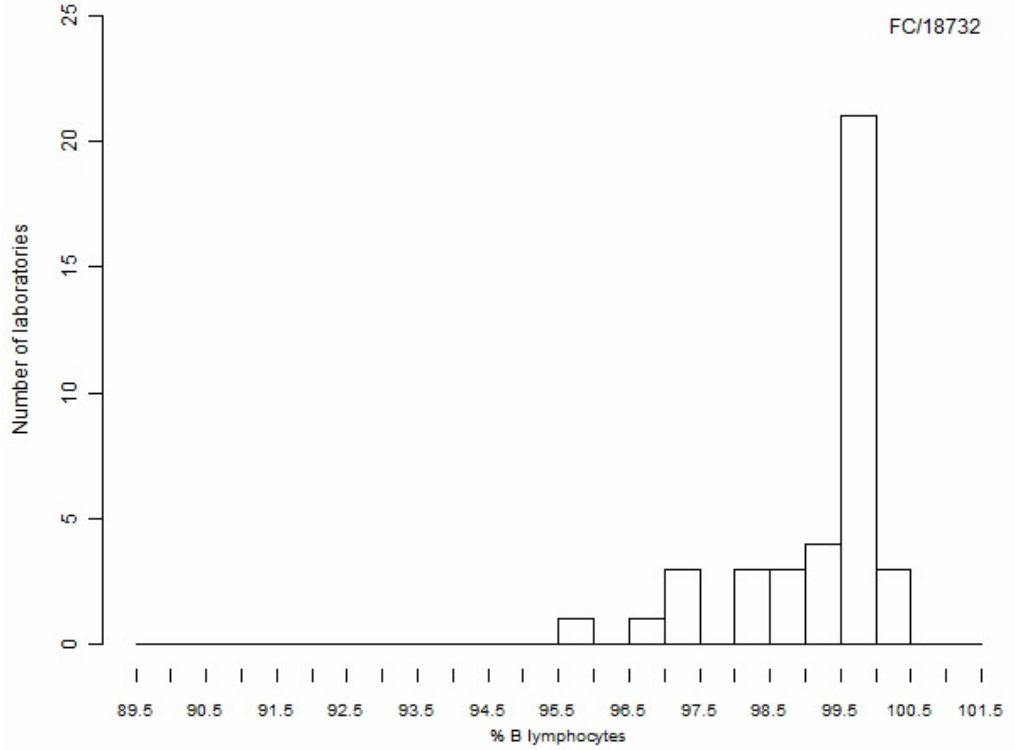


Results not represented on the graph

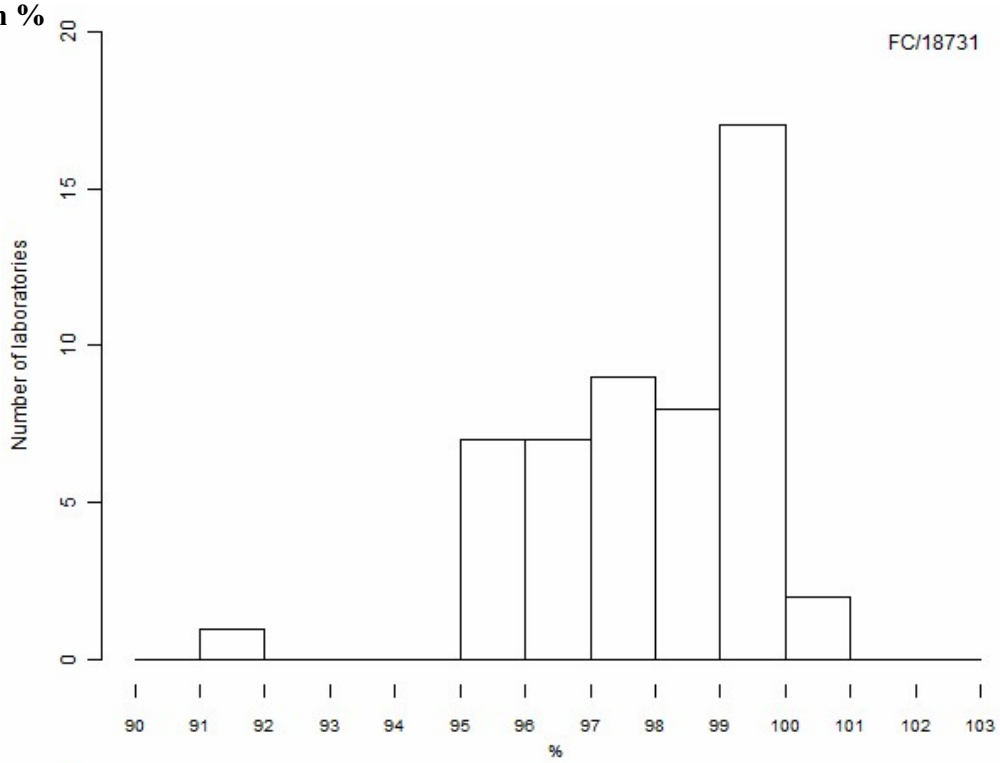
FC/18731 = 2.36
FC/18731 = 3.18
FC/18731 = 29.72
FC/18732 = 2.98
FC/18732 = 3.2
FC/18732 = 499

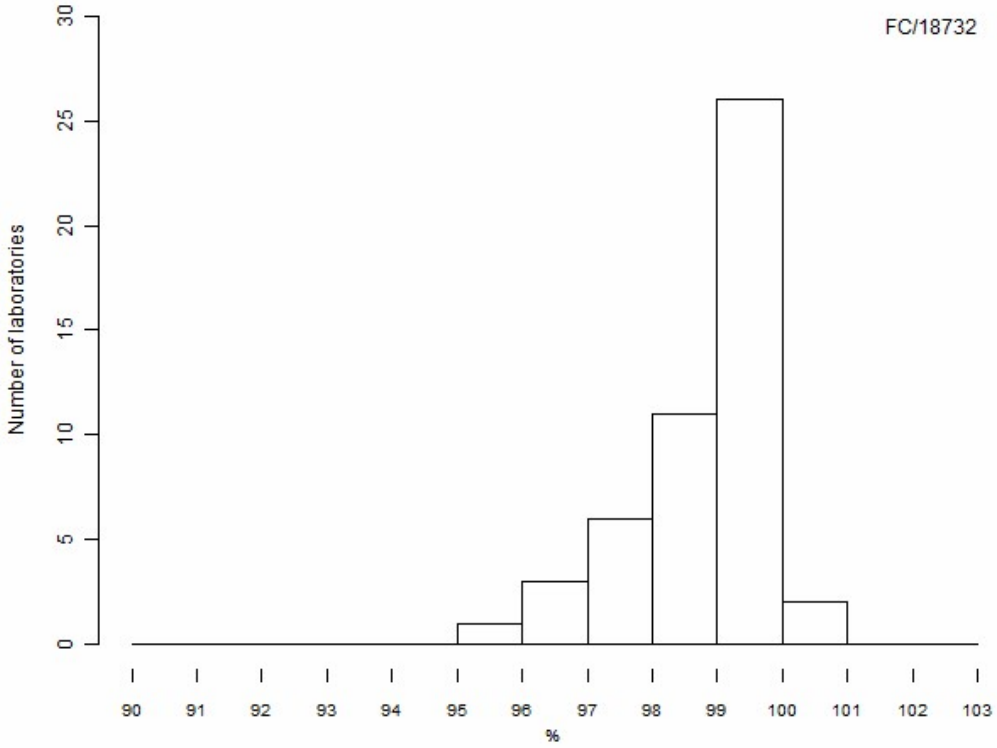
Sum K+L % B lymphocytes



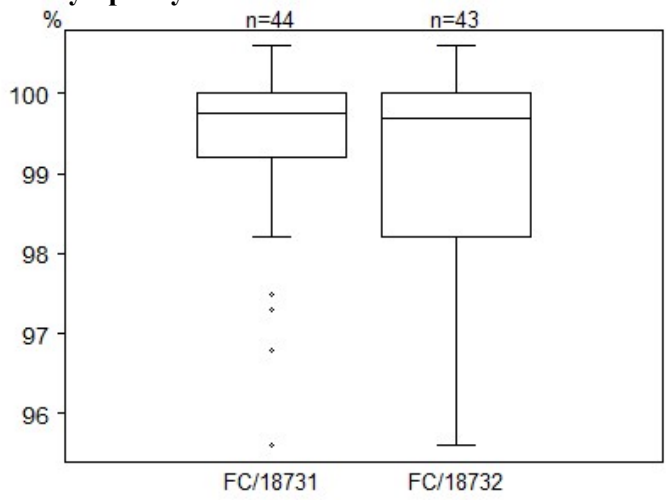


Lymphosum %


FC/18731
Number of laboratories
0
5
10
15
20
90
91
92
93
94
95
96
97
98
99
100
101
102
103
%


FC/18732
Number of laboratories
0
5
10
15
20
25
30
90
91
92
93
94
95
96
97
98
99
100
101
102
103
%

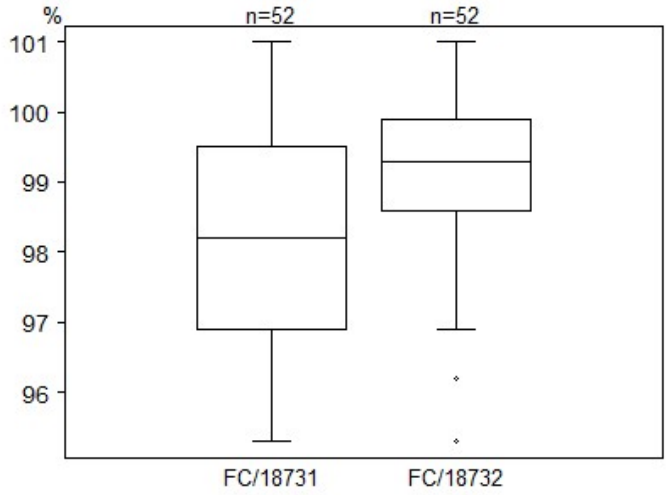
Sum K+L % B lymphocytes



Results not represented on the graph

FC/18731 = 90.3 %
FC/18731 = 92 %
FC/18731 = 93 %
FC/18732 = 33 %
FC/18732 = 35.4 %
FC/18732 = 43.7 %
FC/18732 = 67.6 %

Lymphosum %



Results not represented on the graph

FC/18731 = 36.7 %
FC/18731 = 91.2 %
FC/18732 = 125 %
FC/18732 = 137.8 %
FC/18732 = 147 %

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%.

END

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