

**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 2021/2

Sciensano/Flow cytometry/78-E

Expertise and service provision
Quality of laboratories
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 This report was discussed at the meeting of the committee of experts EQA Flow Cytometry on:
 15/06/2021

Authorization to release the report:	By Lobna Bouacida, scheme coordinator, on 16/06/2021.
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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 ($M \pm 2.7$ SD)

$O_{M/G}$: external limits ($M \pm 4.7$ 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

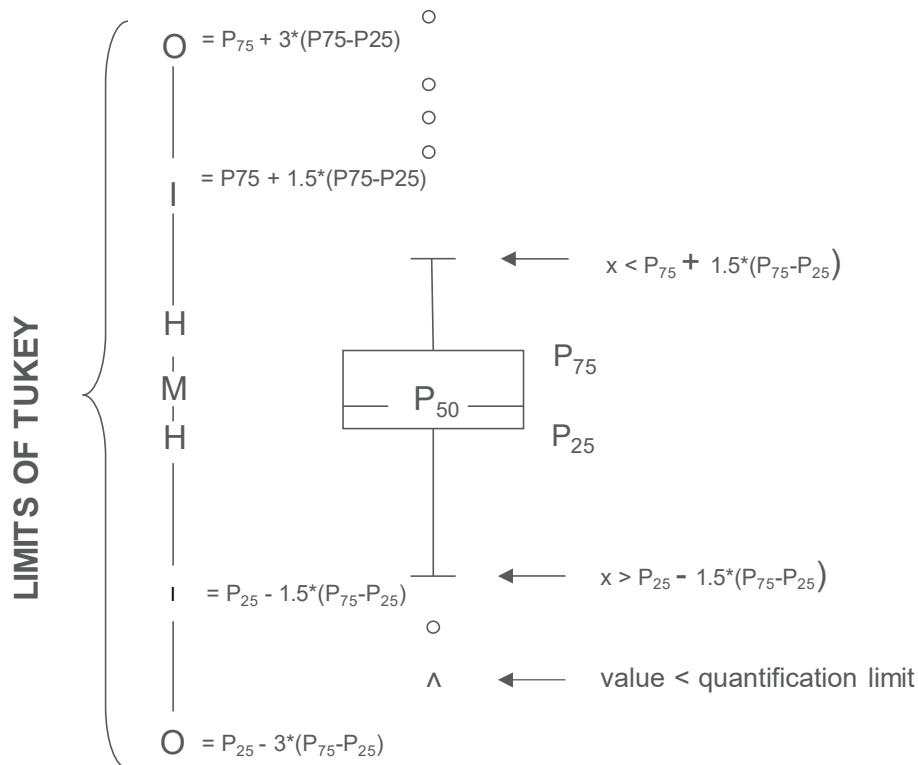
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_fr/brochures.htm

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/18215 and FC/18216) 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).

The samples tested negative for HIV 1 and 2, hepatitis B surface antigen and hepatitis C. Homogeneity was confirmed based on white blood cells determination.

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

FC18215

	%	10 ⁹ /L
Leukocytes		8.3
Lymphocytes	28.8	
CD3⁺ cells	64.5	1.54
CD4⁺CD3⁺ cells	42.4	1.01
CD8⁺CD3⁺ cells	19.6	0.47
CD19⁺ cells	10.5	0.25
NK cells	21.7	0.52
κ % B lymphocytes	54.4	
λ % B lymphocytes	45.5	
κ/λ ratio	1.20	

FC18216

	%	10 ⁹ /L
Leukocytes		5.8
Lymphocytes	20.0	
CD3⁺ cells	68.9	0.80
CD4⁺CD3⁺ cells	47.7	0.55
CD8⁺CD3⁺ cells	20.2	0.23
CD19⁺ cells	12.0	0.14
NK cells	18.0	0.21
κ % B lymphocytes	58.5	
λ % B lymphocytes	41.5	
κ/λ ratio	1.41	

PARTICIPATION

Fifty-two laboratories (1 Canadian and 51 Belgian clinical laboratories) participated in the survey 2021/2 (send-out of blood samples on May 3, 2021 (day 0)).

RESULTS

100% of the Belgian laboratories received the samples on day 1 or 2. 44 laboratories (86%) received the samples on day 1 and seven (14%) received them on day 2.

71% (n=36) of the Belgian laboratories performed the analyses on day 1, 23% (n=12) on day 2 and 6% (n=3) 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.

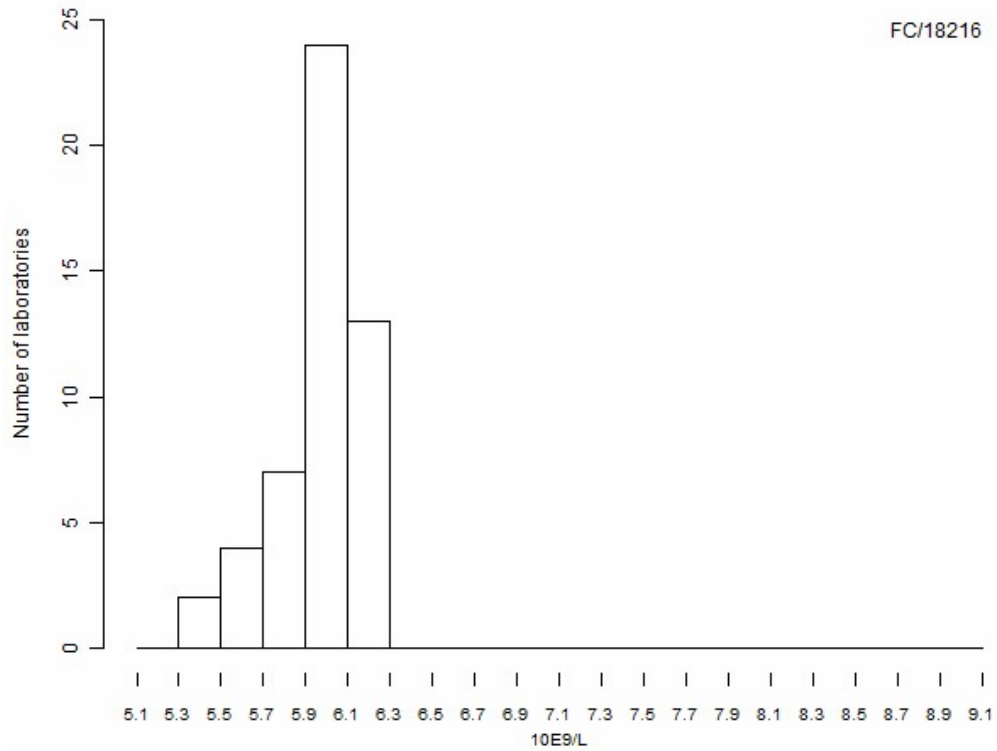
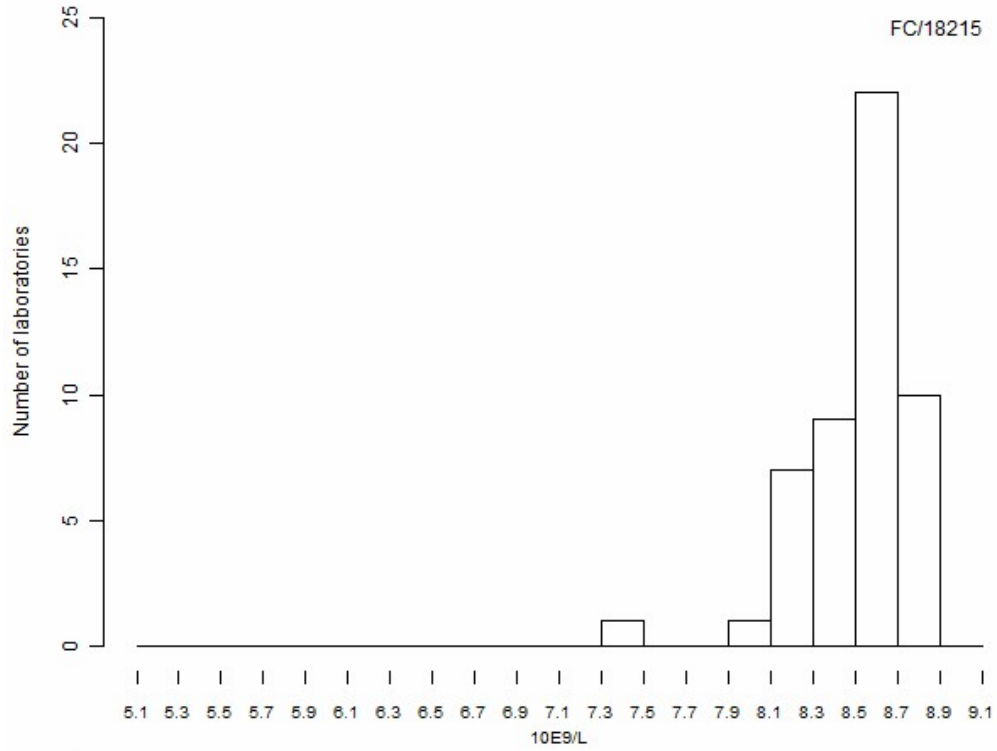
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=51). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=48).

The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/18215 and FC/18216:

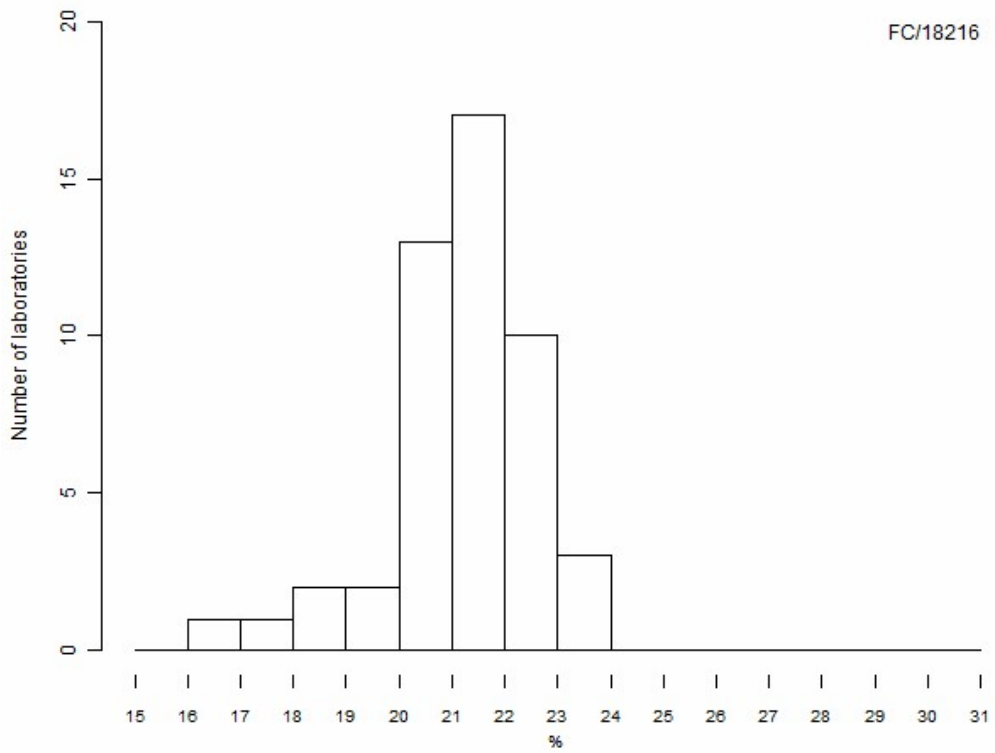
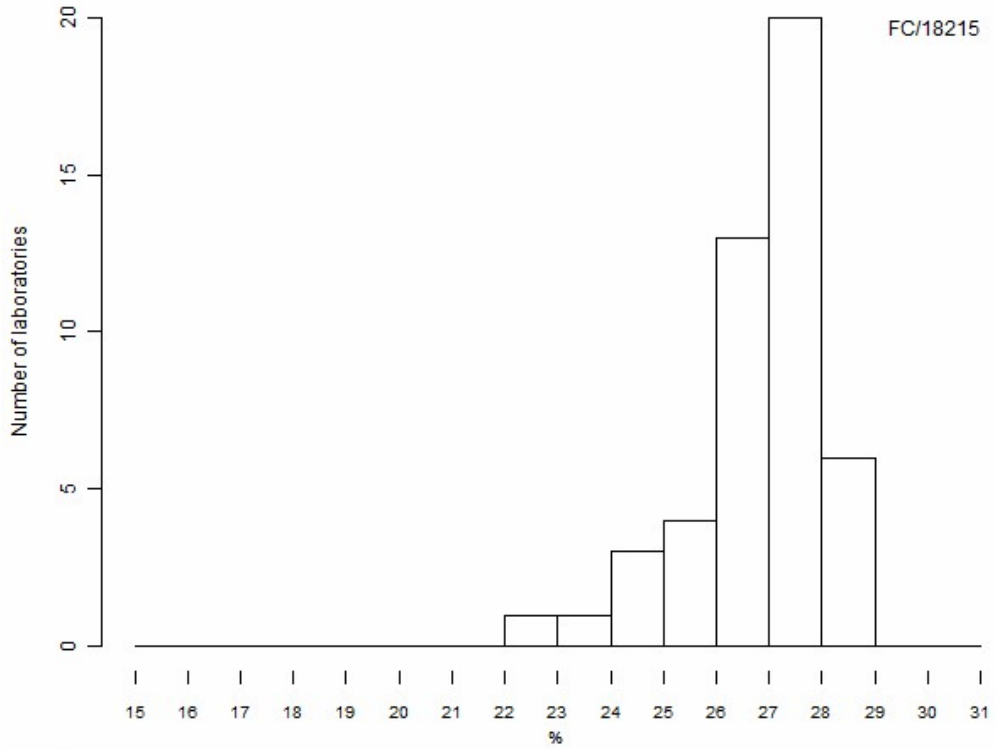
FC/18215	Median	SD	CV,%	N
WBC 10E9/L	8.60	0.22	2.5	47
Lympho% haematology analyser	27.3	0.7	2.7	47
Lympho% flow cytometer	27.3	1.9	6.8	47
CD3 %	67.6	2.5	3.7	51
CD3 10E9/L	1.578	0.111	7.1	47
CD4 %	44.2	2.7	6.2	51
CD4 10E9/L	1.037	0.082	7.9	47
CD8 %	19.3	1.6	8.1	51
CD8 10E9/L	0.447	0.054	12.0	47
CD19 %	10.0	0.9	8.9	51
CD19 10E9/L	0.235	0.039	16.5	47
NKcells %	21.0	2.1	10.2	51
NKcells 10E9/L	0.471	0.080	17.0	47
Kappa % B lymphocytes	56.0	2.0	3.6	45
Lambda % B lymphocytes	43.2	2.4	5.5	45
Kappa/lambda	1.29	0.08	6.3	45
Sum K+L % B lymphocytes	99.4	0.9	0.9	45
Lymphosum %	98.0	1.5	1.5	51

FC/18216	Median	SD	CV,%	N
WBC 10E9/L	5.98	0.15	2.5	47
Lympho% haematology analyser	21.5	1.2	5.7	47
Lympho% flow cytometer	21.4	2.2	10.4	47
CD3 %	69.0	2.4	3.5	51
CD3 10E9/L	0.868	0.068	7.8	47
CD4 %	47.3	2.0	4.2	51
CD4 10E9/L	0.600	0.056	9.3	47
CD8 %	19.0	1.0	5.1	51
CD8 10E9/L	0.240	0.031	12.8	47
CD19 %	12.0	1.1	9.3	51
CD19 10E9/L	0.151	0.021	13.7	47
NKcells %	18.0	2.5	14.0	51
NKcells 10E9/L	0.235	0.029	12.3	47
Kappa % B lymphocytes	58.1	2.9	5.0	45
Lambda % B lymphocytes	41.1	2.6	6.3	45
Kappa/lambda	1.40	0.16	11.1	45
Sum K+L % B lymphocytes	99.8	1.0	1.0	45
Lymphosum %	98.0	1.5	1.5	51

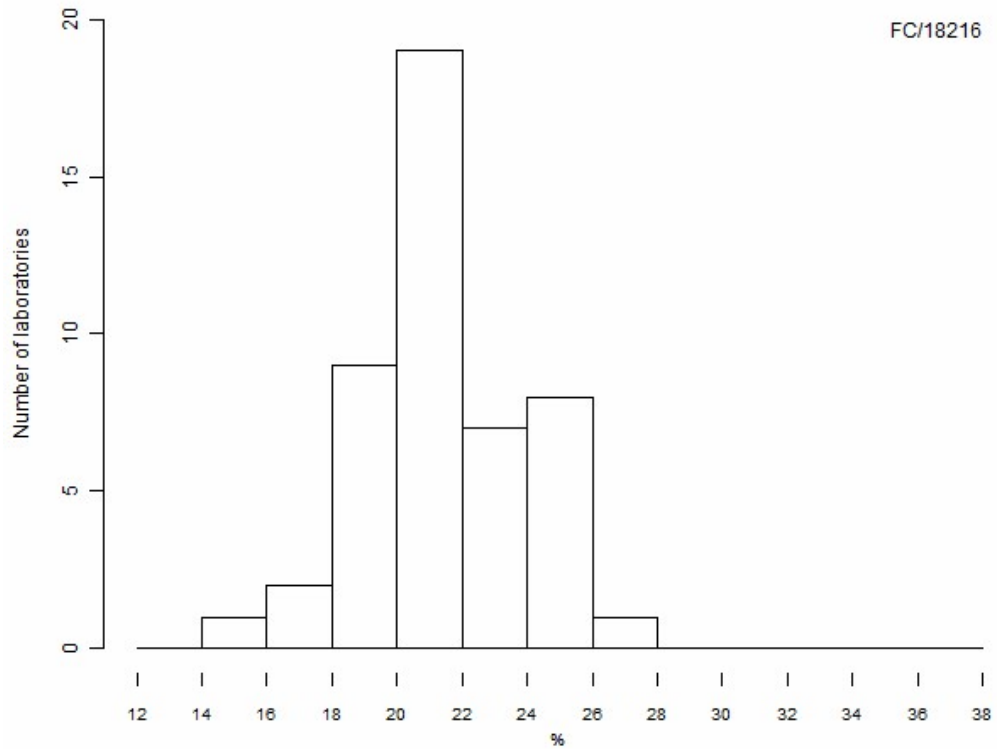
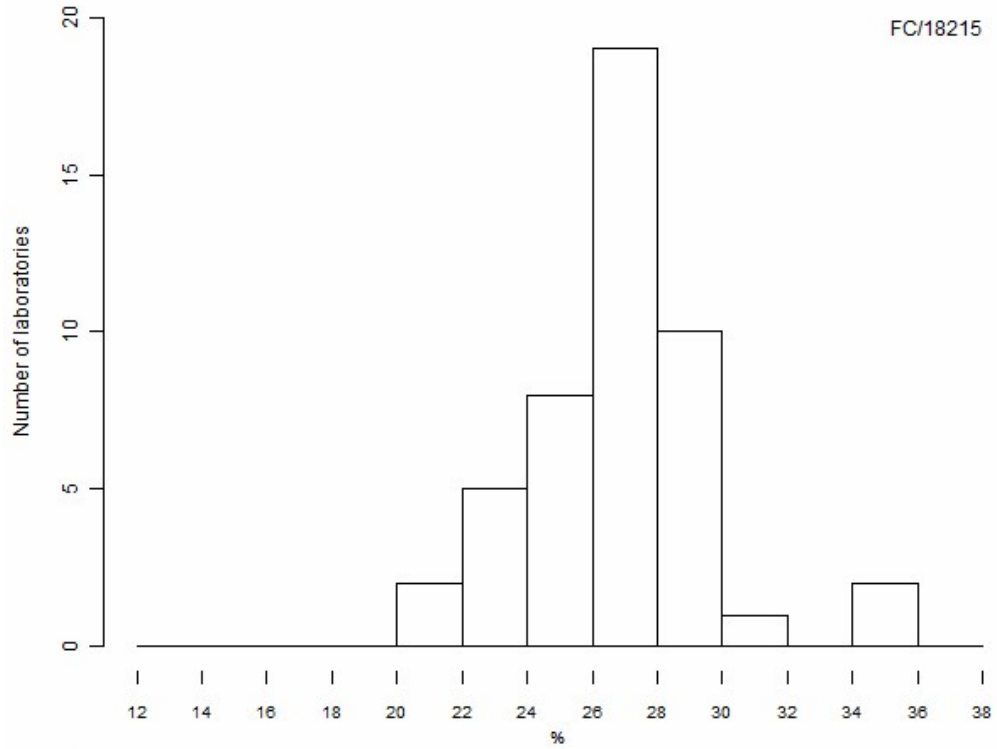
WBC 10E9/L



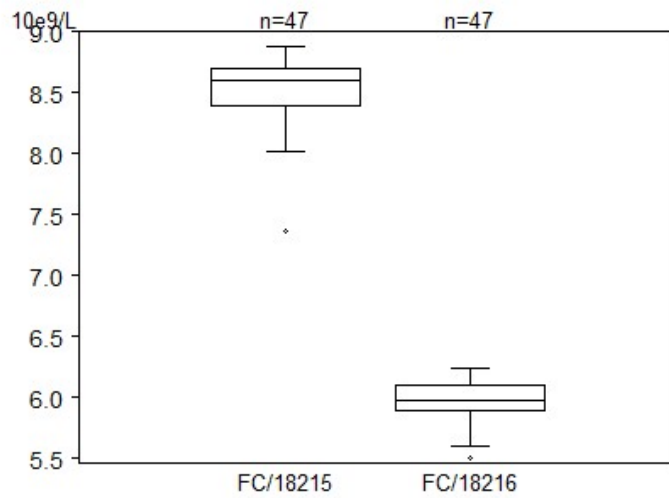
Lympho% haematology analyser



Lympho% flow cytometer

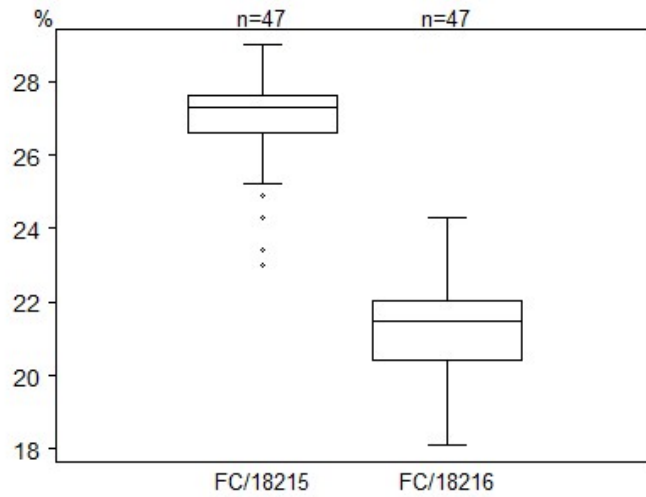


WBC 10E9/L



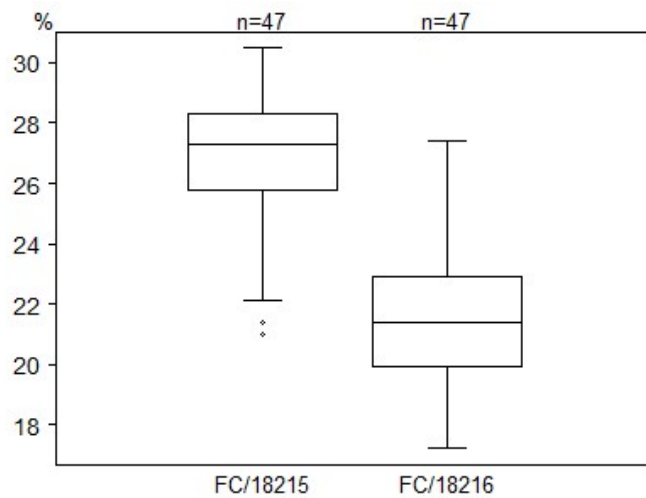
Results not represented
on the graph
FC/18216 = 5.37 10e9/l

Lympho% haematology analyser



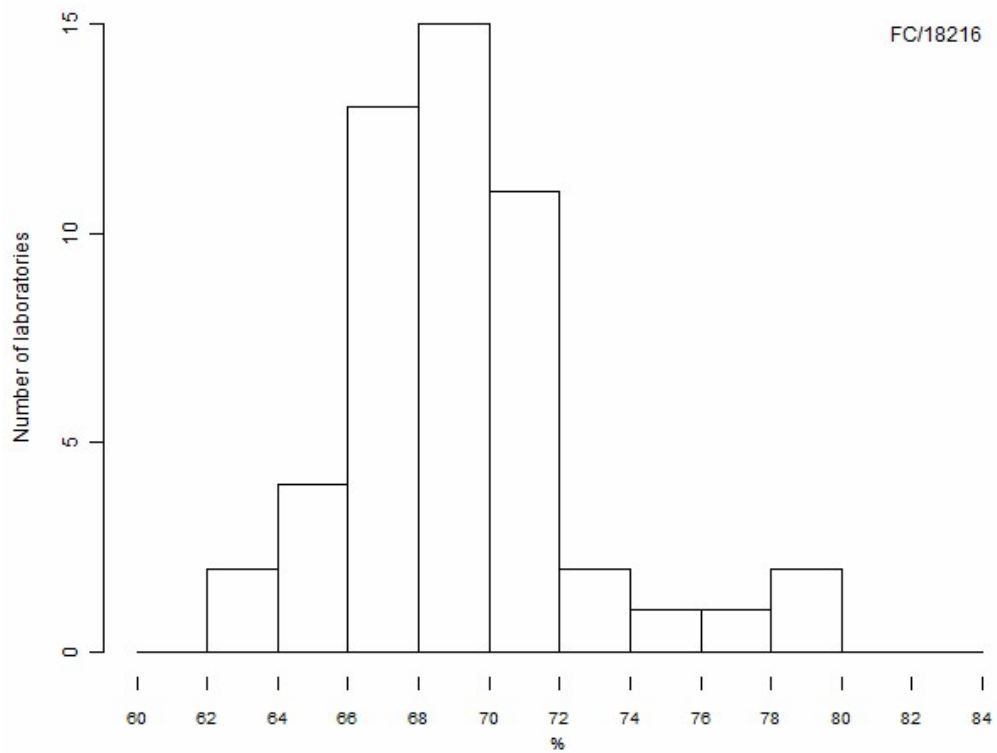
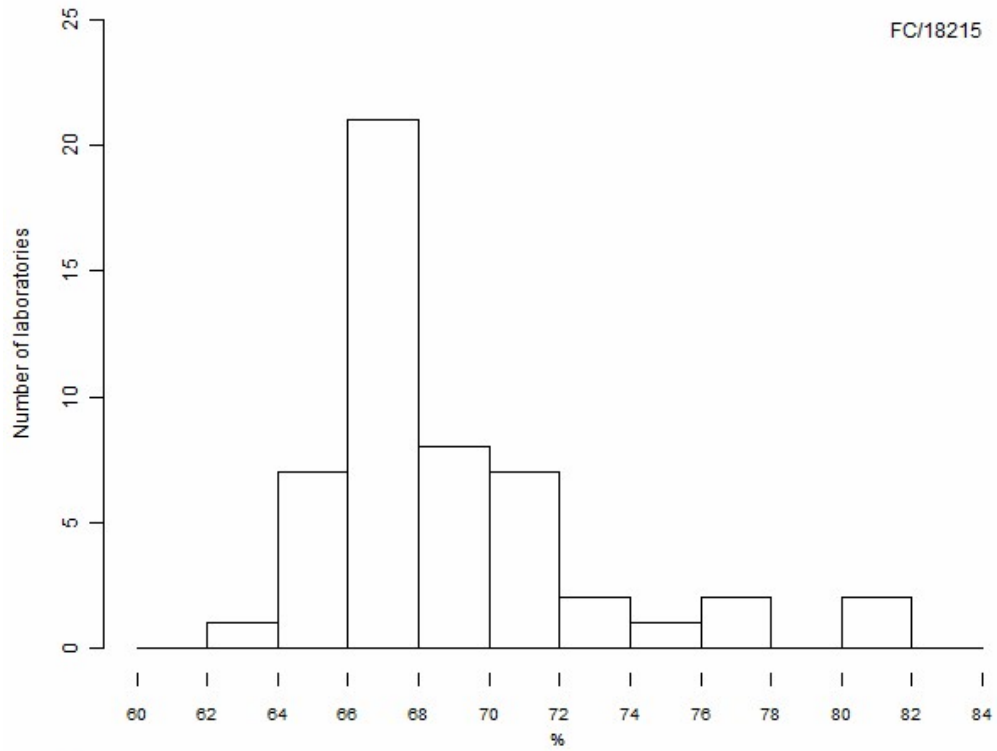
Results not represented
on the graph
FC/18215 = 38.9 %
FC/18216 = 16.3 %

Lympho% flow cytometer

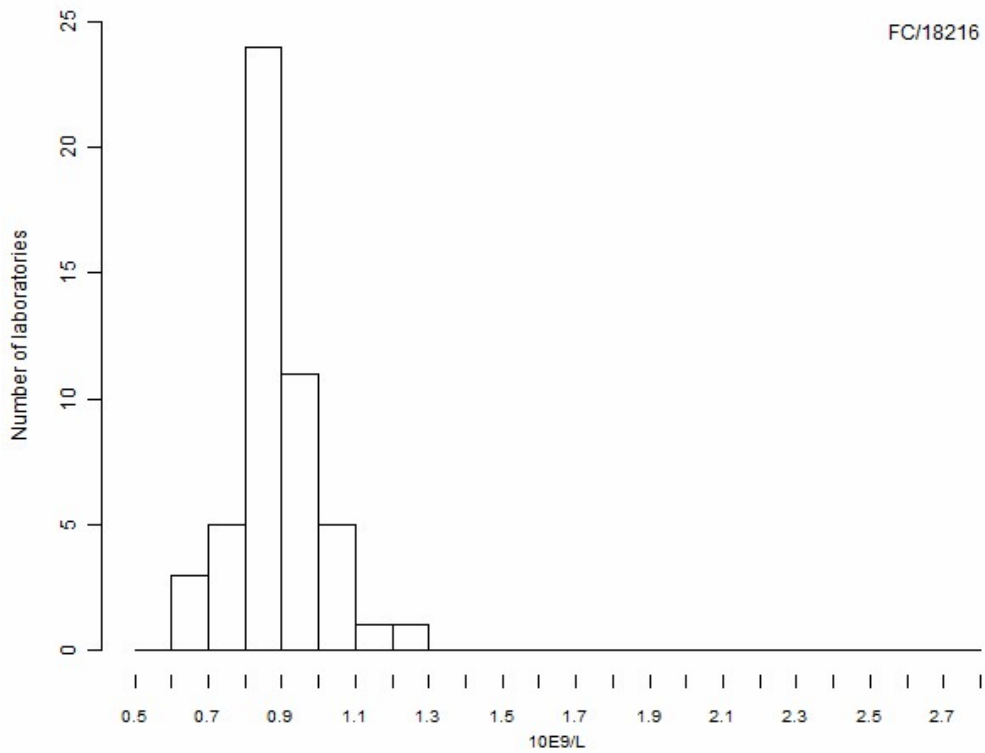
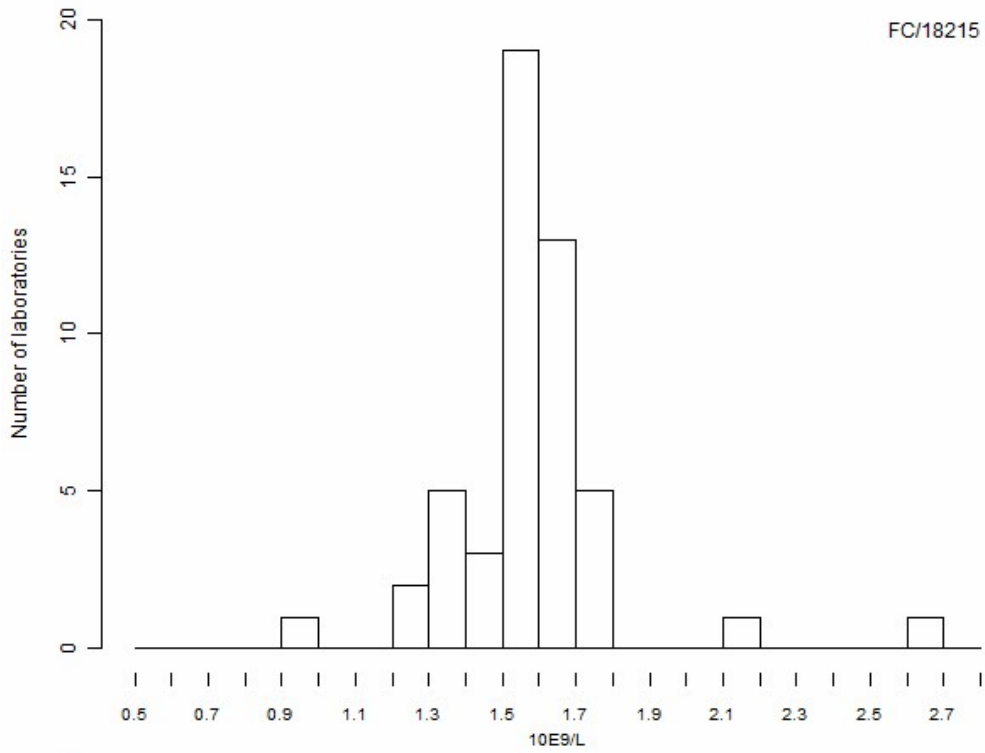


Results not represented
on the graph
FC/18215 = 34.6 %
FC/18215 = 34.8 %
FC/18216 = 14.6 %

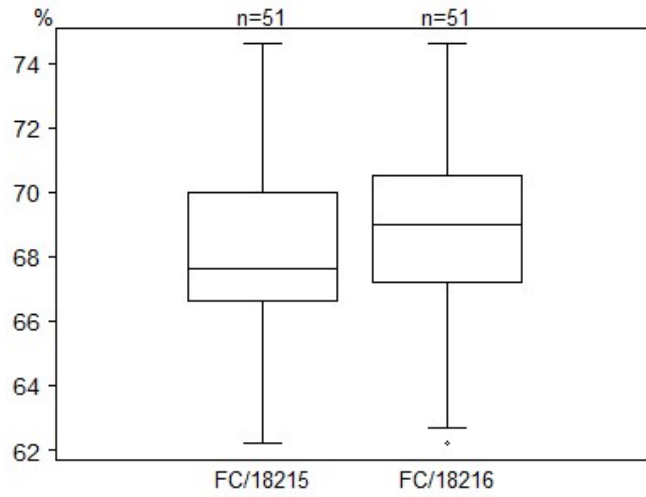
CD3 %



CD3 10E9/L



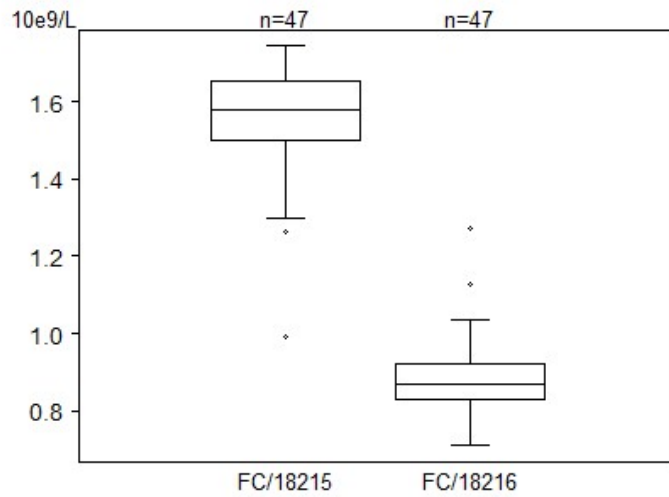
CD3 %



Results not represented on the graph

FC/18215 = 77.2 %
FC/18215 = 77.4 %
FC/18215 = 80.7 %
FC/18215 = 81.5 %
FC/18216 = 76.1 %
FC/18216 = 79.2 %
FC/18216 = 79.4 %

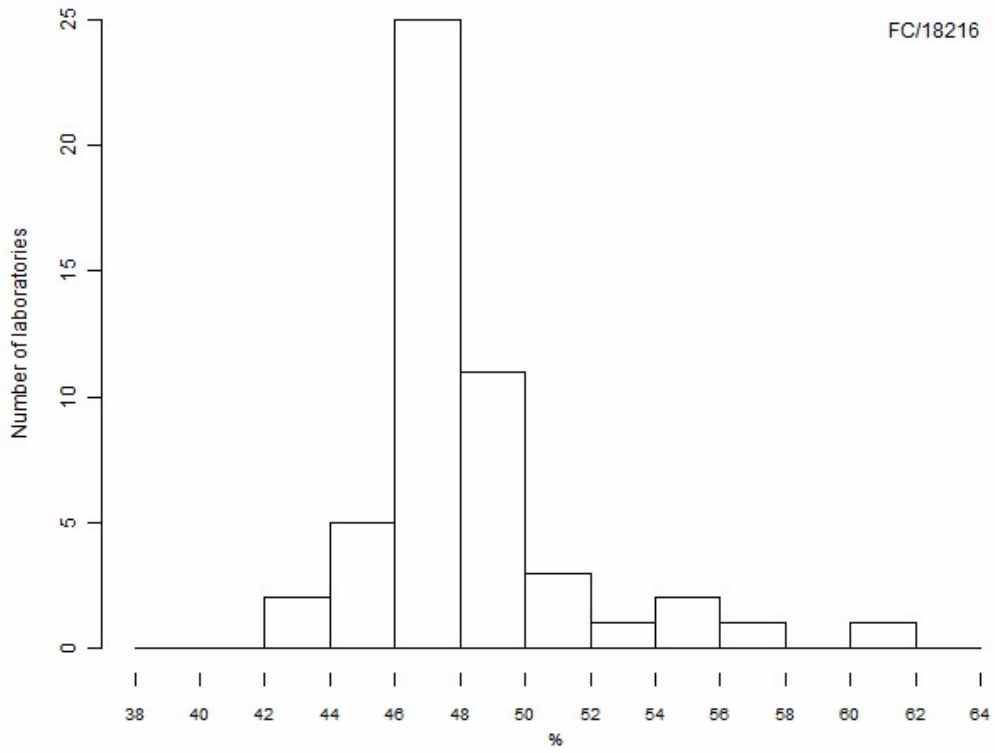
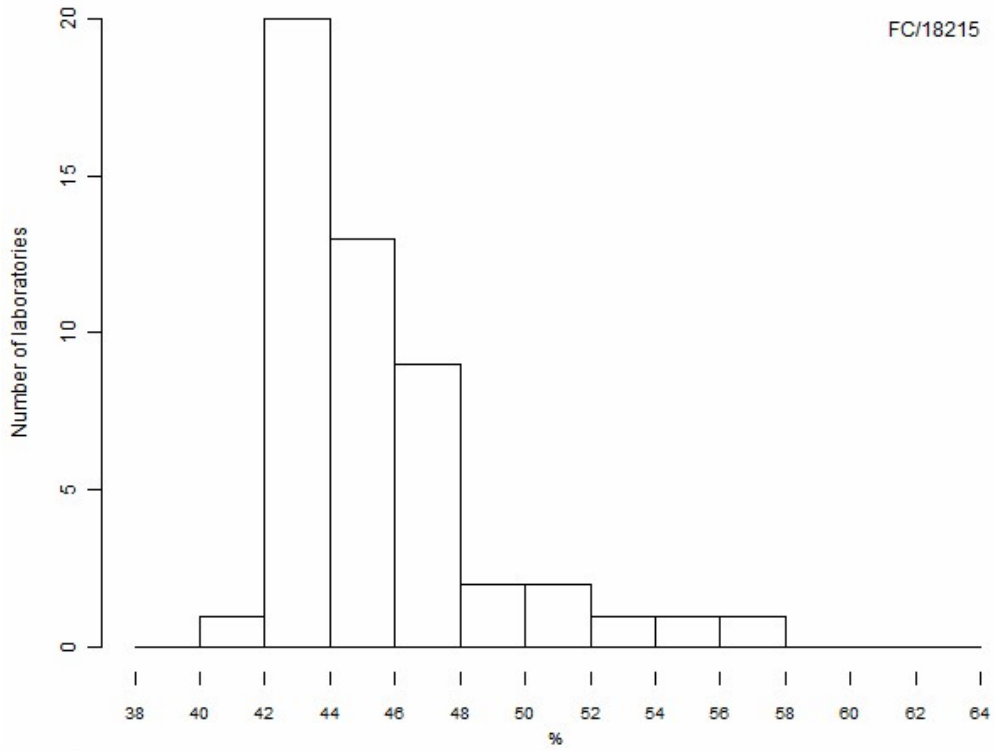
CD3 10E9/L



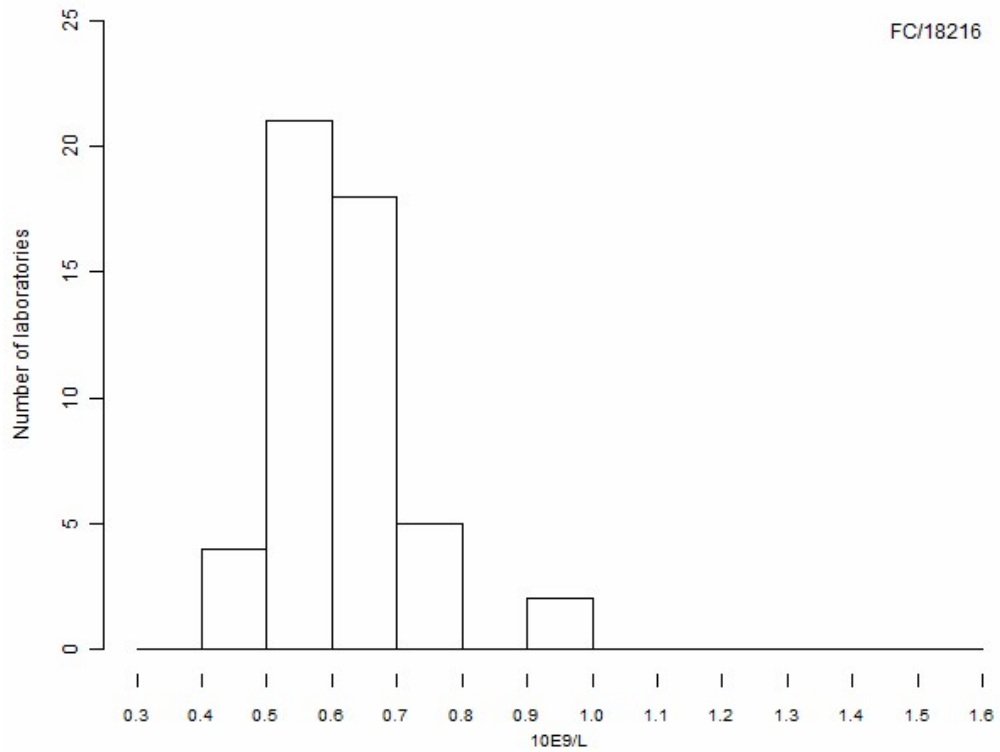
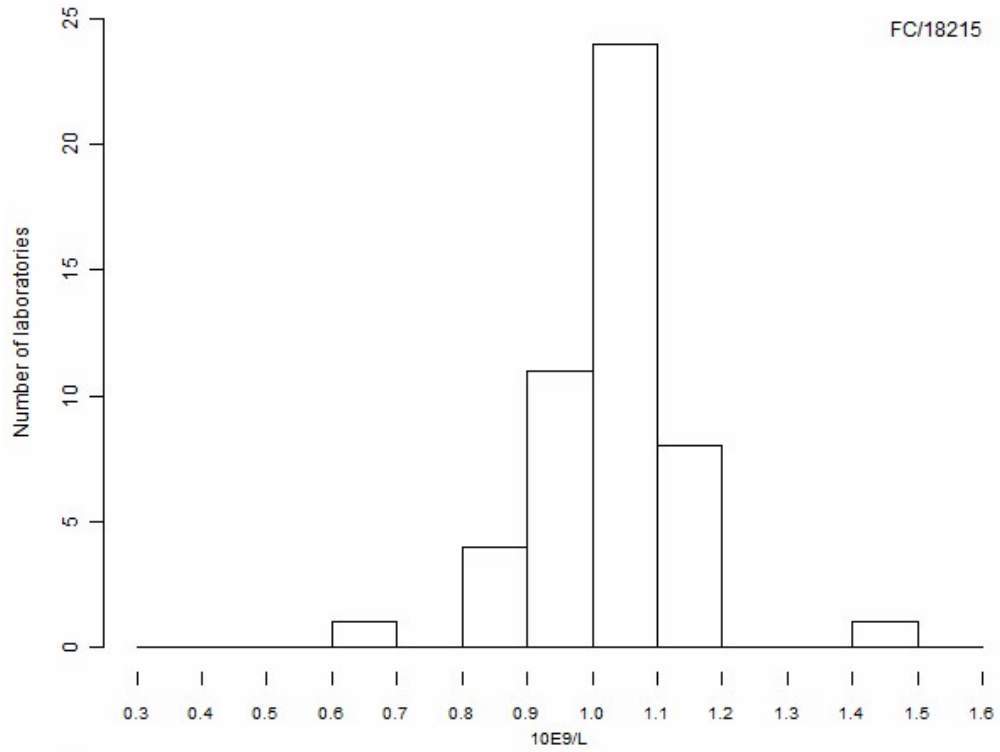
Results not represented on the graph

FC/18215 = 2.118 10e9
FC/18215 = 2.613 10e9
FC/18216 = 0.62 10e9/l
FC/18216 = 0.64 10e9/l
FC/18216 = 0.653 10e9

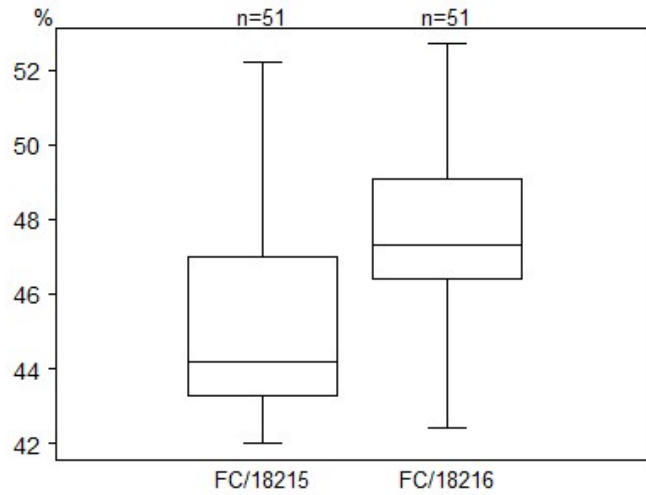
CD4 %



CD4 10E9/L



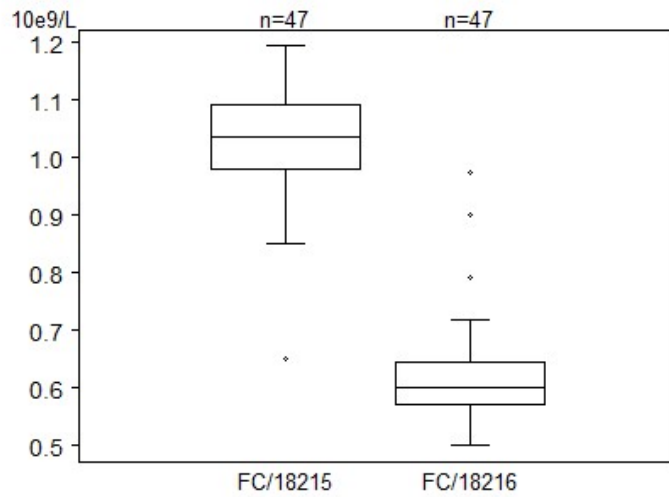
CD4 %



Results not represented on the graph

FC/18215 = 55.2 %
FC/18215 = 57.6 %
FC/18215 = 70.1 %
FC/18216 = 54.5 %
FC/18216 = 55.8 %
FC/18216 = 57 %
FC/18216 = 60.8 %

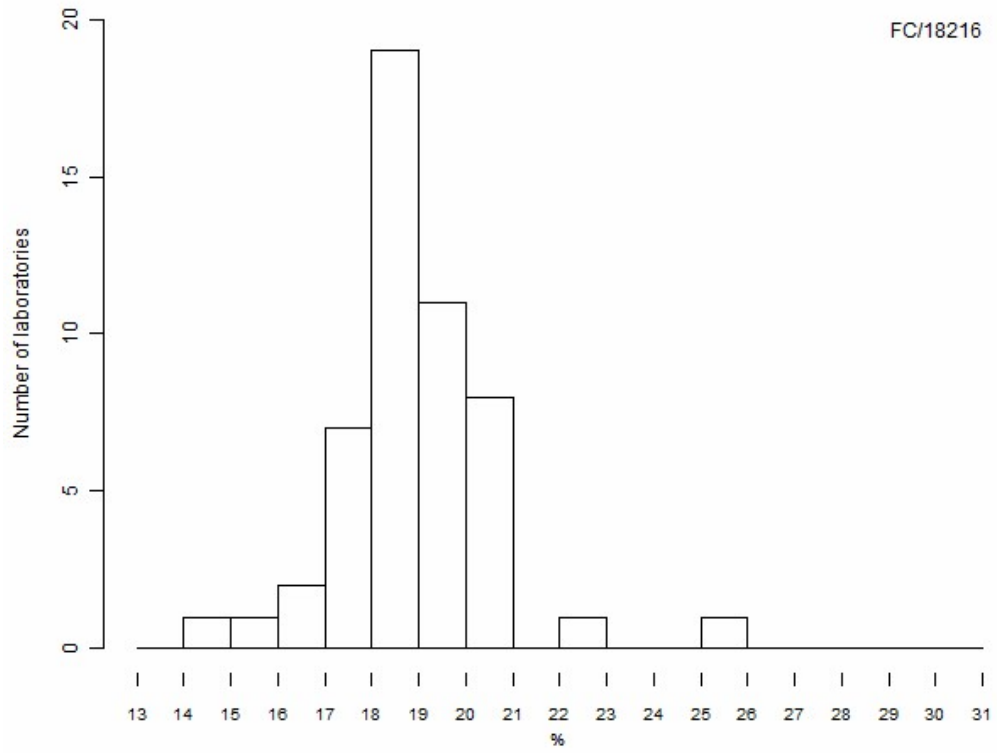
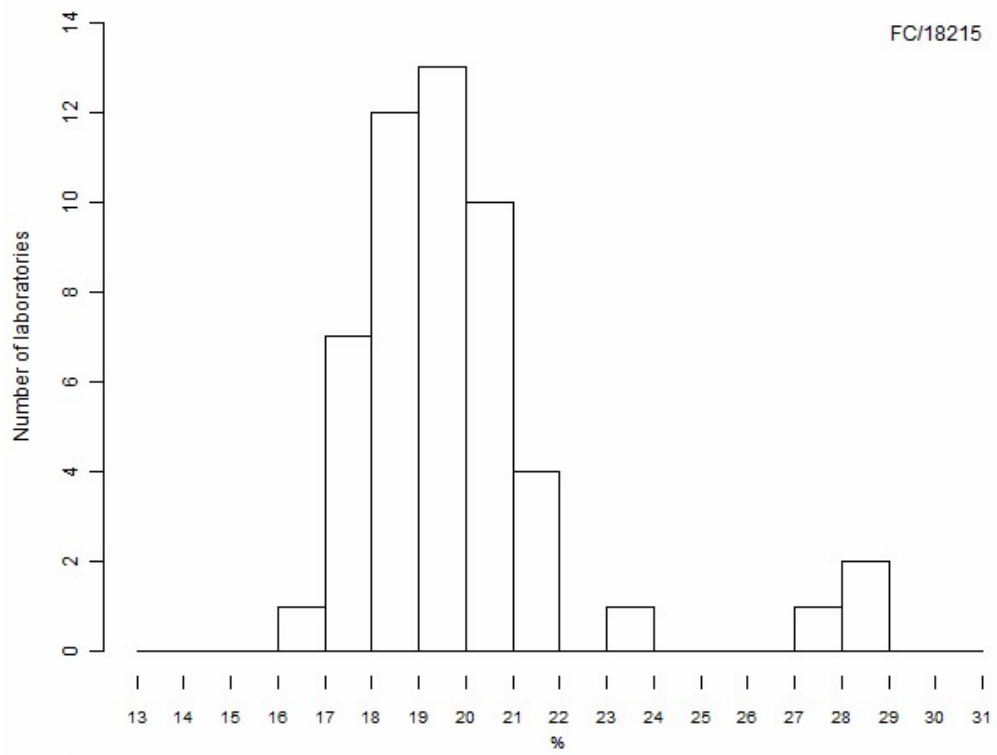
CD4 10E9/L



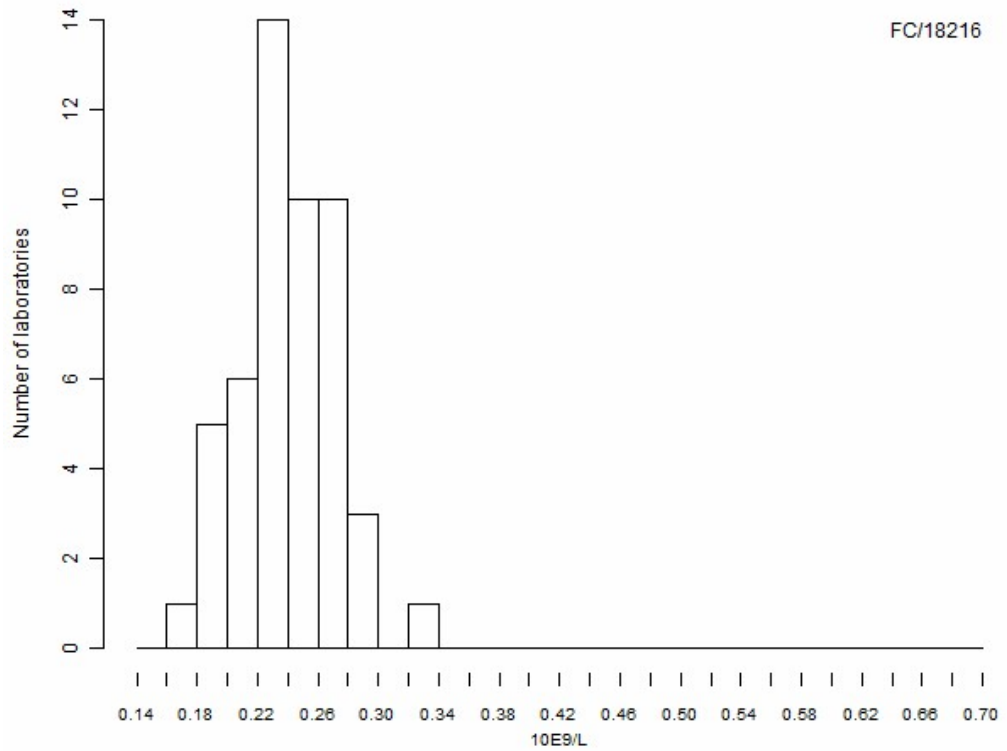
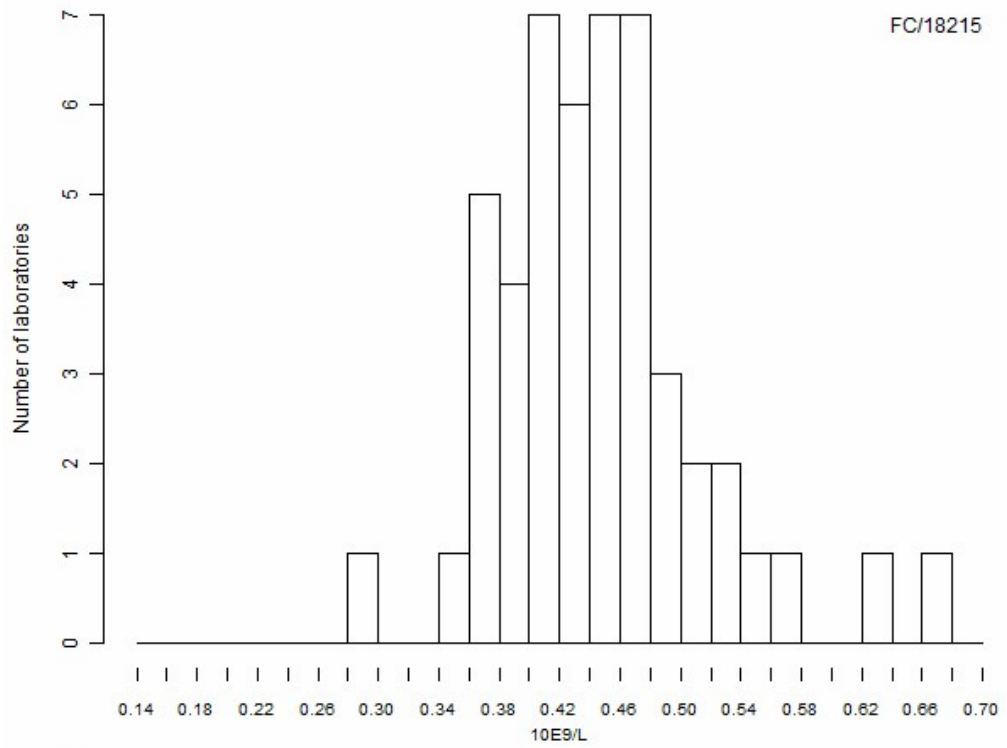
Results not represented on the graph

FC/18215 = 1.497 10e9
FC/18215 = 2.372 10e9
FC/18216 = 0.44 10e9/l
FC/18216 = 0.44 10e9/l
FC/18216 = 0.456 10e9

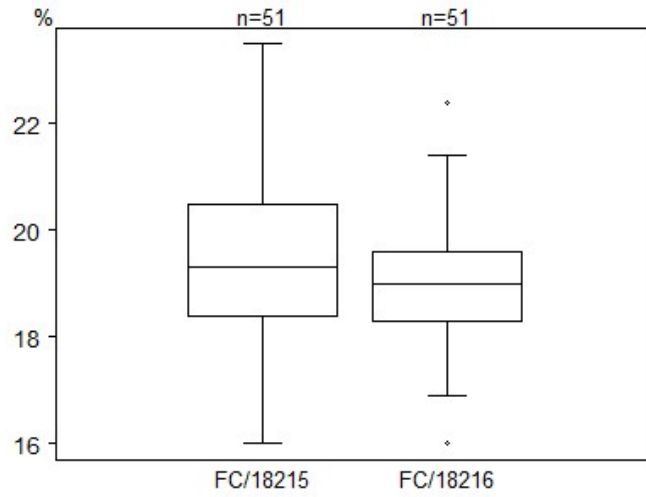
CD8 %



CD8 10E9/L



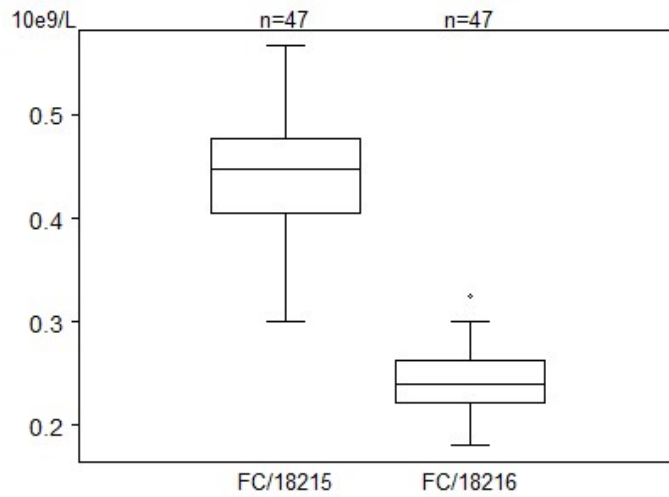
CD8 %



Results not represented on the graph

FC/18215 = 27.3 %
FC/18215 = 28.1 %
FC/18215 = 29 %
FC/18216 = 14.6 %
FC/18216 = 25.9 %

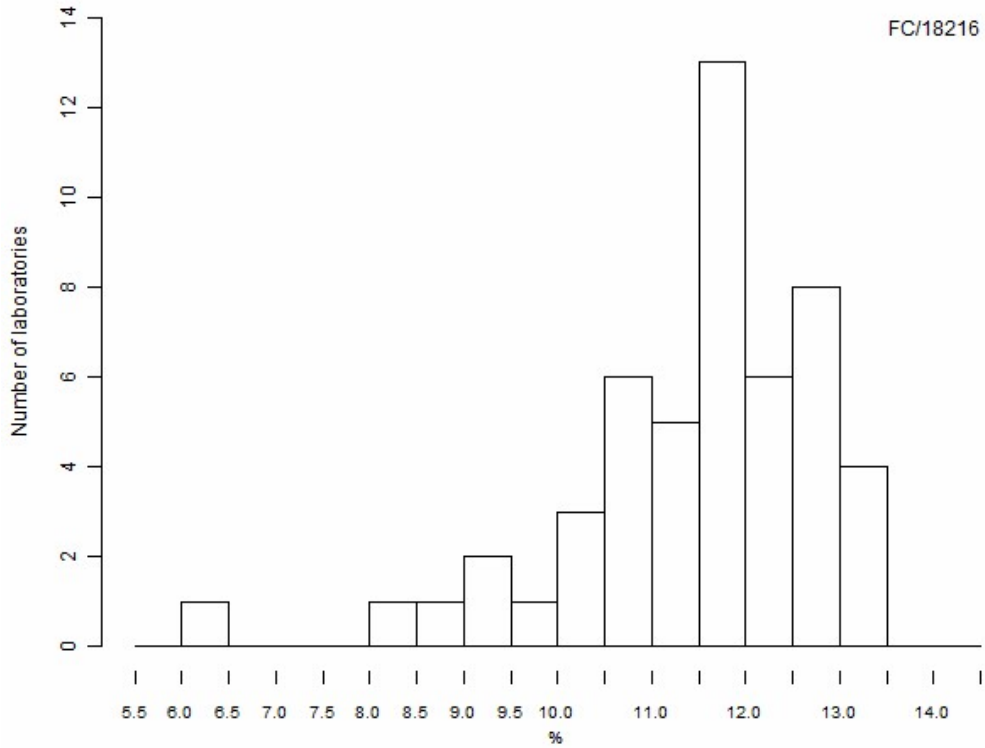
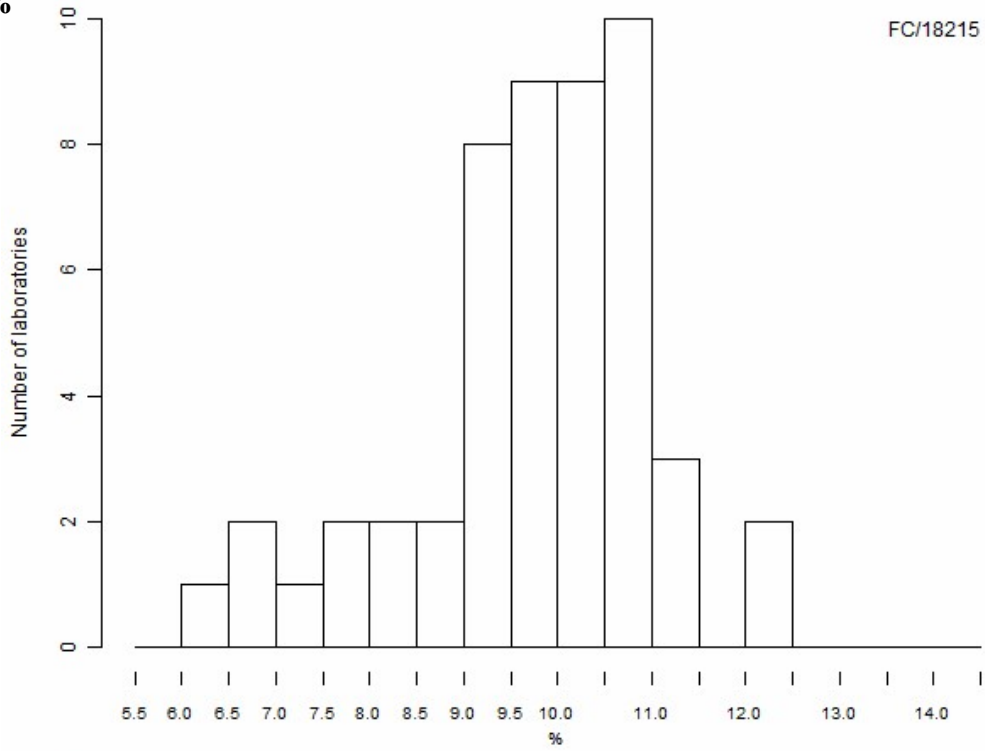
CD8 10E9/L



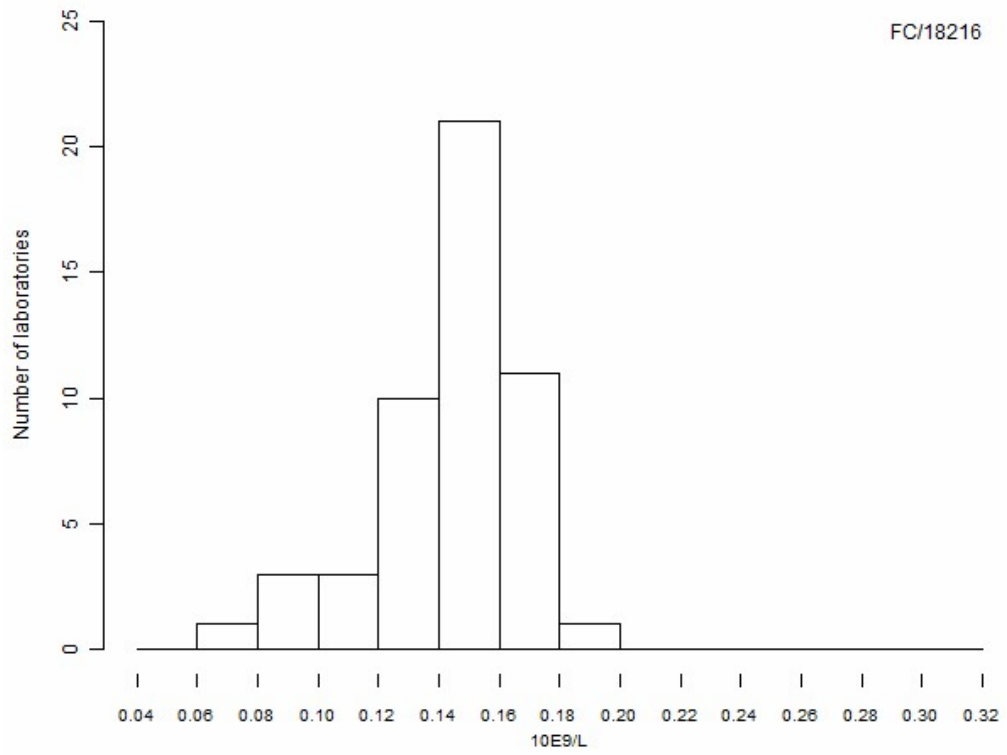
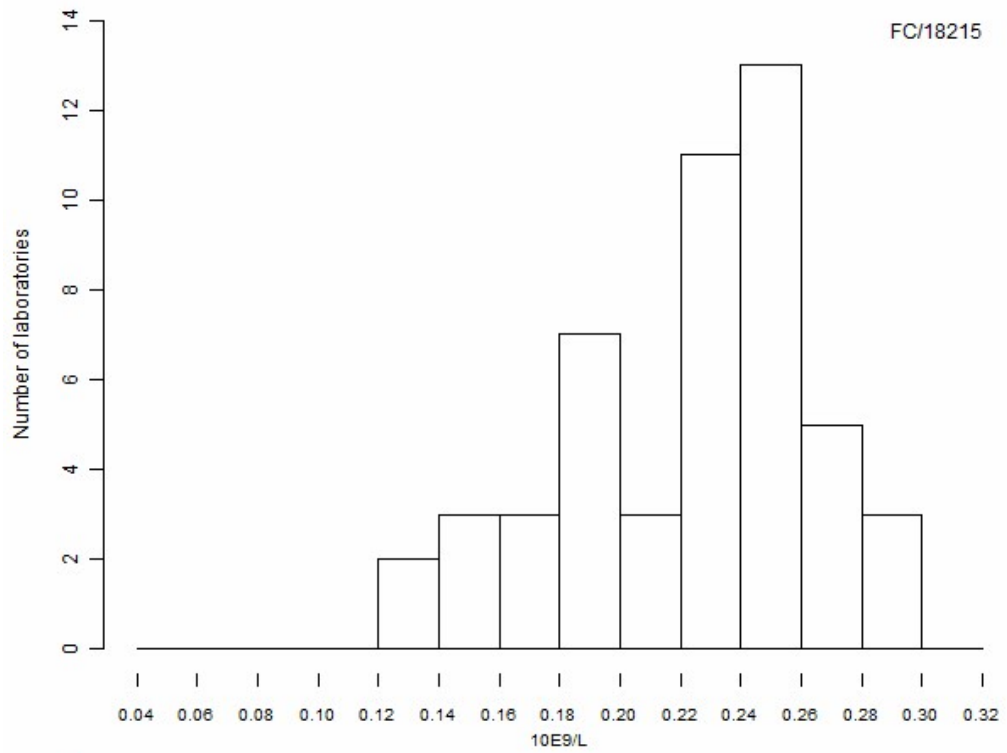
Results not represented on the graph

FC/18215 = 0.629 10e9
FC/18215 = 0.667 10e9
FC/18215 = 0.981 10e9

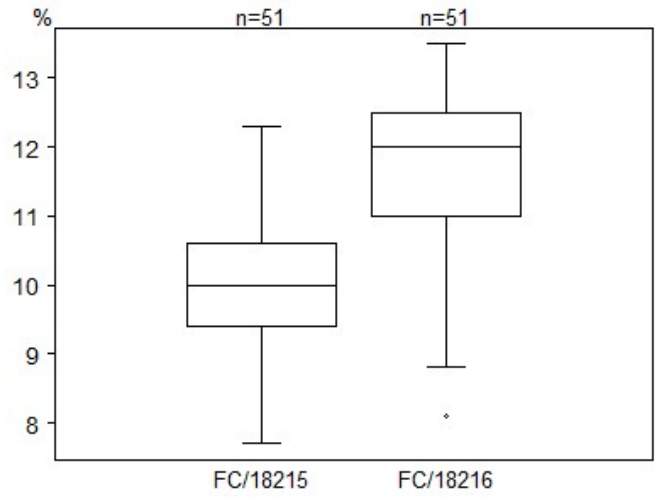
CD19 %



CD19 10E9/L



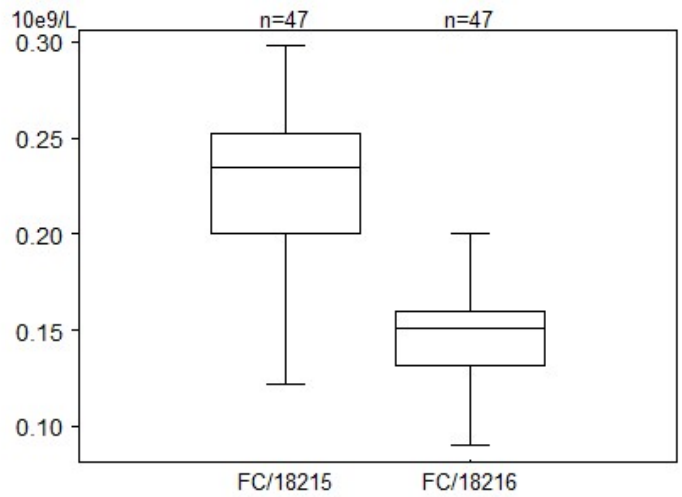
CD19 %



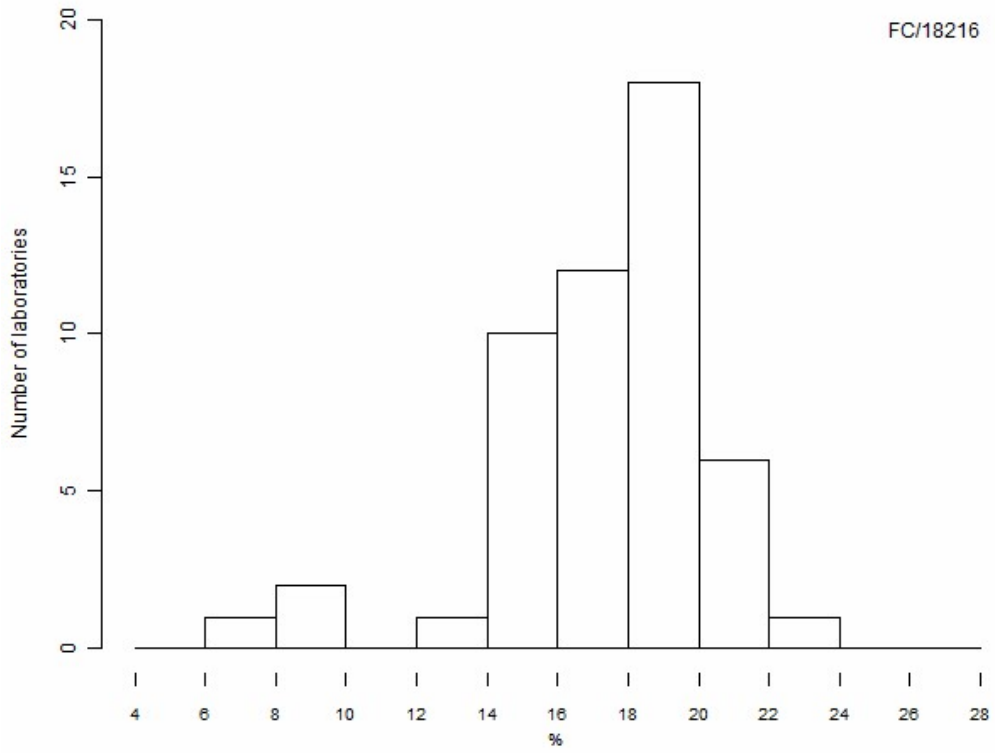
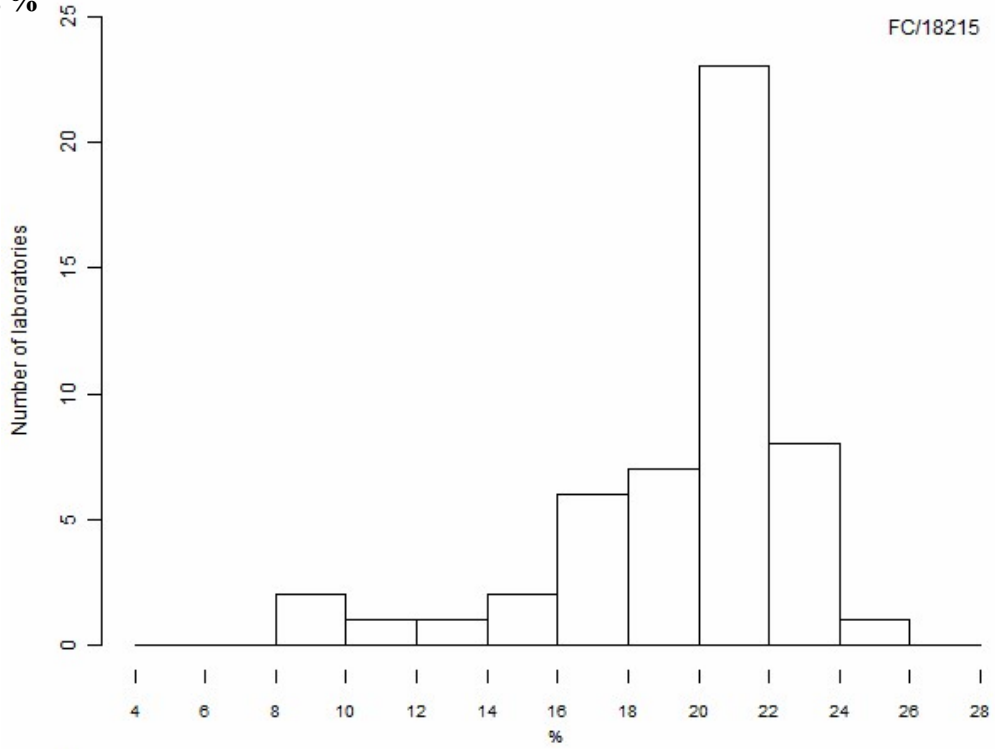
Results not represented on the graph

- FC/18215 = 6.4 %
- FC/18215 = 6.7 %
- FC/18215 = 6.9 %
- FC/18215 = 7.2 %
- FC/18216 = 6.1 %

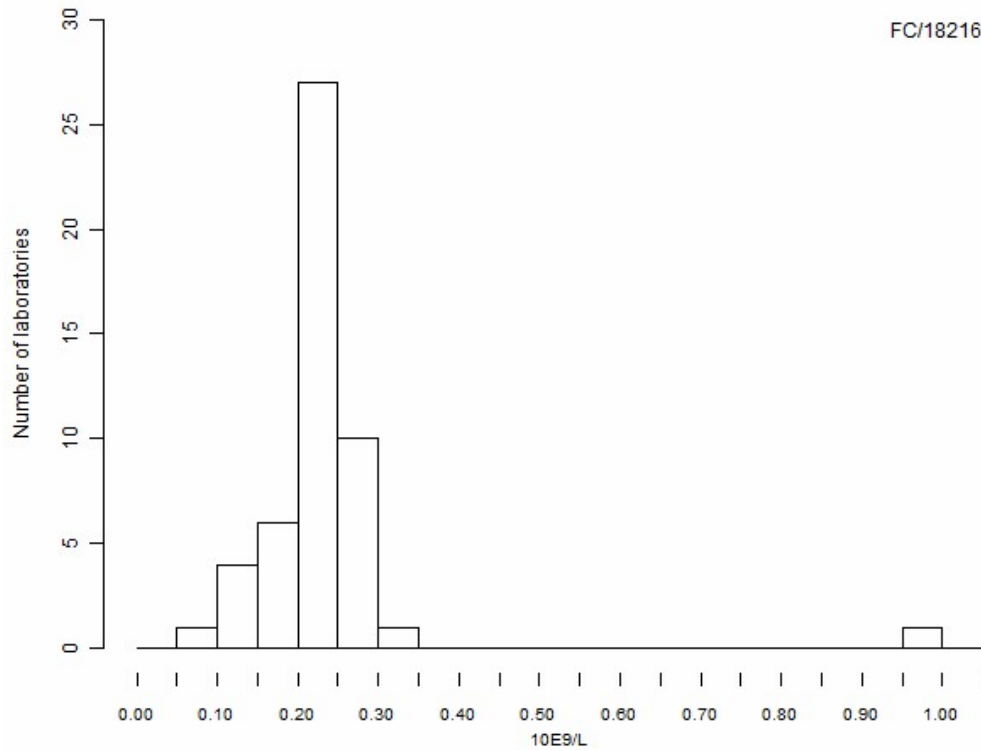
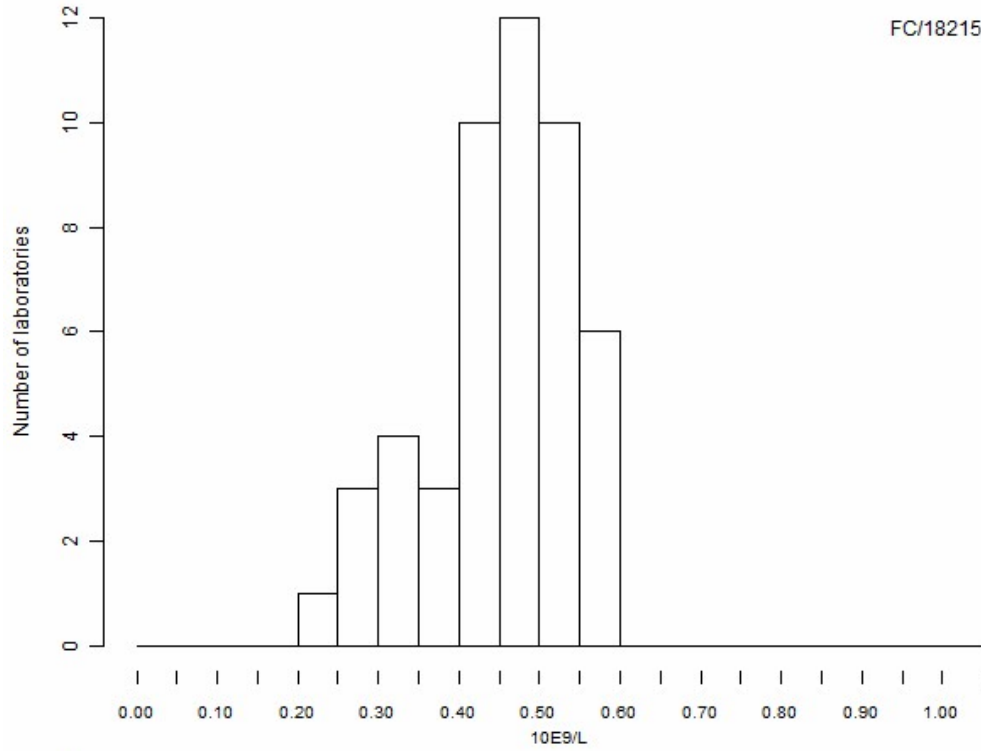
CD19 10E9/L



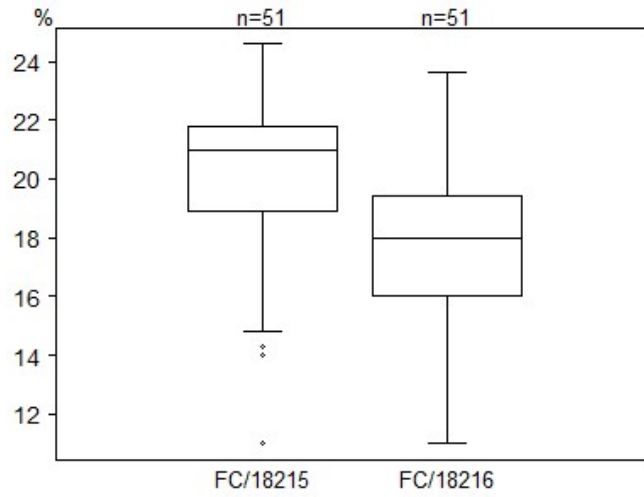
NKcells %



NKcells 10E9/L



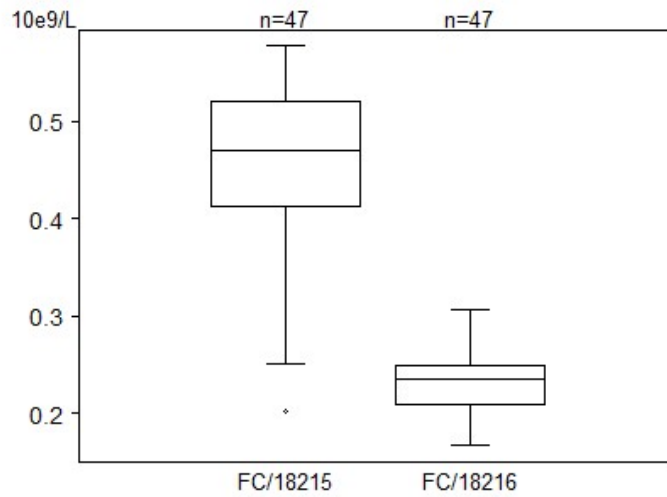
NKcells %



Results not represented on the graph

FC/18215 = 9.4 %
FC/18215 = 9.5 %
FC/18216 = 7.2 %
FC/18216 = 9 %
FC/18216 = 9.6 %

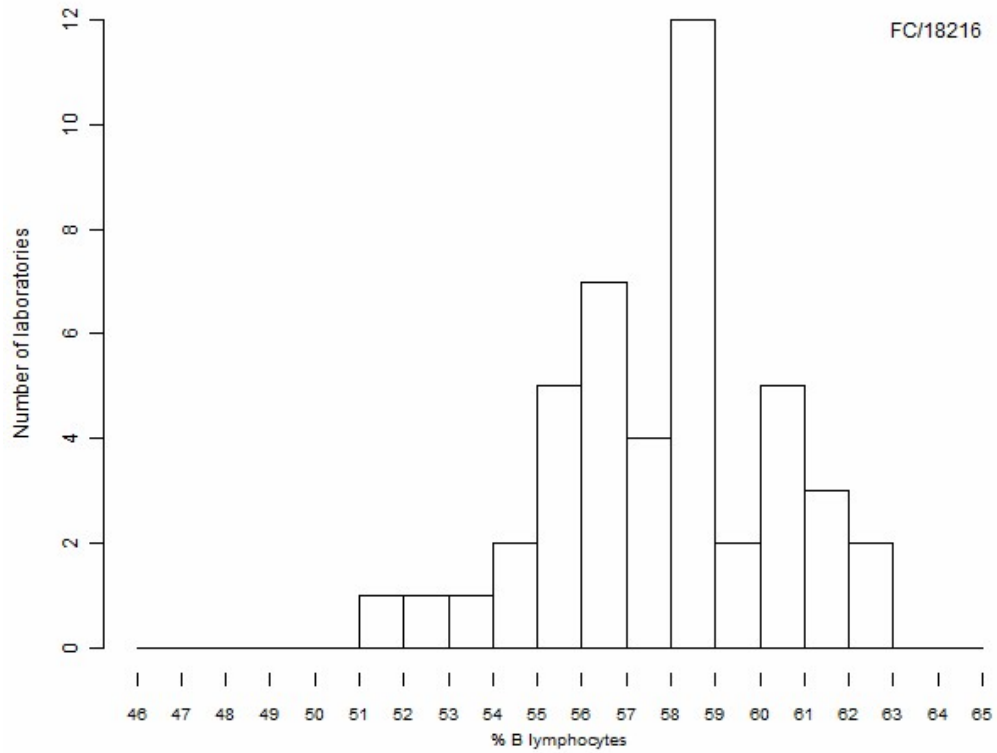
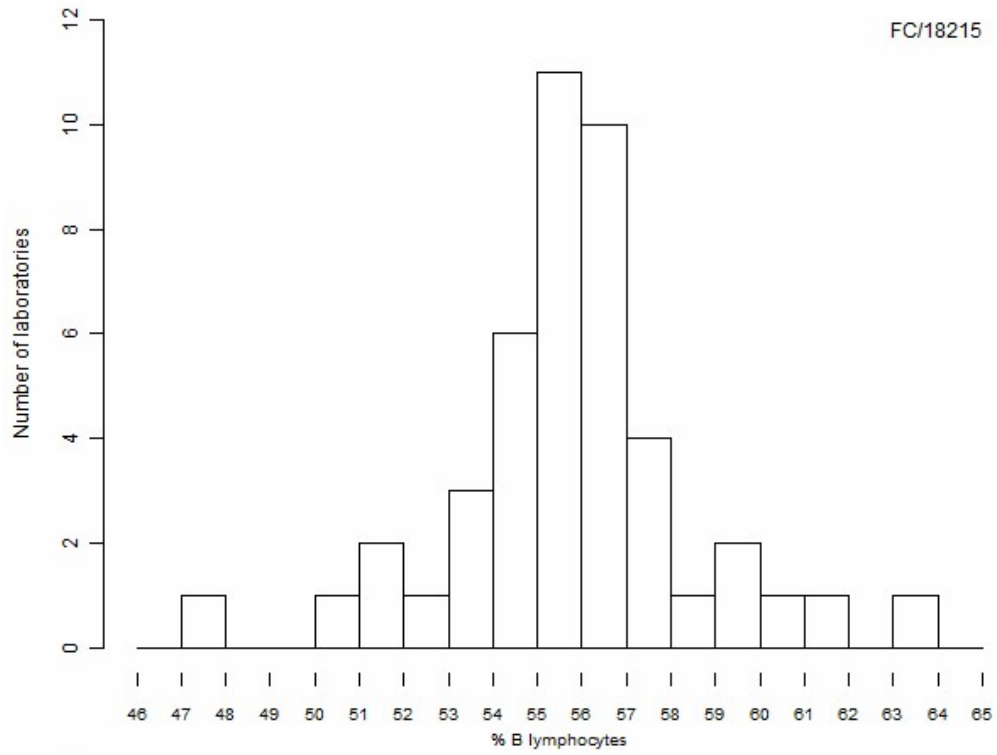
NKcells 10E9/L



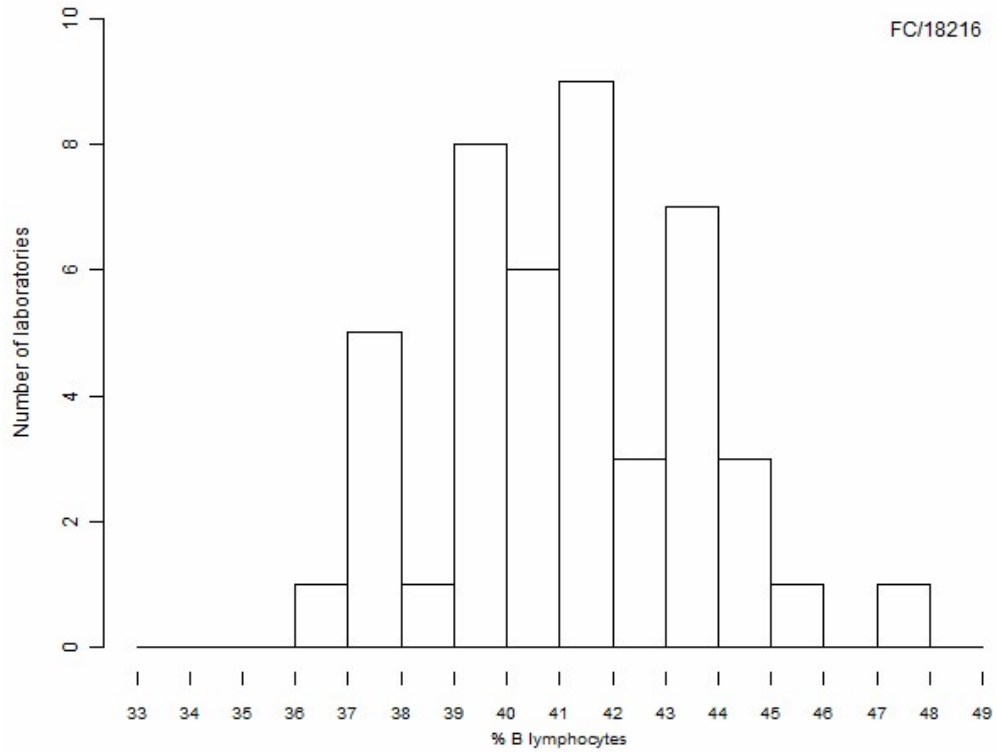
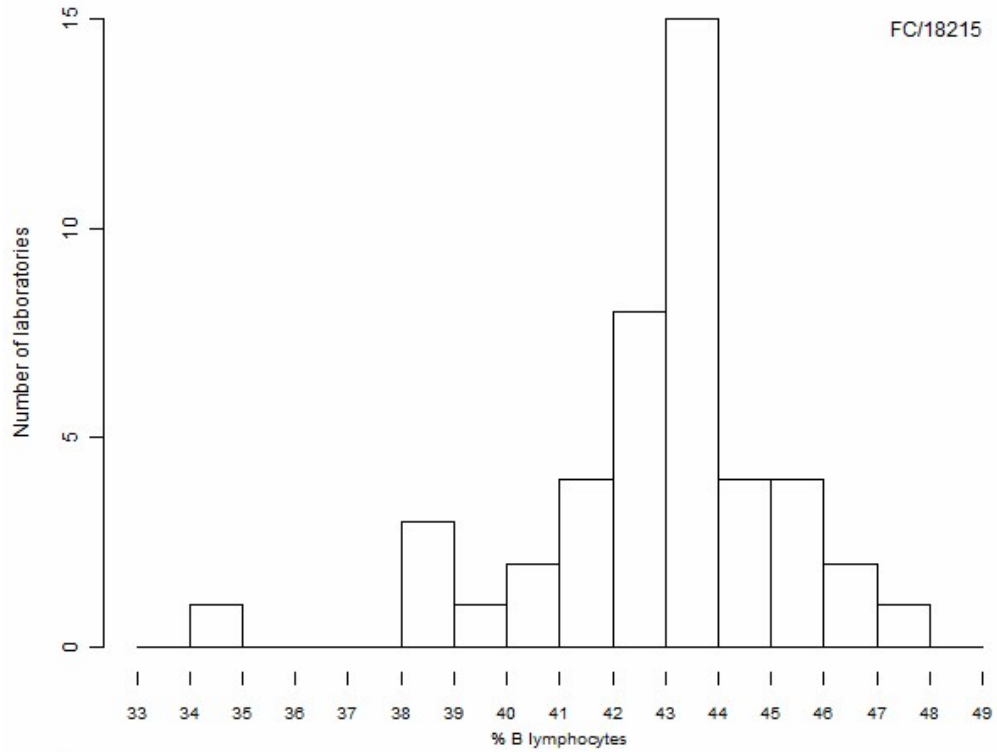
Results not represented on the graph

FC/18215 = 1.724 10e9
FC/18216 = 0.09 10e9/l
FC/18216 = 0.11 10e9/l
FC/18216 = 0.13 10e9/l
FC/18216 = 0.13 10e9/l
FC/18216 = 0.144 10e9
FC/18216 = 0.986 10e9

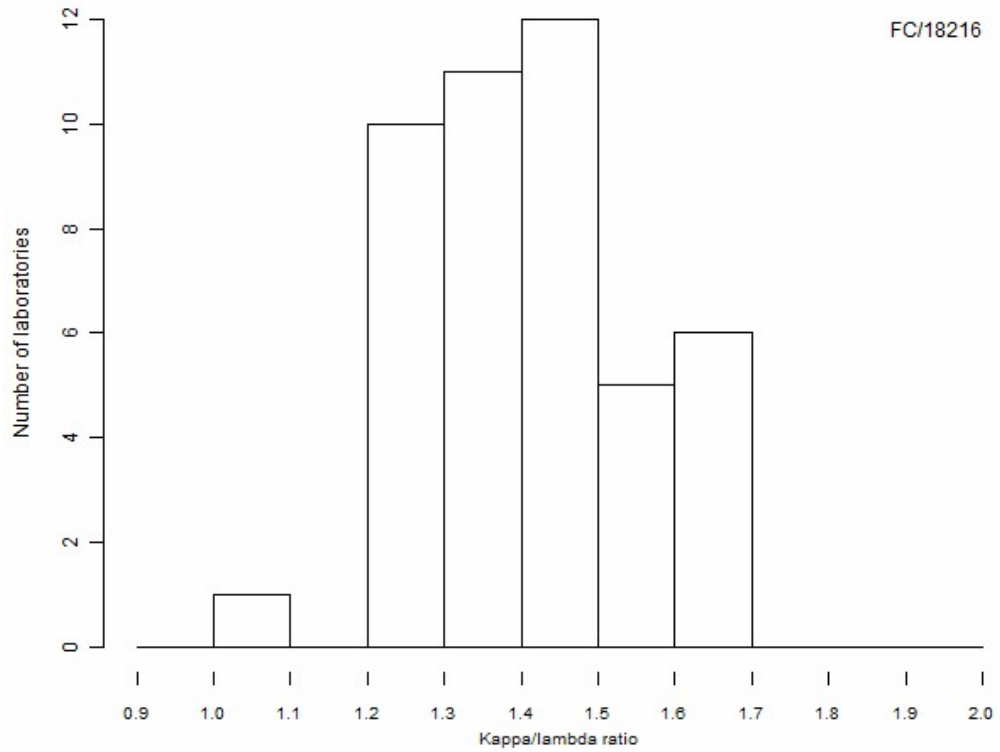
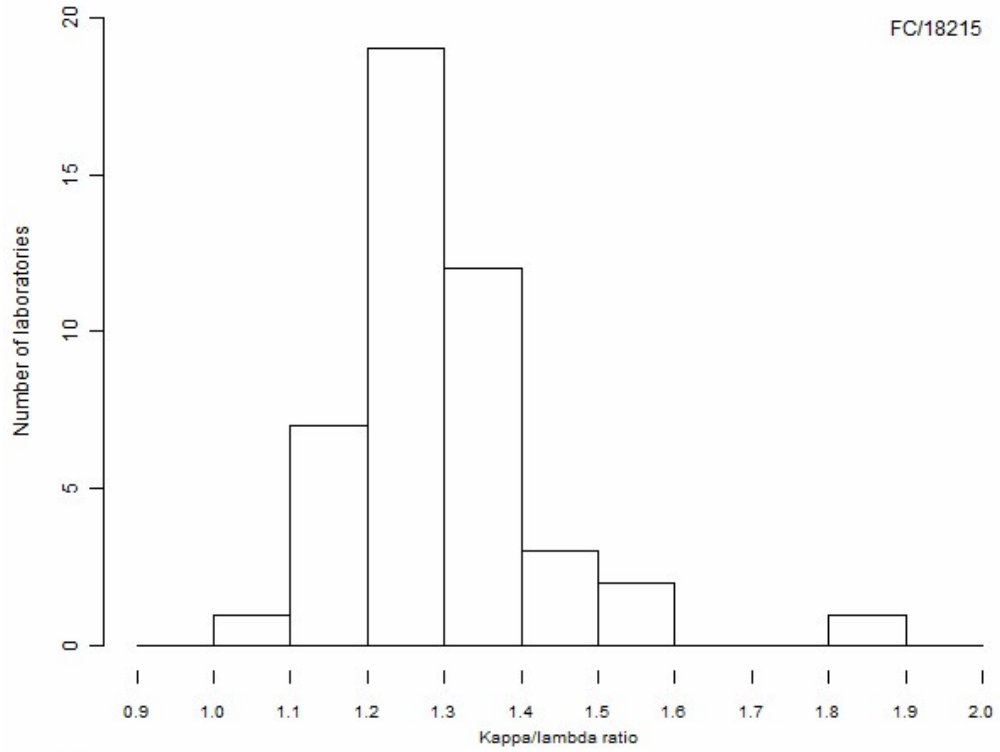
Kappa % B lymphocytes



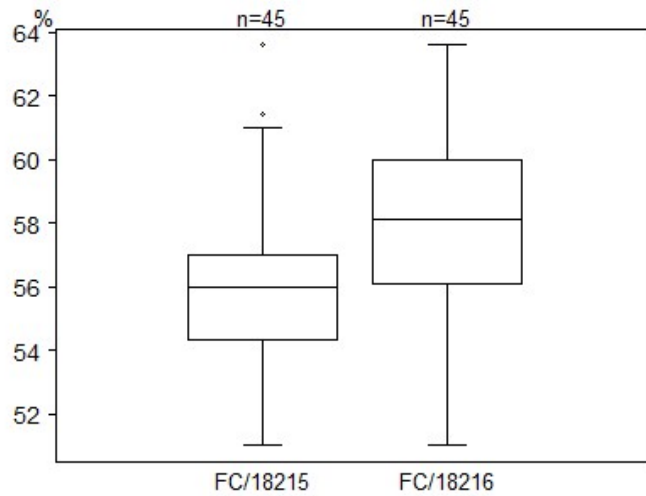
Lambda % B lymphocytes



Kappa/lambda

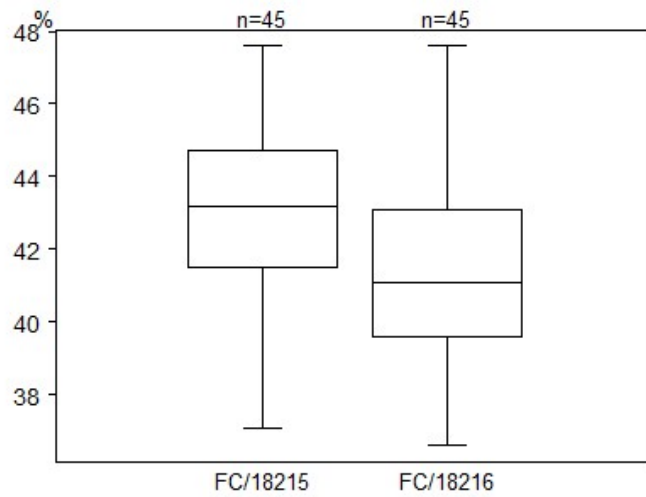


Kappa % B lymphocytes



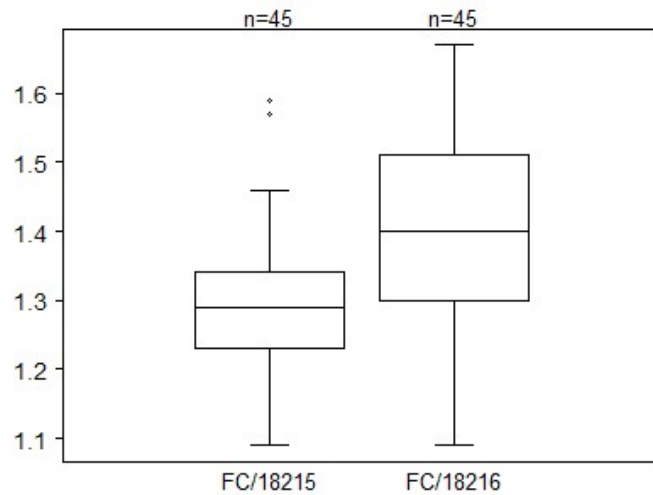
Results not represented
on the graph
FC/18215 = 48 %

Lambda % B lymphocytes



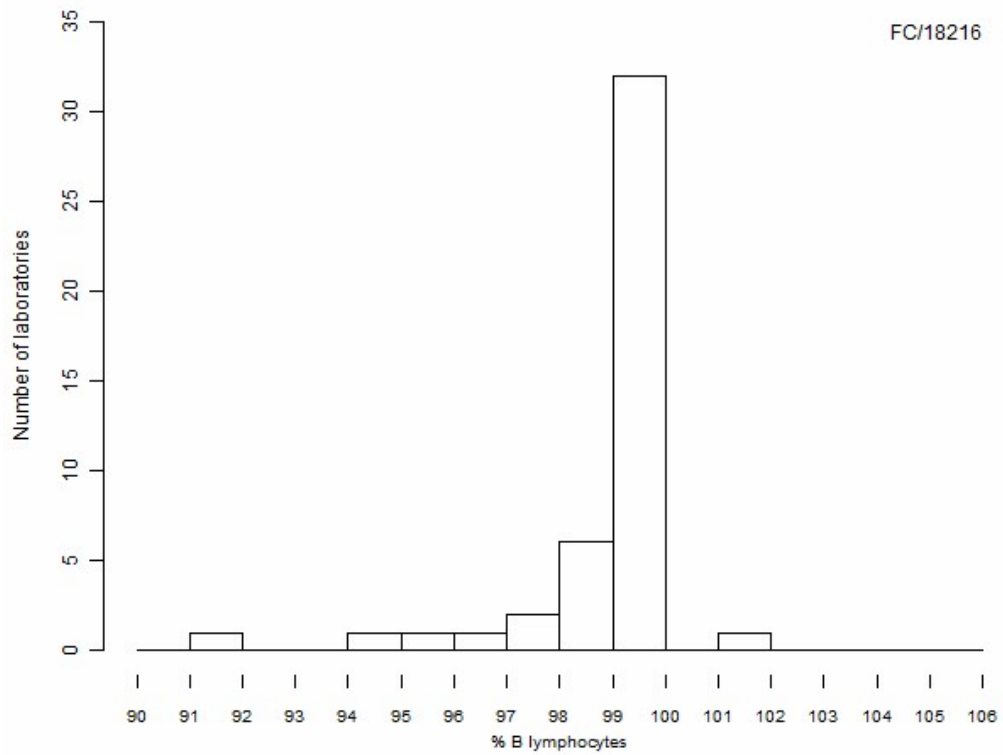
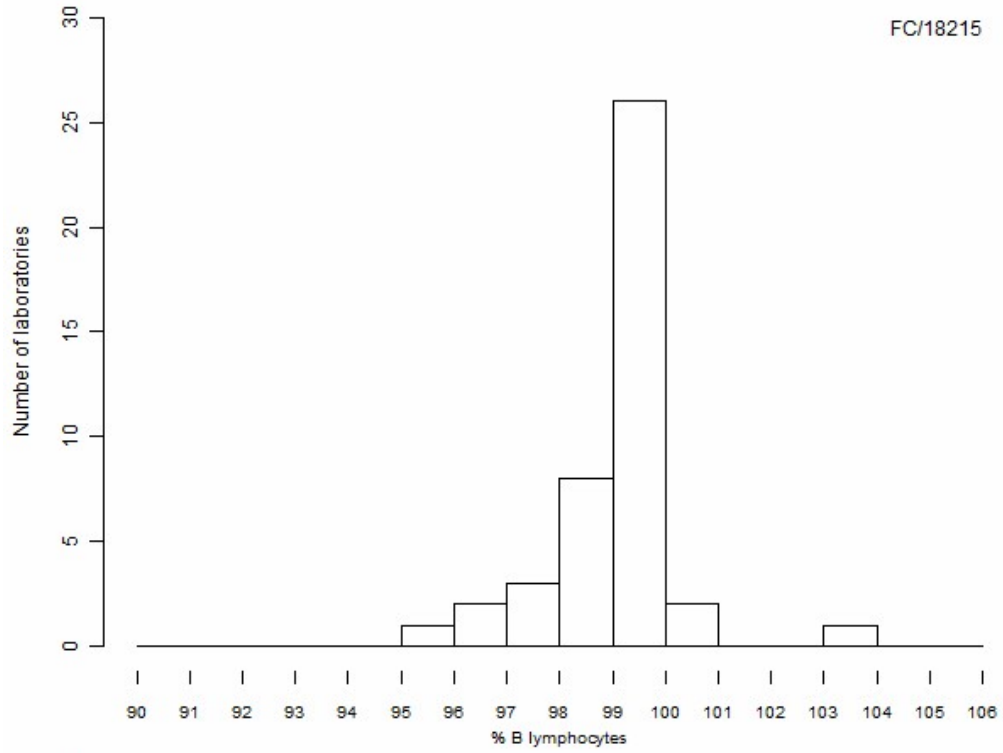
Results not represented
on the graph
FC/18215 = 34.3 %

Kappa/lambda

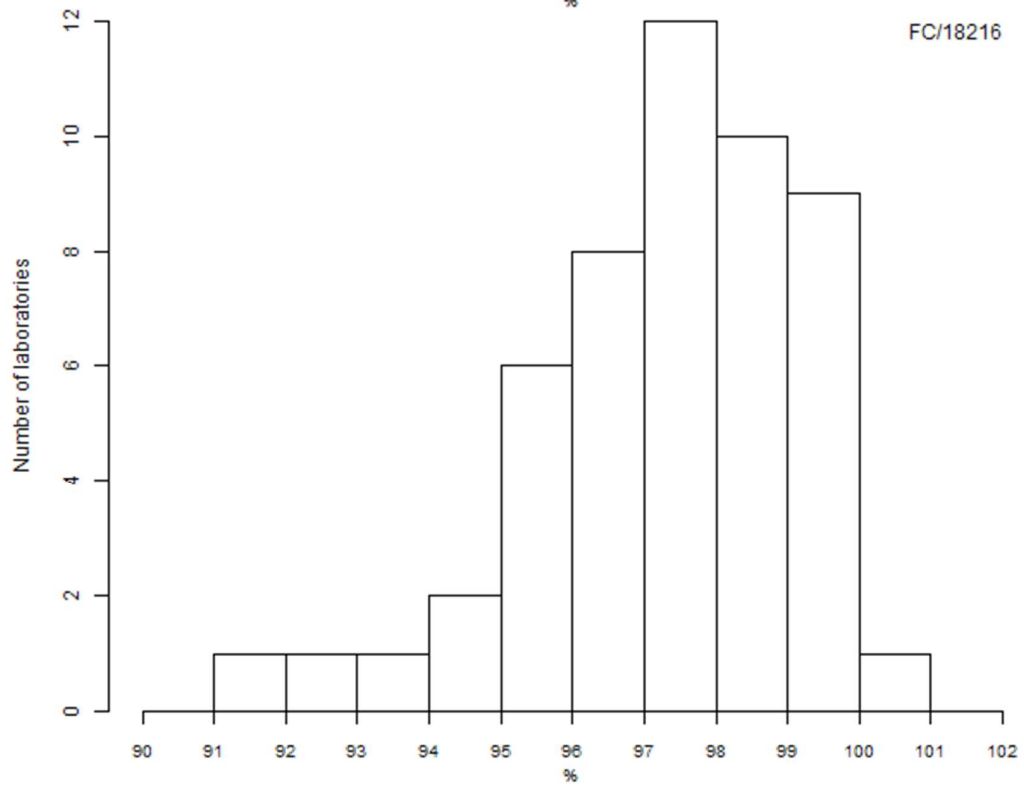
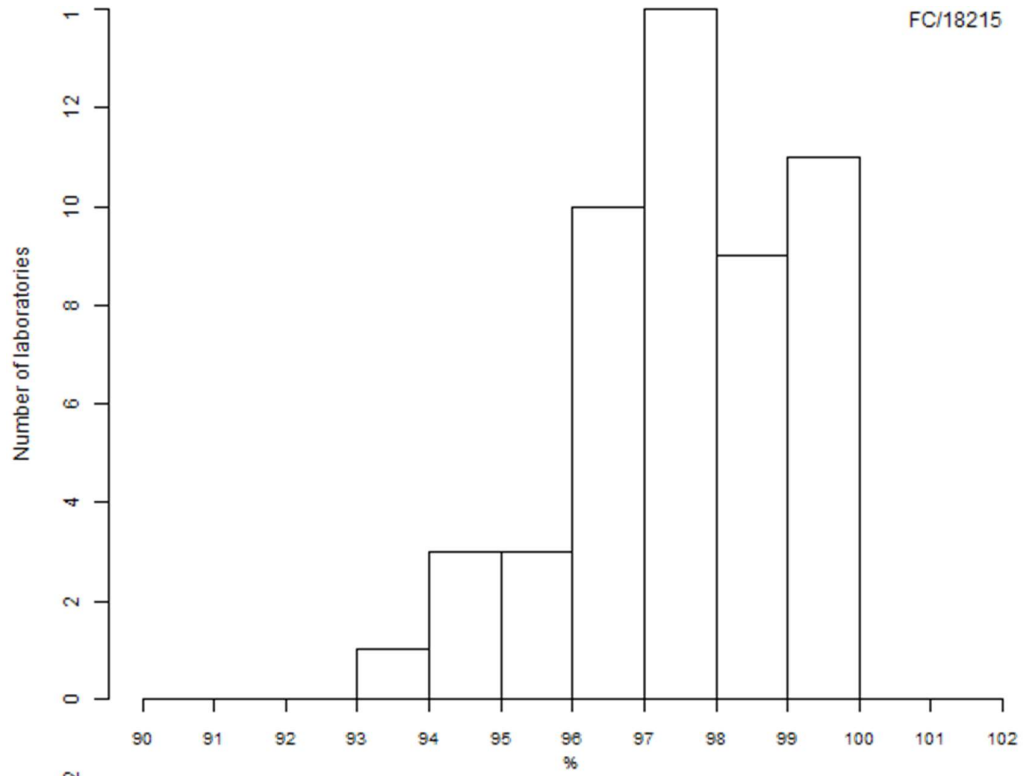


Results not represented
on the graph
FC/18215 = 1.85

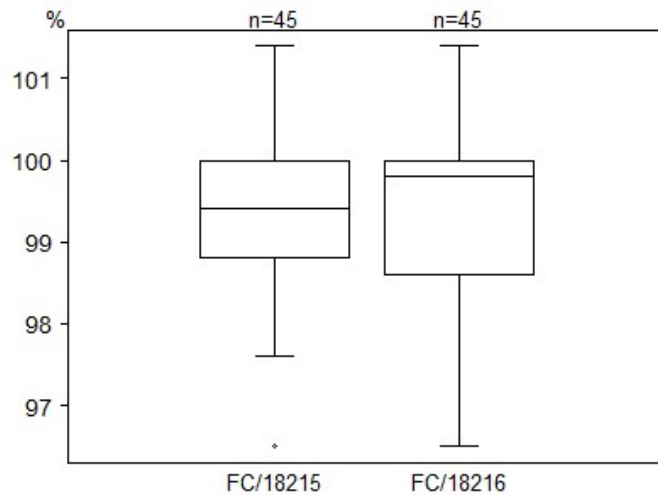
Sum K+L % B lymphocytes



Lymphosum %



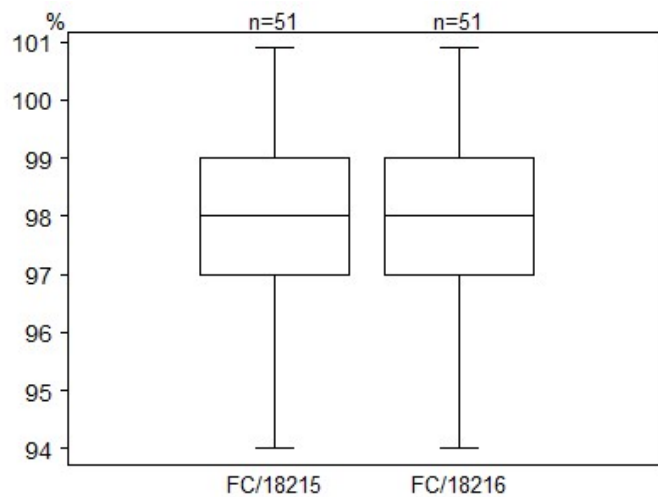
Sum K+L % B lymphocytes



Results not represented on the graph

FC/18215 = 88.7 %
FC/18215 = 90 %
FC/18215 = 95.6 %
FC/18215 = 104 %
FC/18216 = 92 %
FC/18216 = 94.1 %
FC/18216 = 96 %

Lymphosum %



Results not represented on the graph

FC/18216 = 92 %
FC/18216 = 93 %

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 \pm 5%, with a maximum variability of \leq 10%.

END

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