

**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 2022/1

Sciensano/Flow cytometry/81-E

Biological health risks
Quality of laboratories
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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 the brochures available on our website (only in Dutch and French):

https://www.wiv-isp.be/QML/index_nl.htm

→ select in the proposed menu :

ALGEMENE INFORMATIEBROCHURE EKE

→ select in the proposed menu “Brochures”:

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

- Statistische methoden gebruikt voor EKE
- Verwerking van gecensureerde waarden

https://www.wiv-isp.be/QML/index_fr.htm

→ select in the proposed menu :

BROCHURE D'INFORMATION GENERALE EEQ

→ select in the proposed menu “Brochures”:

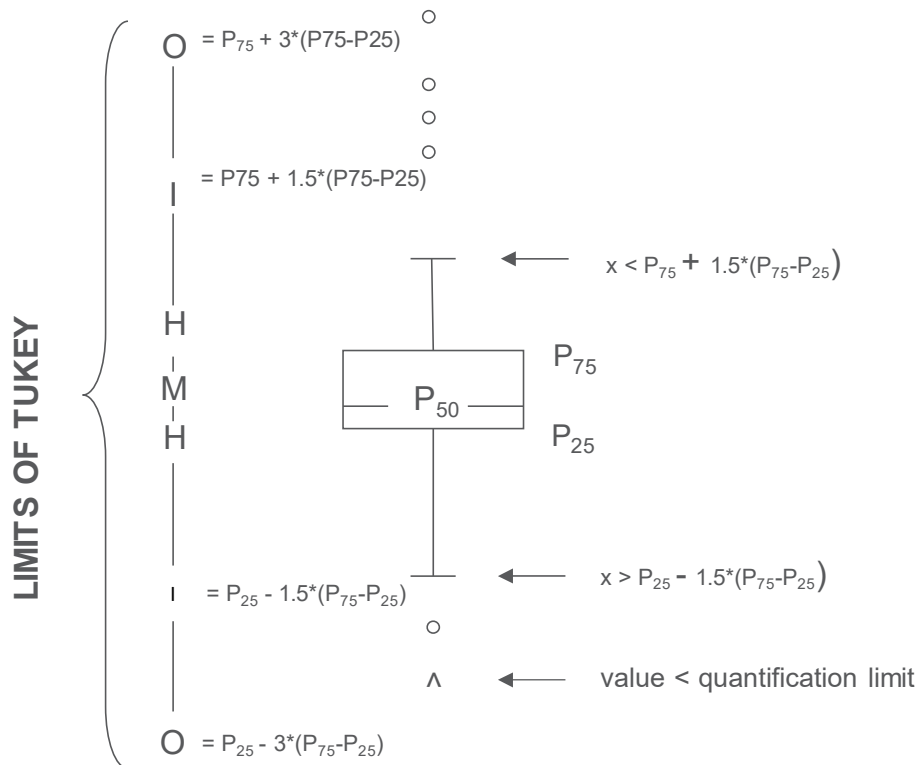
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_fr/brochures.htm

- 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/18847 and FC/18848) 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):

FC18847

	%	10 ⁹ /L
Leukocytes		9.4
Lymphocytes	26.9	
CD3⁺ cells	61.9	1.57
CD4⁺CD3⁺ cells	32.5	0.82
CD8⁺CD3⁺ cells	28.5	0.72
CD19⁺ cells	13.1	0.33
NK cells	22.7	0.57
κ % B lymphocytes	63.3	
λ % B lymphocytes	36.5	
κ/λ ratio	1.73	

FC18848

	%	10 ⁹ /L
Leukocytes		5.7
Lymphocytes	40.7	
CD3⁺ cells	78.3	1.82
CD4⁺CD3⁺ cells	64.1	1.49
CD8⁺CD3⁺ cells	13.5	0.31
CD19⁺ cells	12.8	0.30
NK cells	7.7	0.18
κ % B lymphocytes	58.8	
λ % B lymphocytes	41.2	
κ/λ ratio	1.43	

PARTICIPATION

Fifty-two laboratories (1 Canadian and 51 Belgian clinical laboratories) participated in the survey 2022/1 (send-out of blood samples on 21st February 2022 (day 0)).

RESULTS

100% of the Belgian laboratories received the samples on day 1 or 2. 42 laboratories (82%) received the samples on day 1 and nine (18%) received them on day 2.

71% (n=36) of the Belgian laboratories performed the analyses on day 1, 27% (n=14) on day 2 and 2% (n=1) 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.

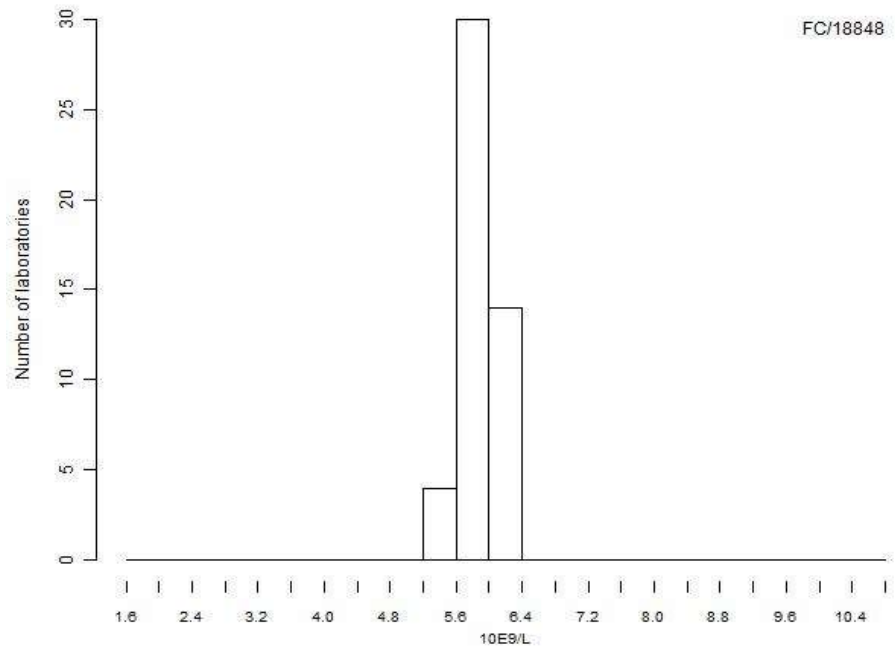
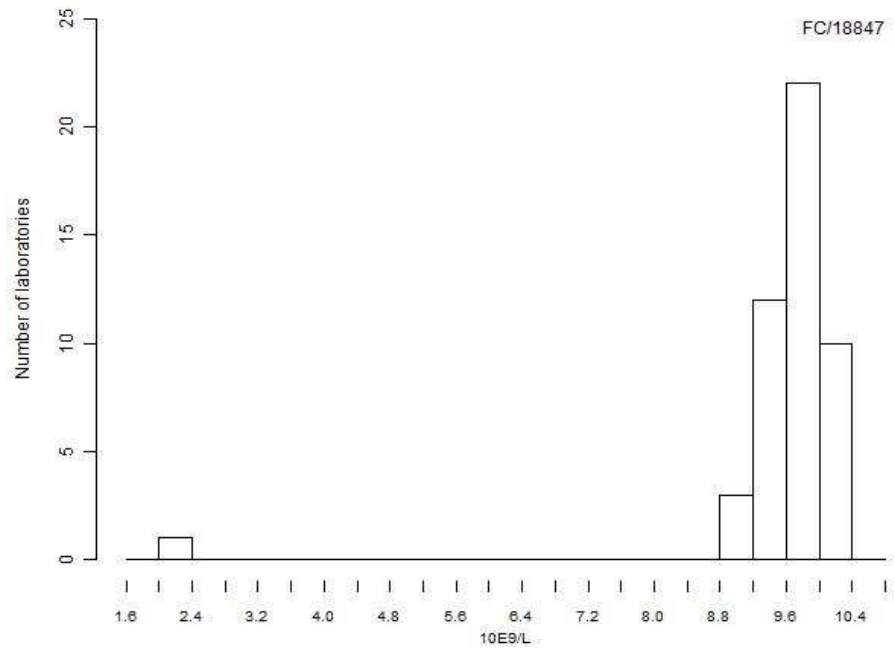
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=51). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=50).

The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/18847 and FC/18848:

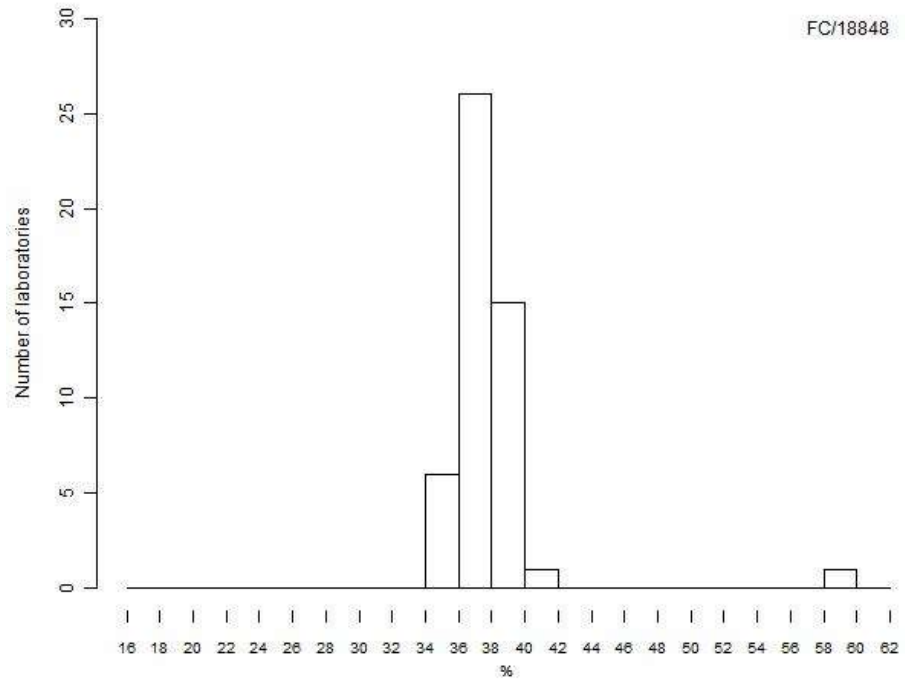
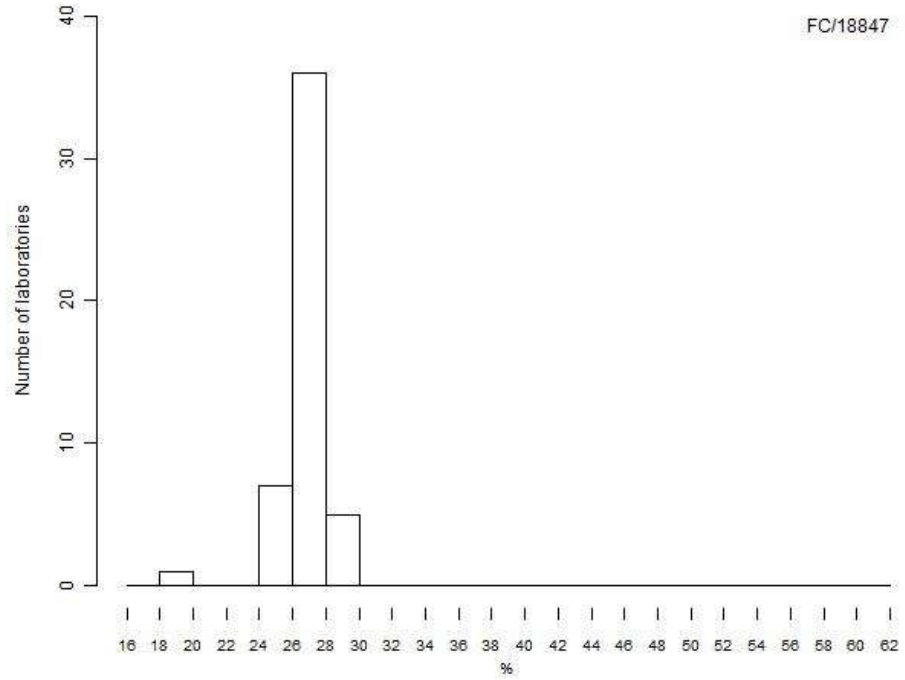
FC/18847	Median	SD	CV,%	N
WBC 10E9/L	9.78	0.34	3.5	48
Lympho% haematology analyser	26.9	0.9	3.4	47
Lympho% flow cytometer	26.7	2.1	8.0	45
CD3 %	64.0	2.6	4.0	50
CD3 10E9/L	1.689	0.107	6.4	48
CD4 %	34.9	2.6	7.4	50
CD4 10E9/L	0.920	0.073	7.9	48
CD8 %	27.4	0.8	3.0	50
CD8 10E9/L	0.719	0.050	6.9	48
CD19 %	12.7	1.3	9.9	50
CD19 10E9/L	0.331	0.048	14.5	48
NKcells %	22.0	2.4	11.1	50
NKcells 10E9/L	0.571	0.090	15.7	48
Kappa % B lymphocytes	62.1	3.2	5.1	43
Lambda % B lymphocytes	37.1	2.7	7.2	43
Kappa/lambda	1.67	0.20	12.0	43
Sum K+L % B lymphocytes	99.6	1.0	1.0	43
Lymphosum %	98.8	1.9	1.9	50

FC/18848	Median	SD	CV,%	N
WBC 10E9/L	5.92	0.18	3.0	48
Lympho% haematology analyser	37.6	1.1	3.0	47
Lympho% flow cytometer	36.9	3.6	9.6	45
CD3 %	79.5	2.5	3.2	50
CD3 10E9/L	1.779	0.118	6.6	48
CD4 %	65.4	2.4	3.7	50
CD4 10E9/L	1.460	0.109	7.5	48
CD8 %	12.8	0.5	4.0	50
CD8 10E9/L	0.286	0.019	6.7	48
CD19 %	12.5	1.6	12.4	50
CD19 10E9/L	0.280	0.028	10.0	48
NKcells %	7.3	1.8	24.3	50
NKcells 10E9/L	0.164	0.030	18.0	48
Kappa % B lymphocytes	58.9	1.9	3.3	43
Lambda % B lymphocytes	40.3	1.7	4.2	43
Kappa/lambda	1.46	0.11	7.6	43
Sum K+L % B lymphocytes	99.8	0.7	0.7	43
Lymphosum %	99.5	0.6	0.6	50

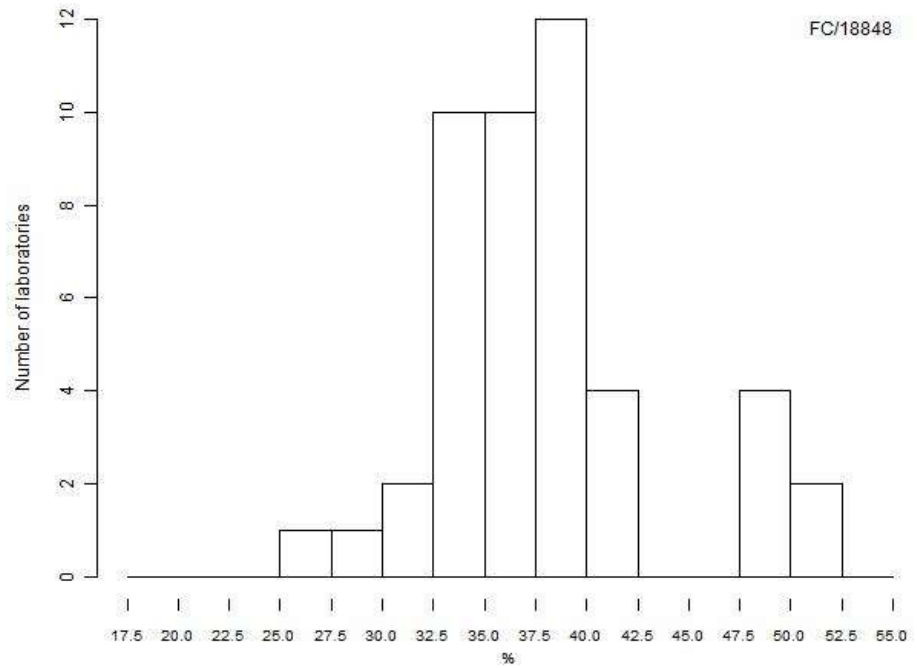
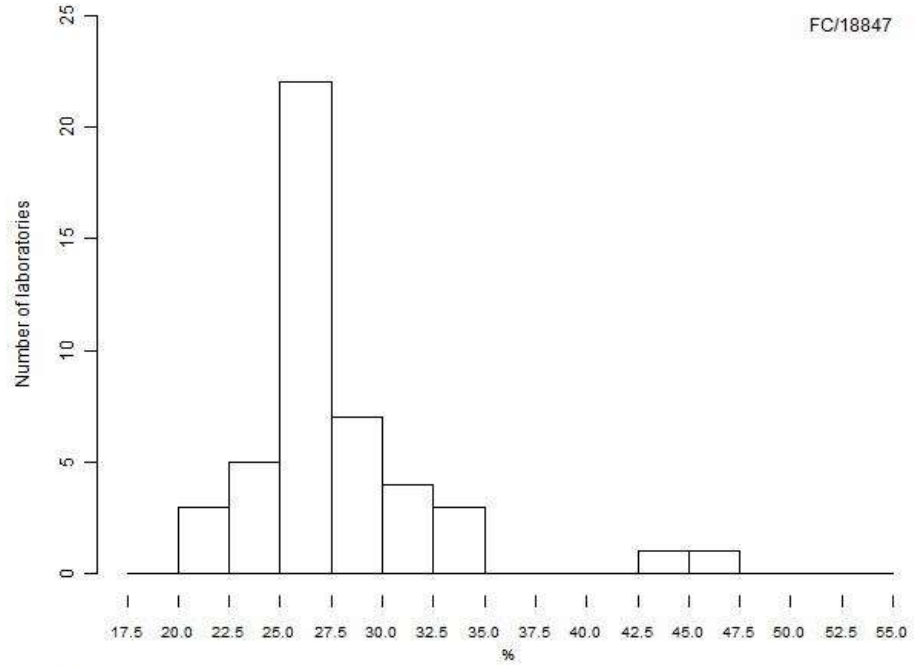
WBC 10E9/L



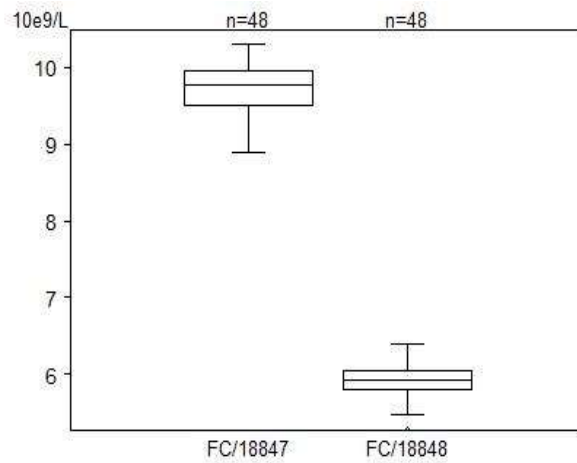
Lympho% haematology analyser



Lympho% flow cytometer



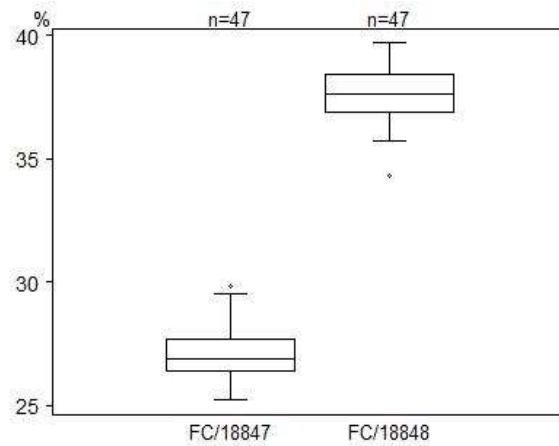
WBC 10E9/L



Results not represented on the graph

FC/18847 = 2.17 10e9/l
 FC/18847 = 9660 10e9/
 FC/18848 = 5900 10e9/

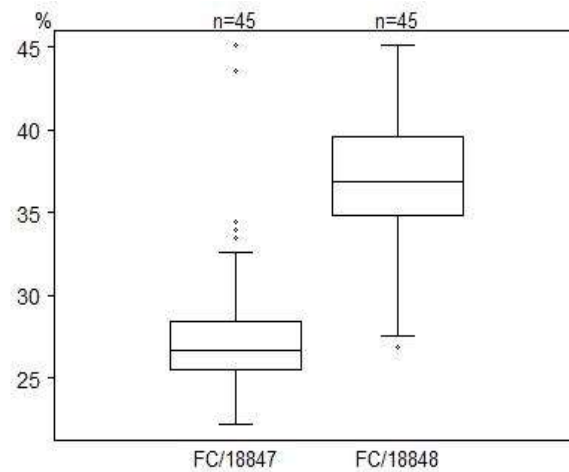
Lympho% haematology analyser



Results not represented on the graph

FC/18847 = 18.9 %
 FC/18847 = 24.2 %
 FC/18847 = 24.3 %
 FC/18847 = 24.4 %
 FC/18848 = 41.8 %
 FC/18848 = 58.2 %

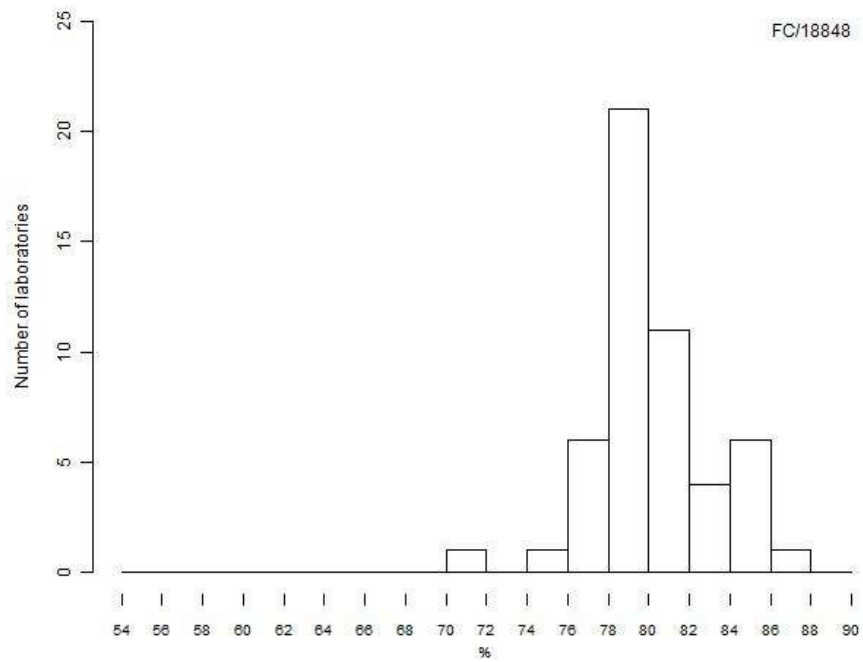
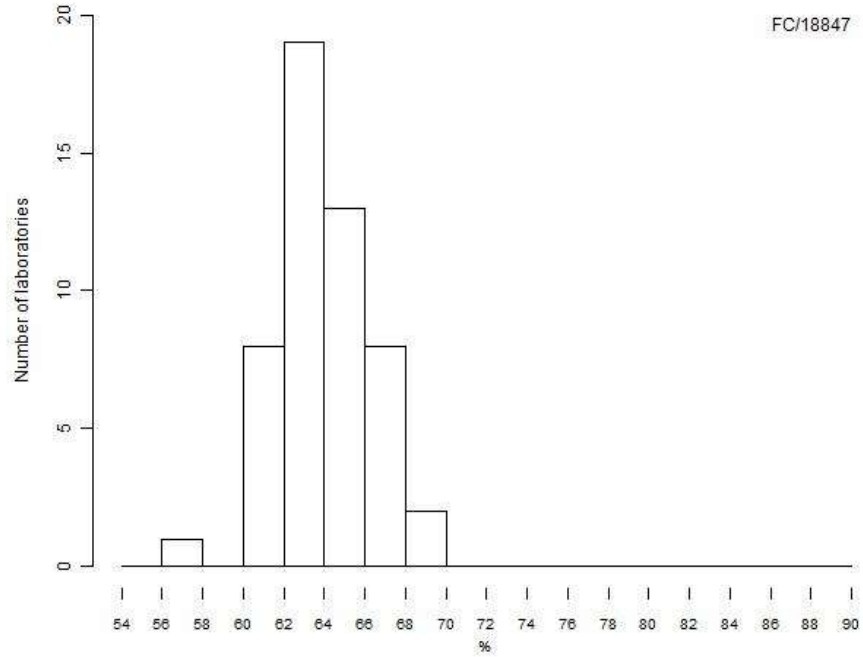
Lympho% flow cytometer



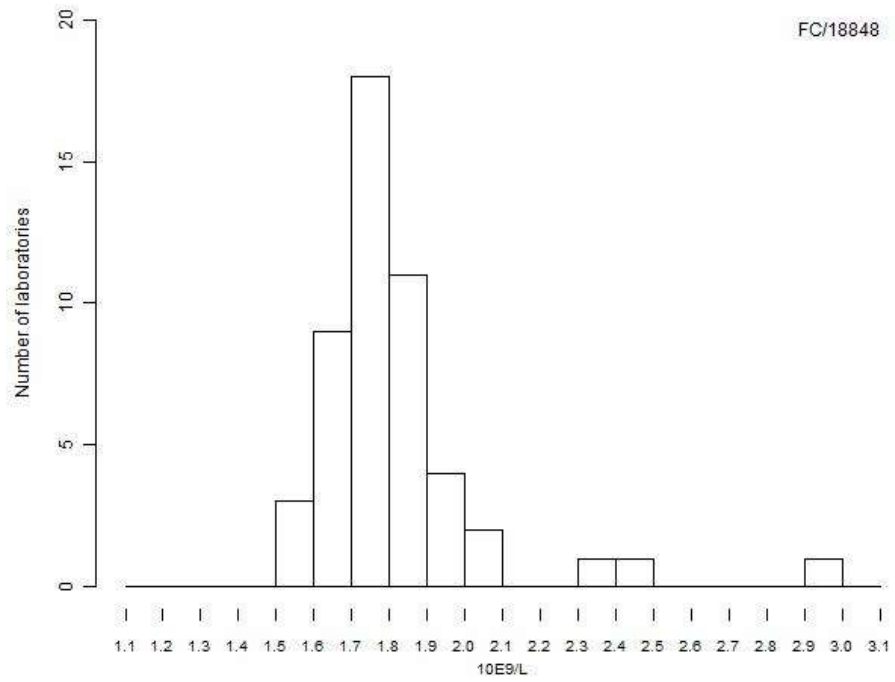
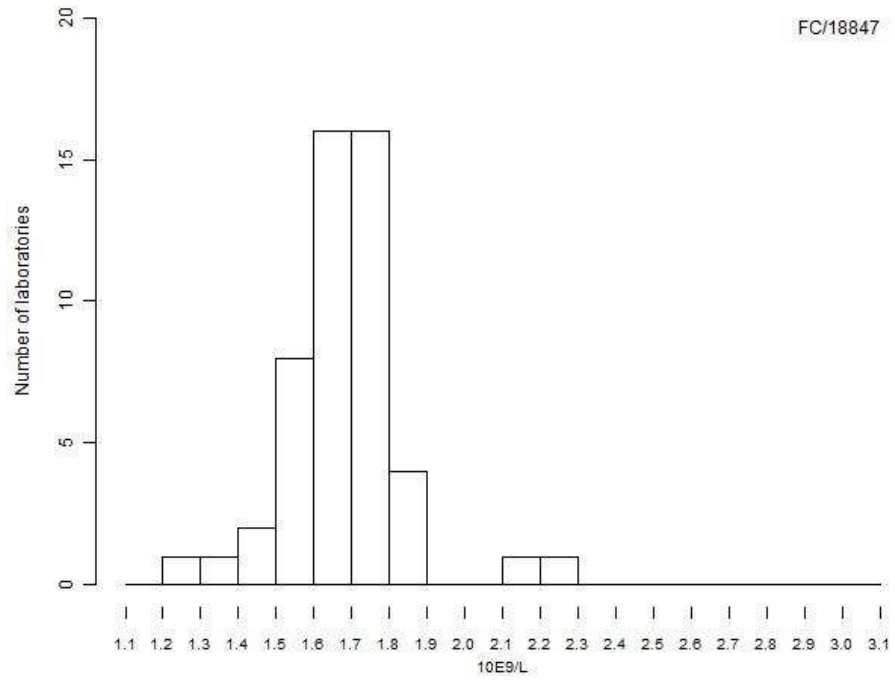
Results not represented on the graph

FC/18847 = 20.6 %
 FC/18848 = 47.8 %
 FC/18848 = 48.4 %
 FC/18848 = 49.5 %
 FC/18848 = 49.6 %
 FC/18848 = 51 %
 FC/18848 = 52.1 %

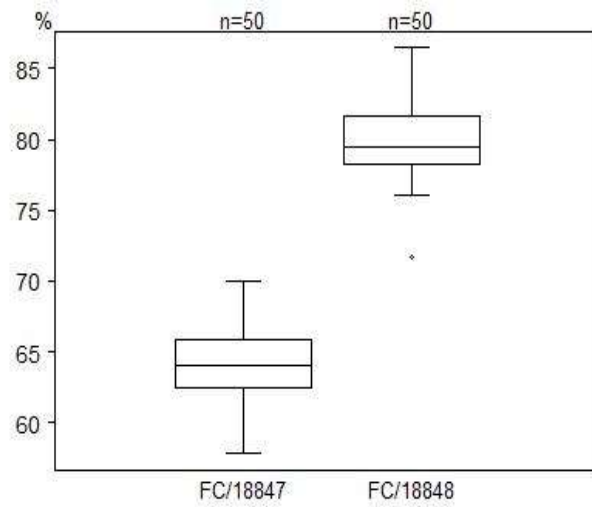
CD3 %



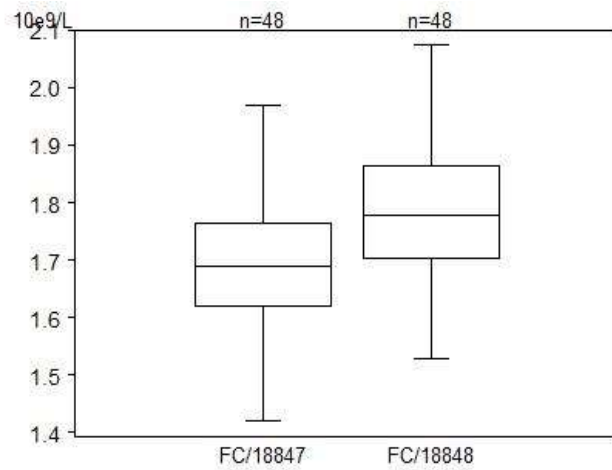
CD3 10E9/L



CD3 %



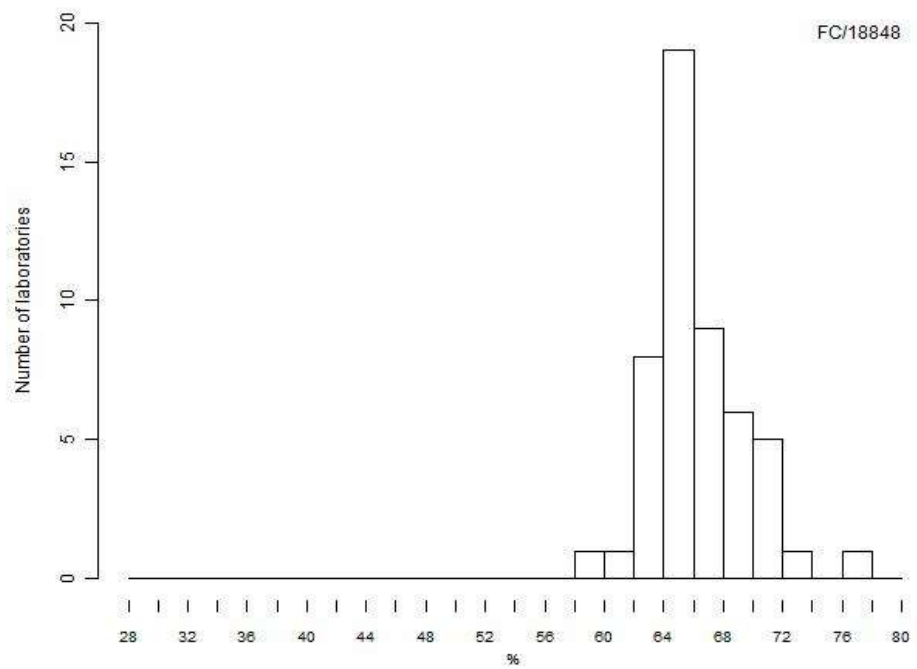
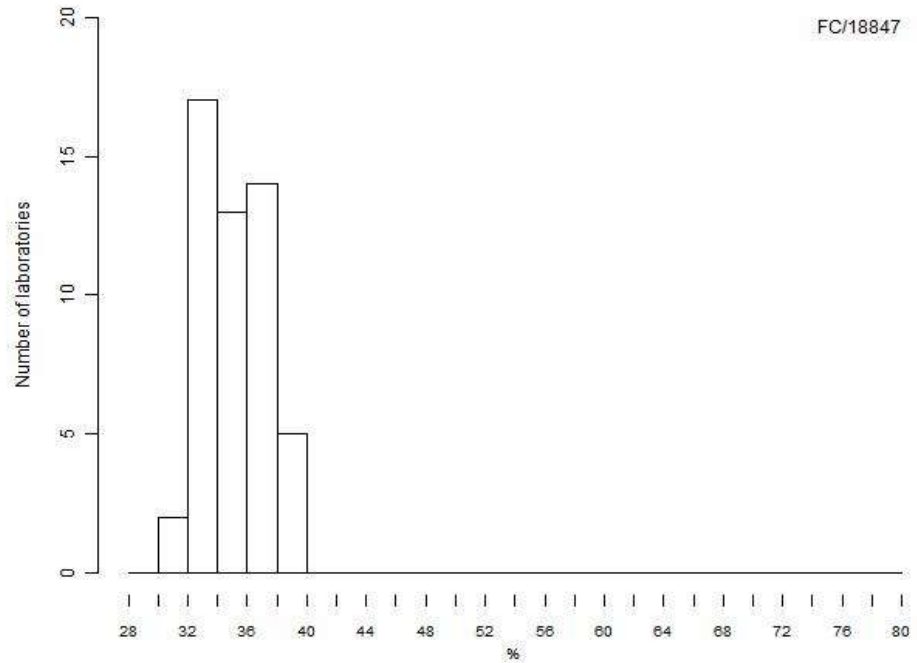
CD3 10E9/L



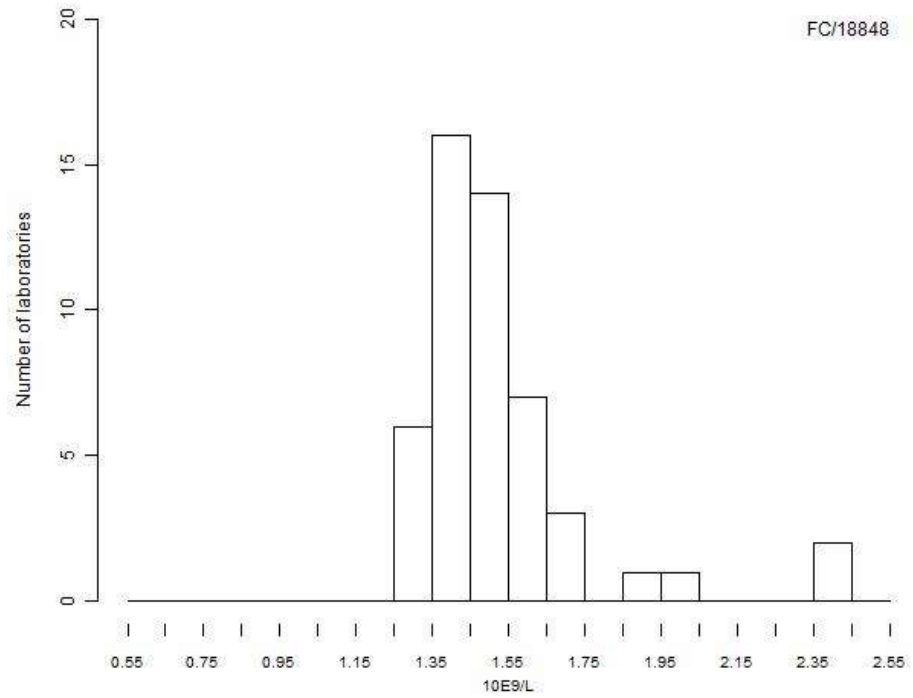
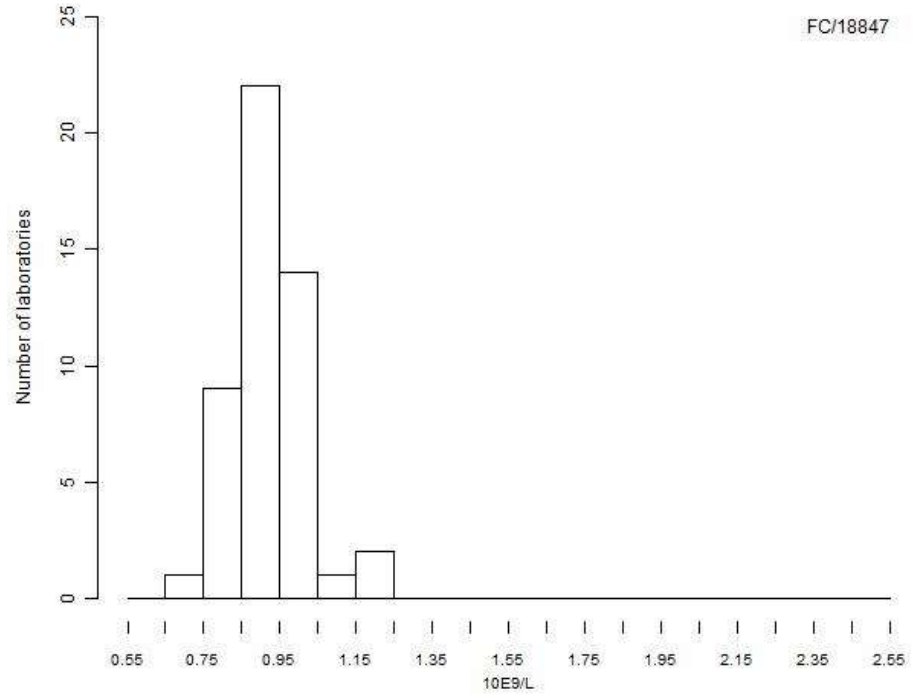
Results not represented
on the graph

FC/18847 = 1.29 10e9/l
FC/18847 = 2.14 10e9/l
FC/18847 = 2.203 10e9
FC/18848 = 2.35 10e9/l
FC/18848 = 2.421 10e9
FC/18848 = 2.915 10e9

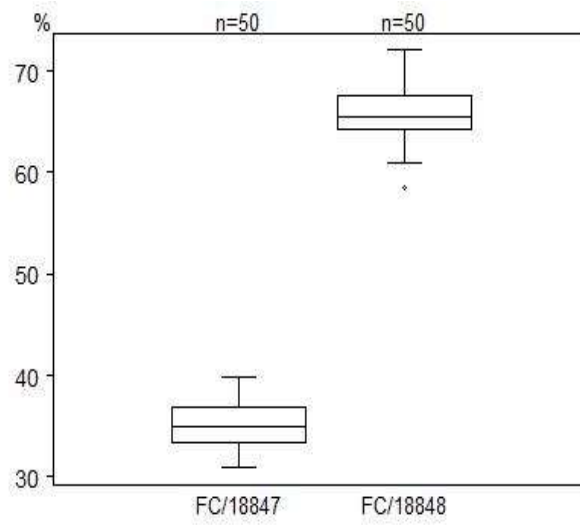
CD4 %



CD4 10E9/L

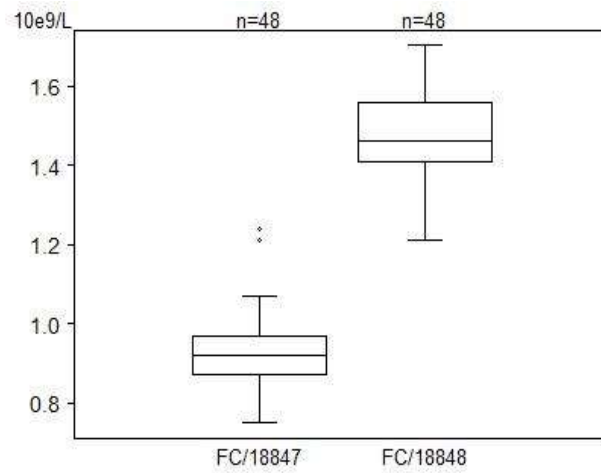


CD4 %



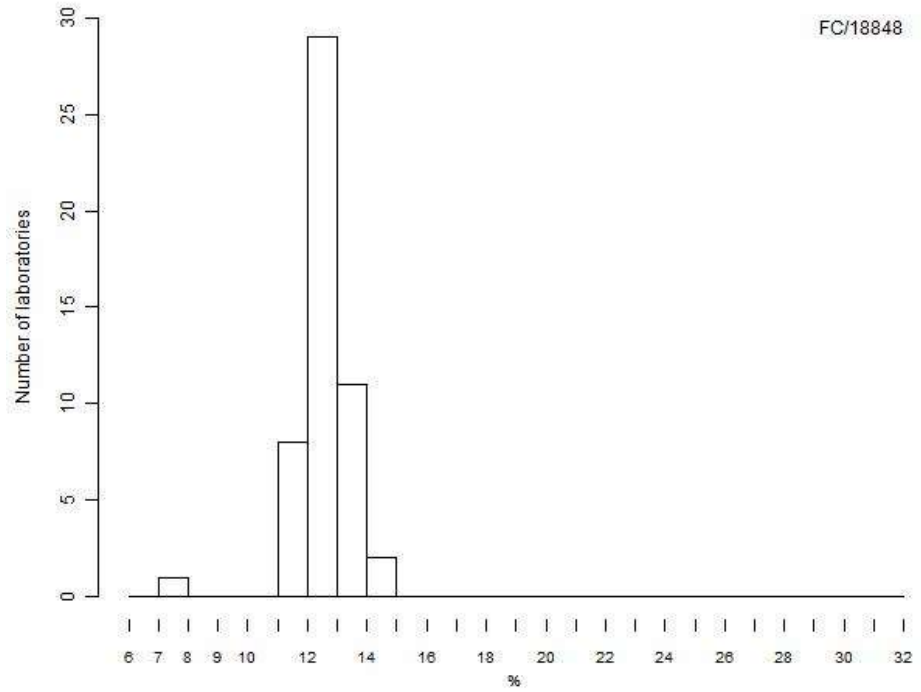
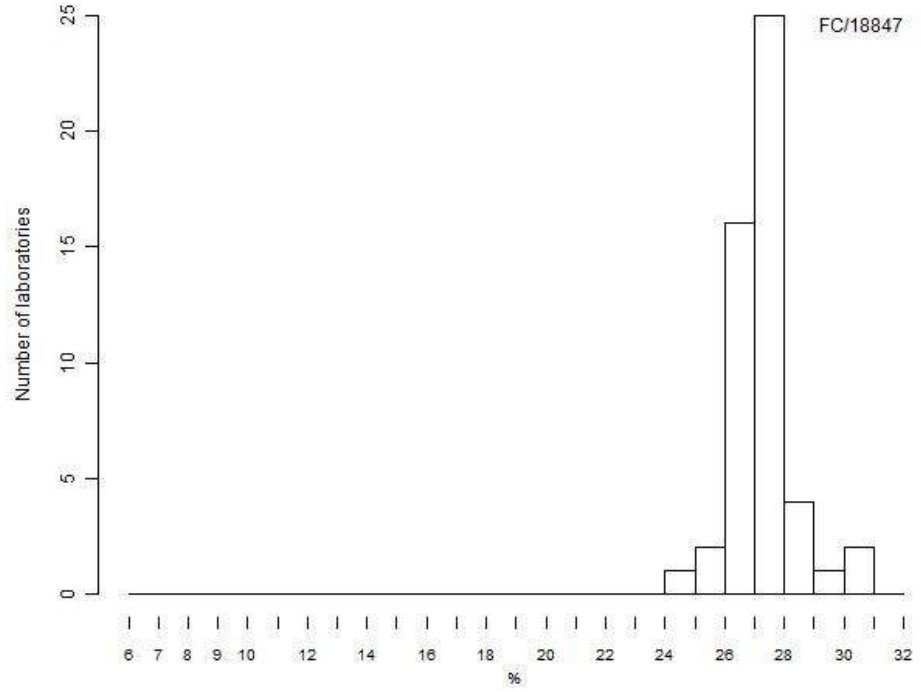
Results not represented
on the graph
FC/18848 = 76.1 %

CD4 10E9/L

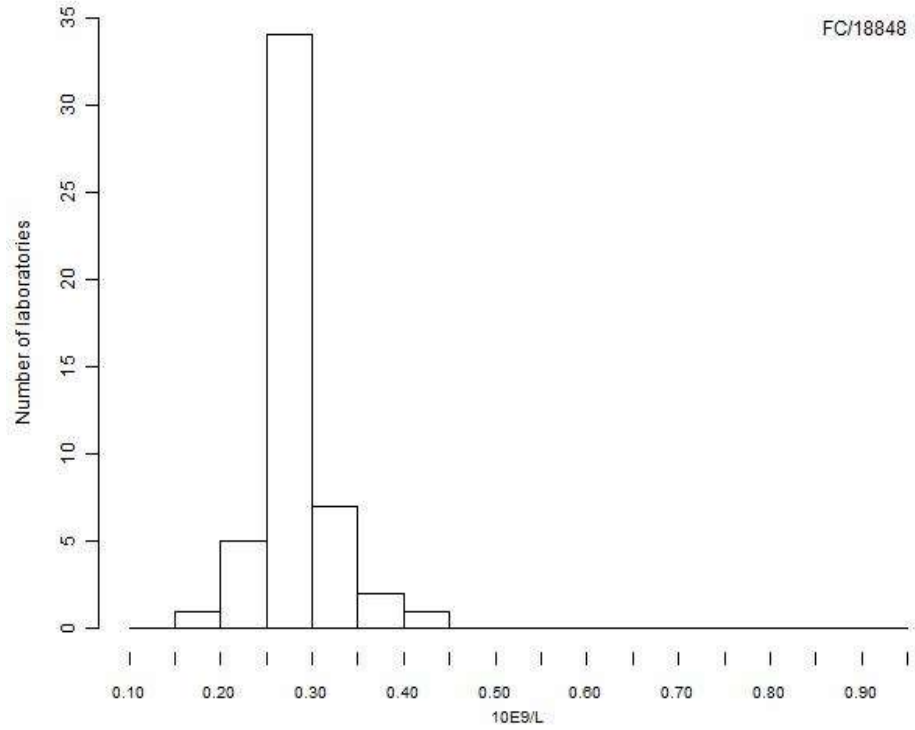
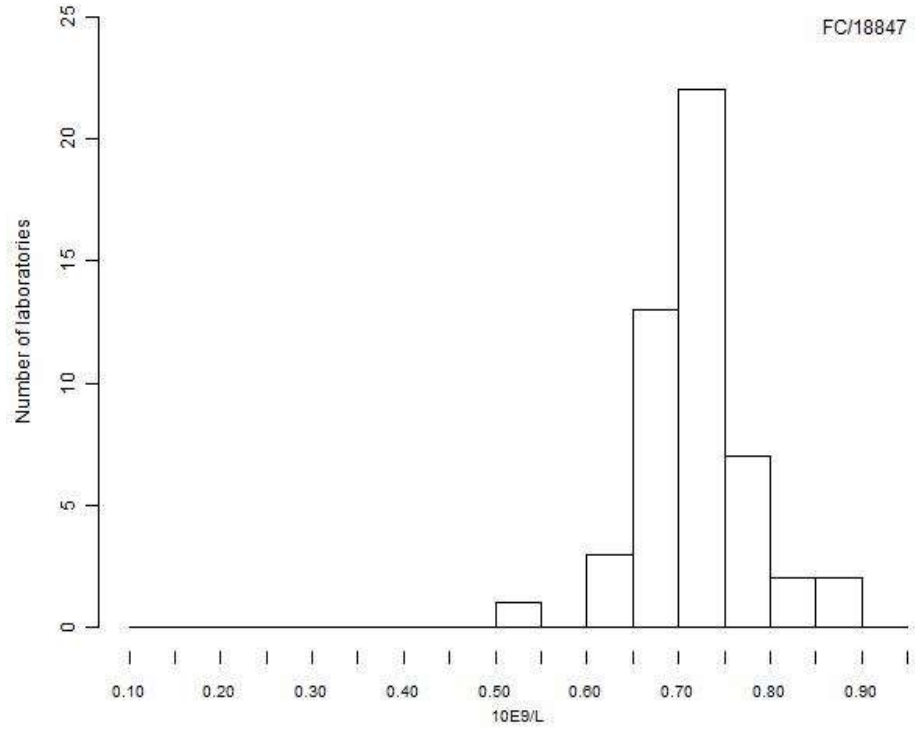


Results not represented
on the graph
FC/18847 = 9.37 10e9/l
FC/18848 = 1.95 10e9/l
FC/18848 = 2.015 10e9
FC/18848 = 2.374 10e9
FC/18848 = 2.443 10e9

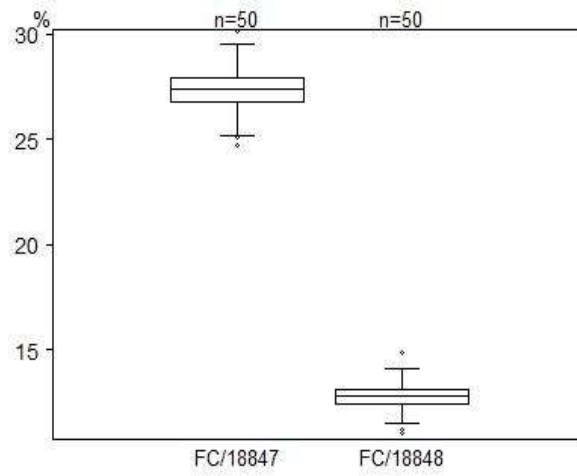
CD8 %



CD8 10E9/L



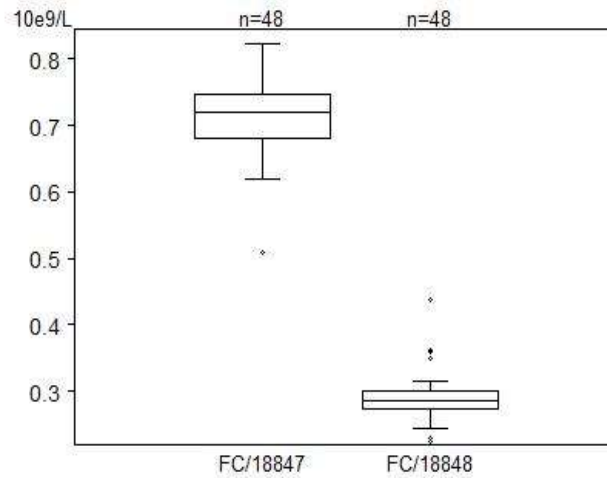
CD8 %



Results not represented on the graph

FC/18847 = 30.1 %
FC/18847 = 30.3 %
FC/18848 = 7.3 %

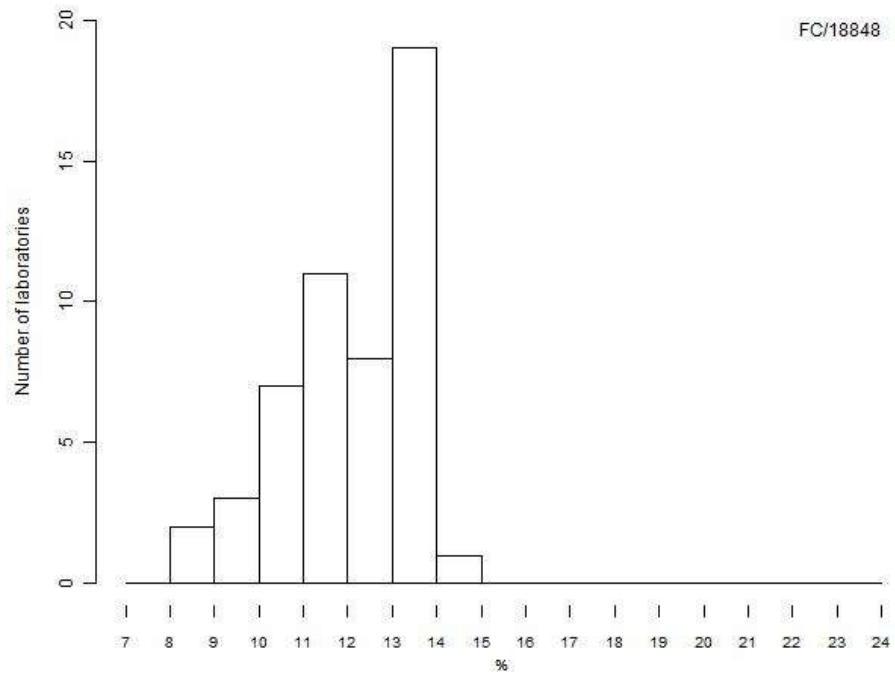
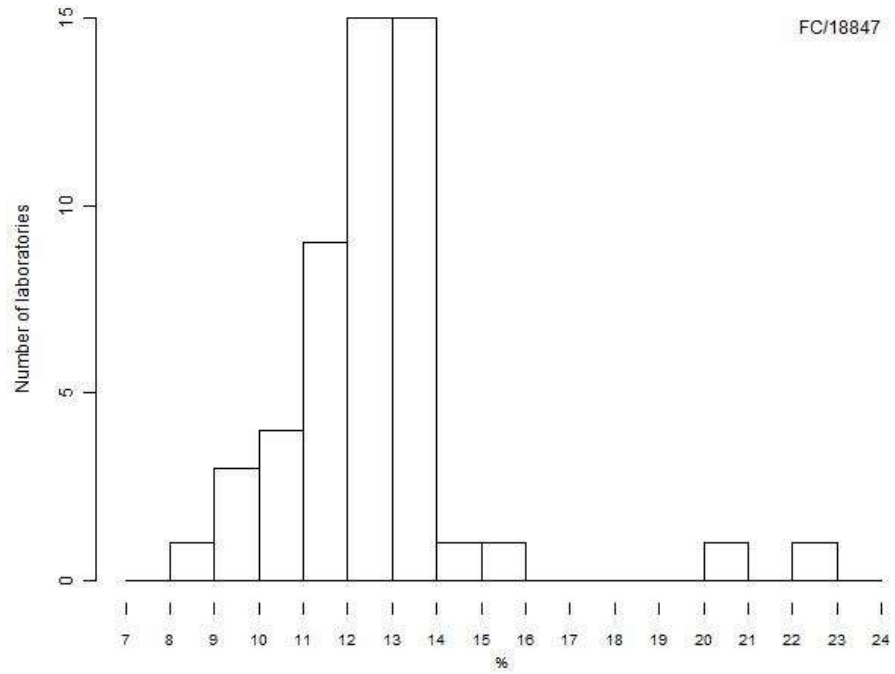
CD8 10E9/L



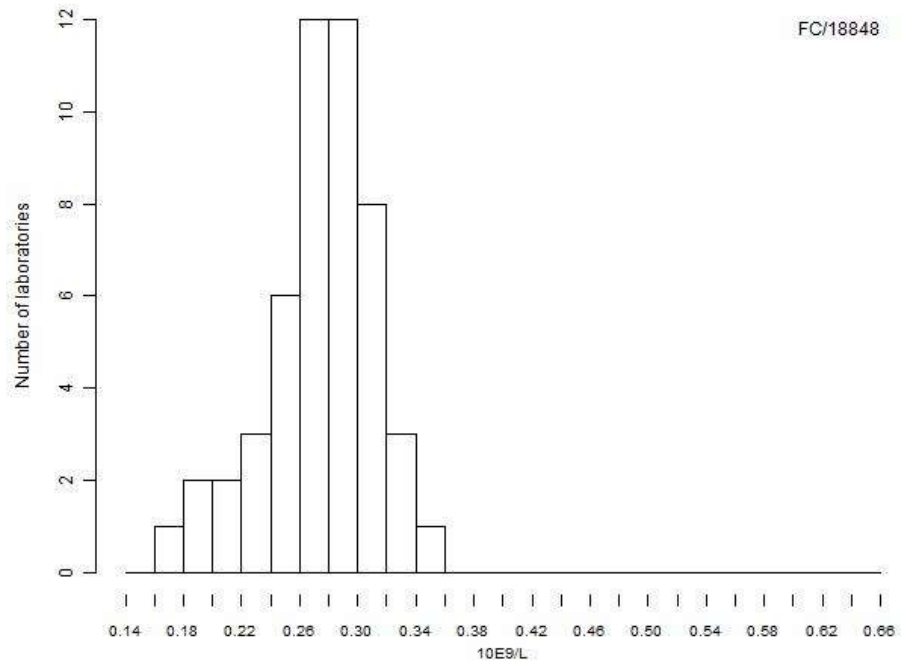
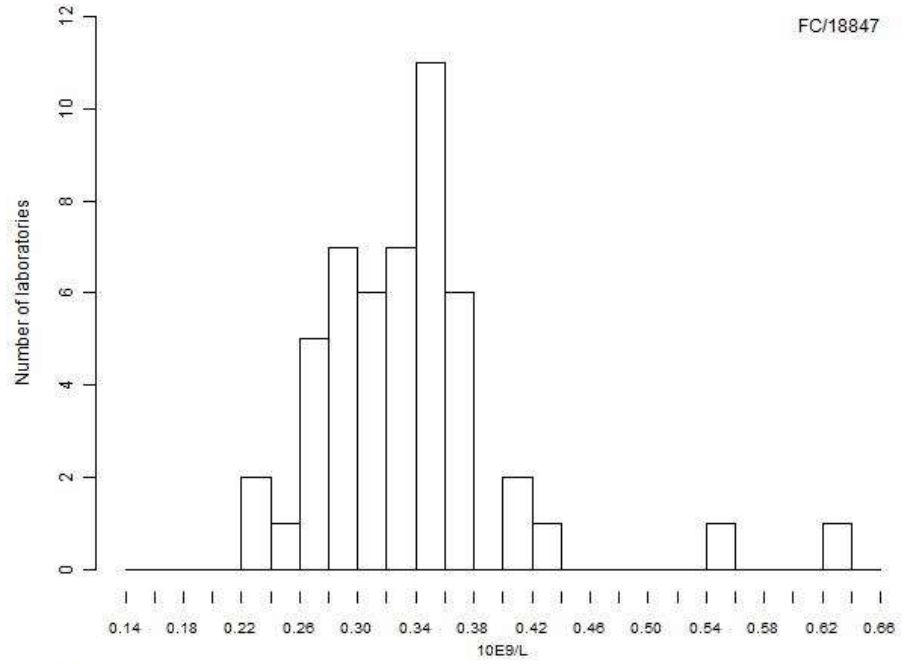
Results not represented on the graph

FC/18847 = 0.88 10e9/L
FC/18847 = 0.892 10e9
FC/18848 = 0.164 10e9

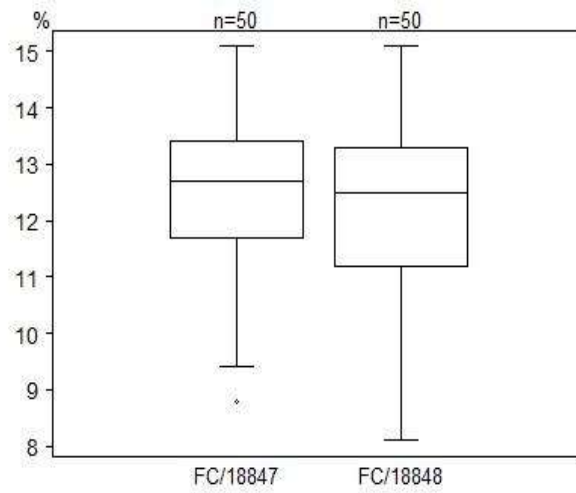
CD19 %



CD19 10E9/L



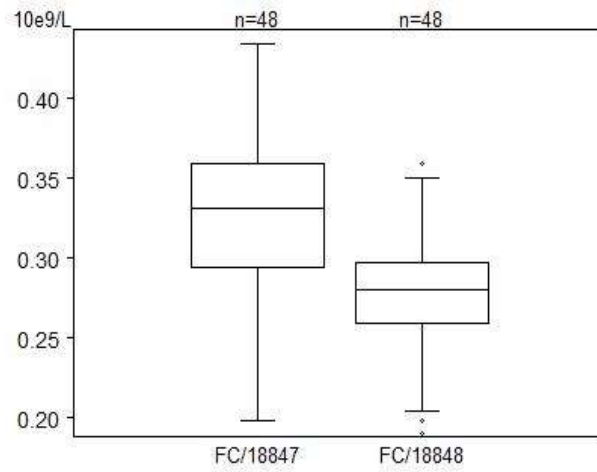
CD19 %



Results not represented on the graph

FC/18847 = 20.4 %
FC/18847 = 22.4 %

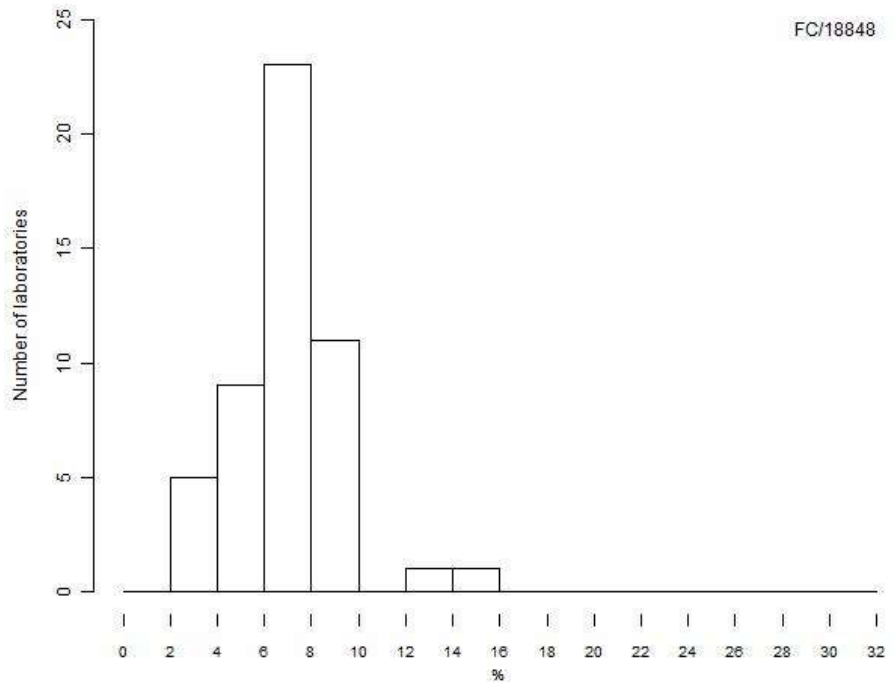
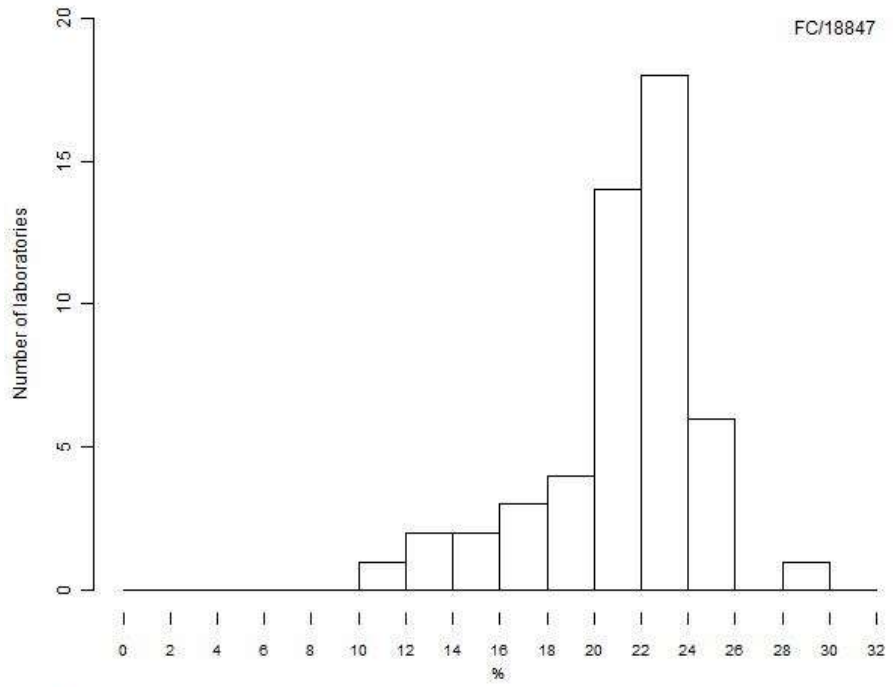
CD19 10E9/L



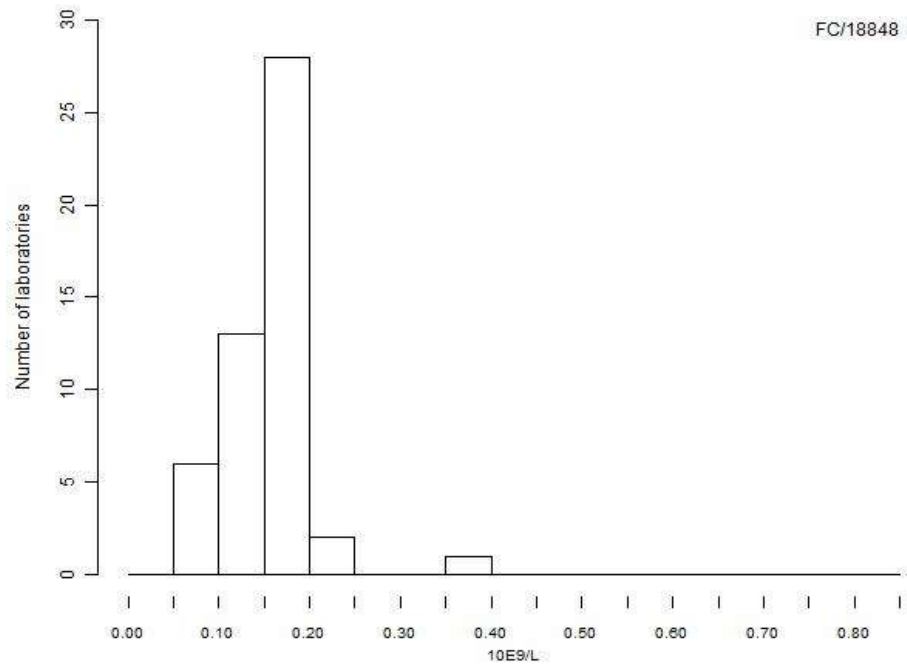
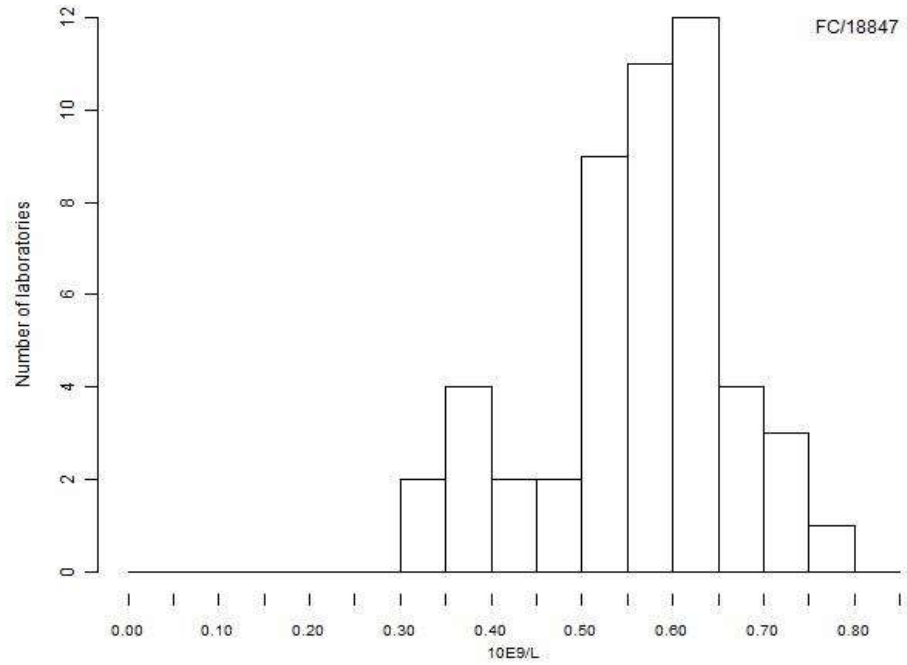
Results not represented on the graph

FC/18847 = 0.55 10e9/L
FC/18847 = 0.635 10e9
FC/18848 = 0.176 10e9

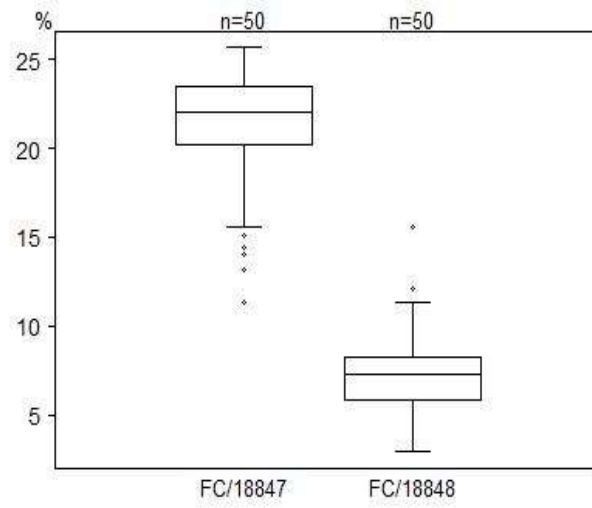
NKcells %



NKcells 10E9/L



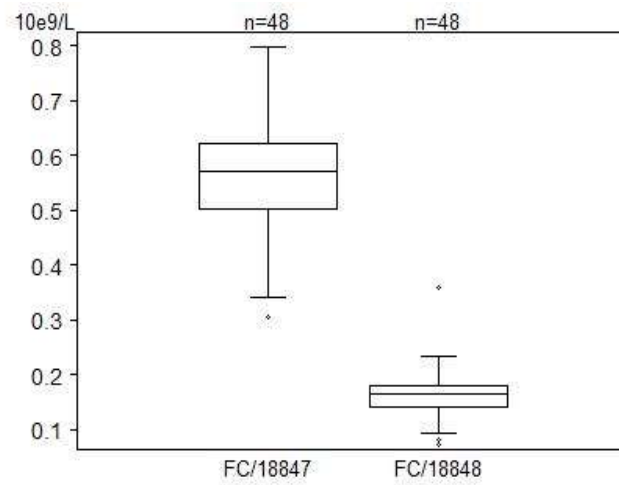
NKcells %



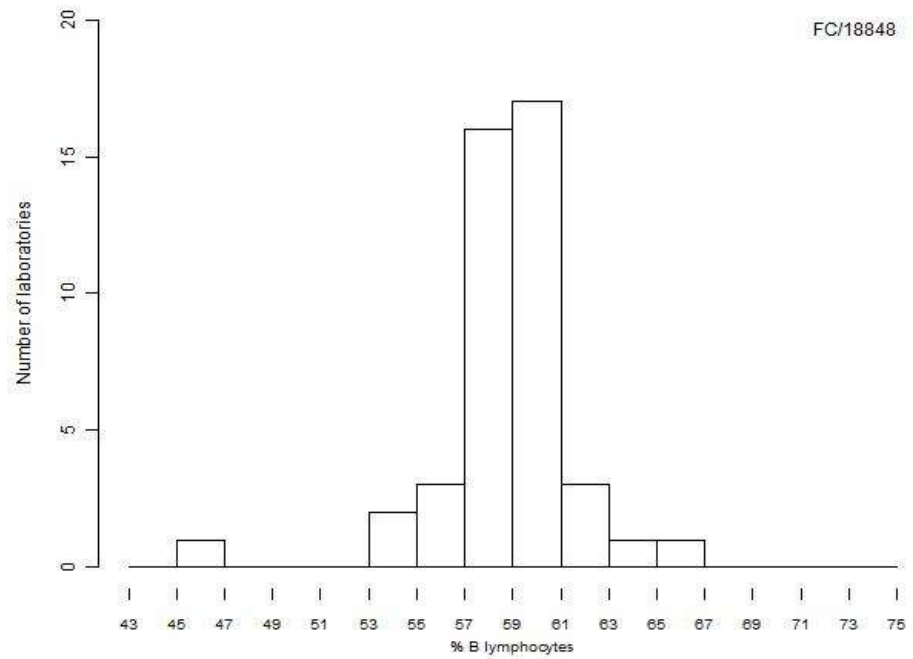
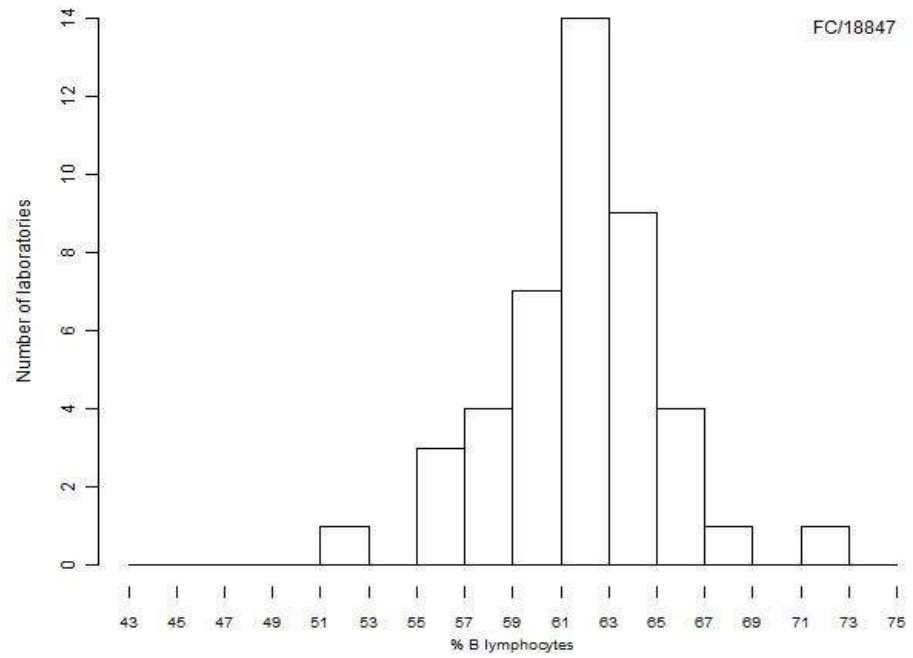
Results not represented
on the graph

FC/18847 = 29.2 %
FC/18848 = 67 %

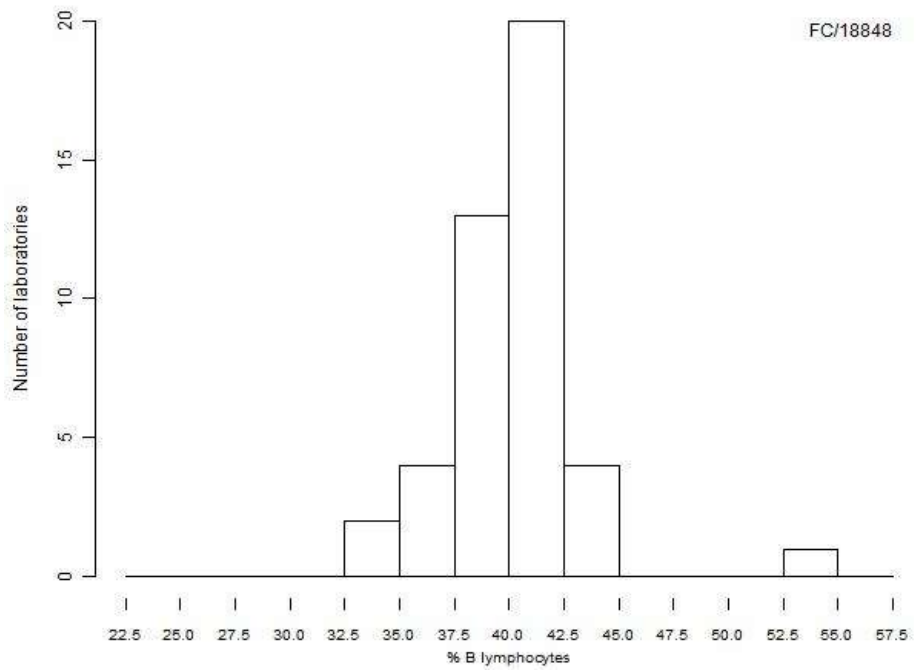
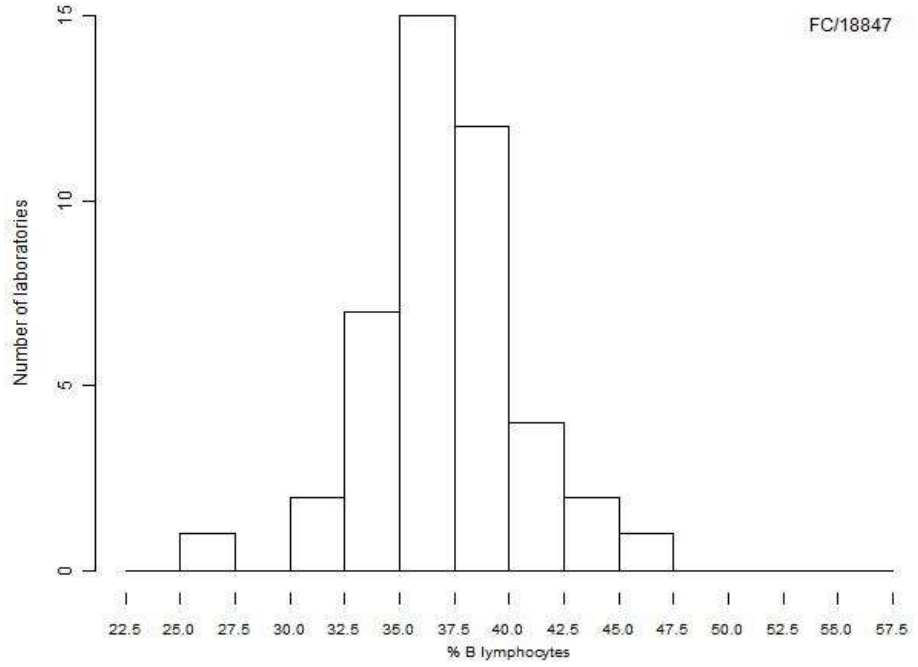
NKcells 10E9/L



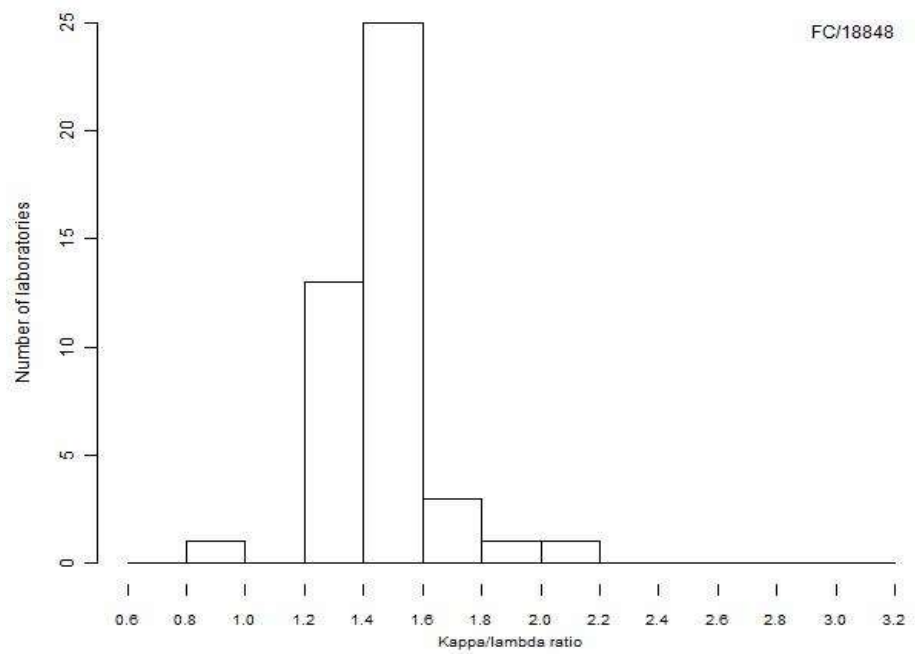
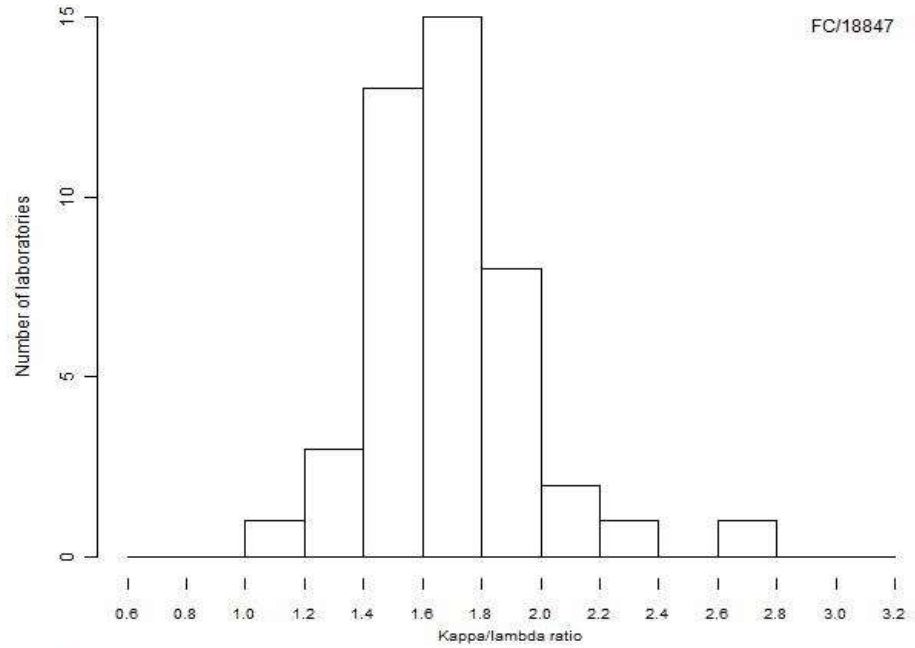
Kappa % B lymphocytes



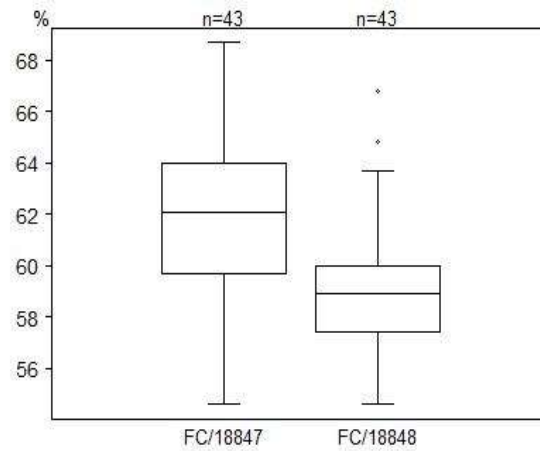
Lambda % B lymphocytes



Kappa/lambda



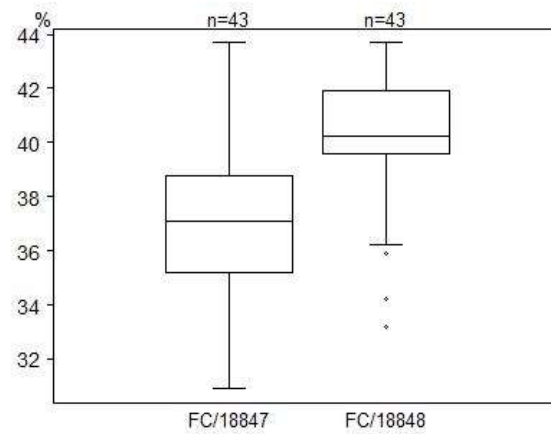
Kappa % B lymphocytes



Results not represented on the graph

FC/18847 = 52.1 %
FC/18847 = 71.2 %
FC/18848 = 46.1 %

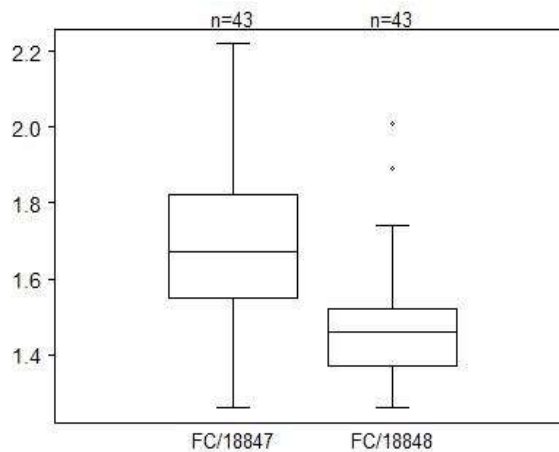
Lambda % B lymphocytes



Results not represented on the graph

FC/18847 = 25.4 %
FC/18847 = 46.5 %
FC/18848 = 53.8 %

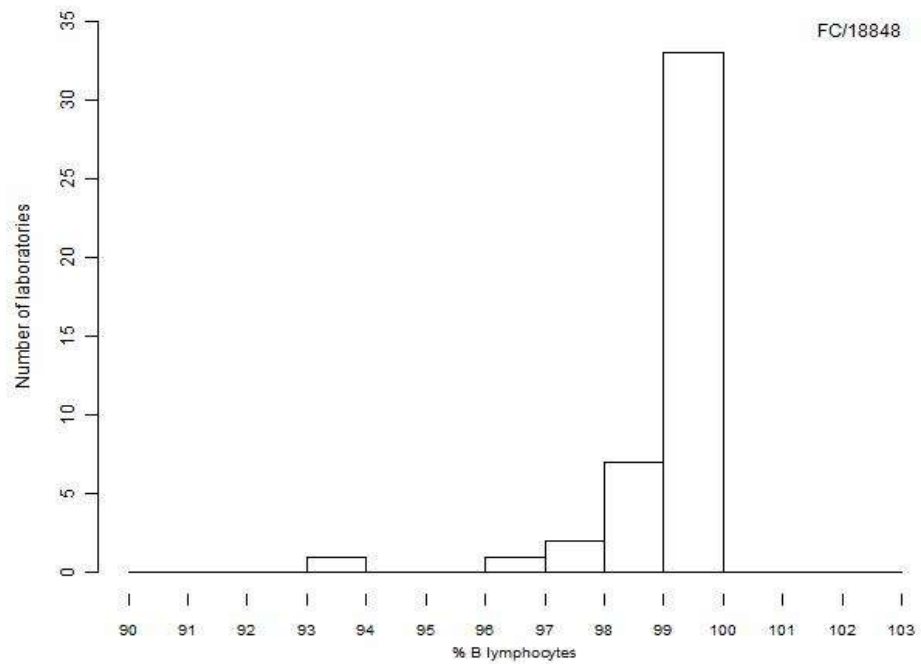
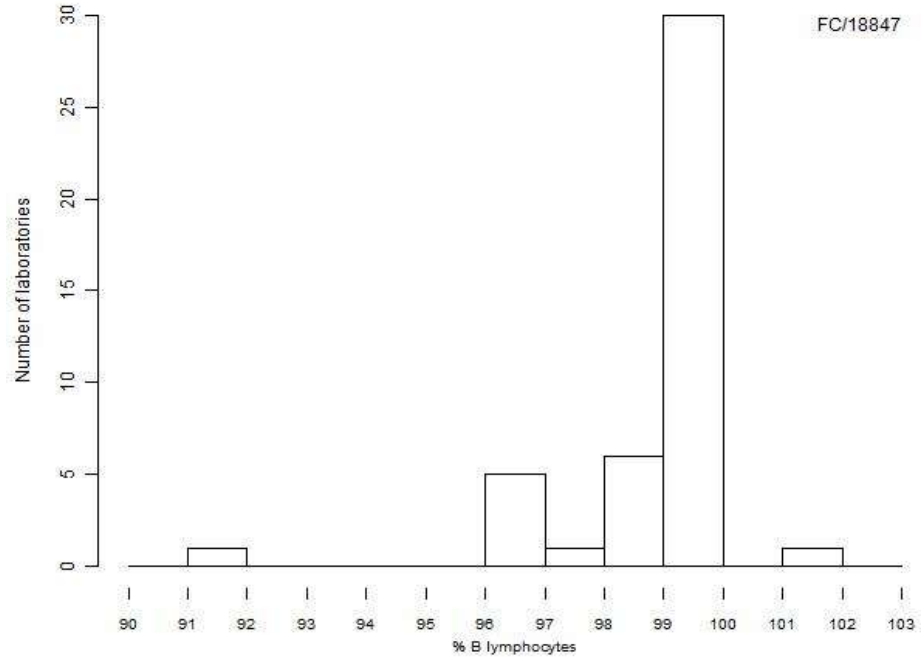
Kappa/lambda



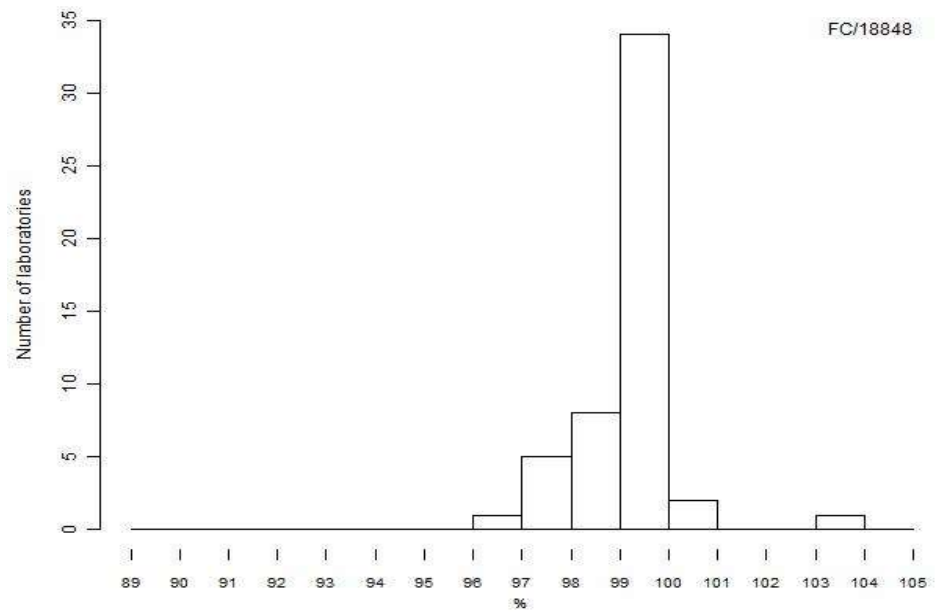
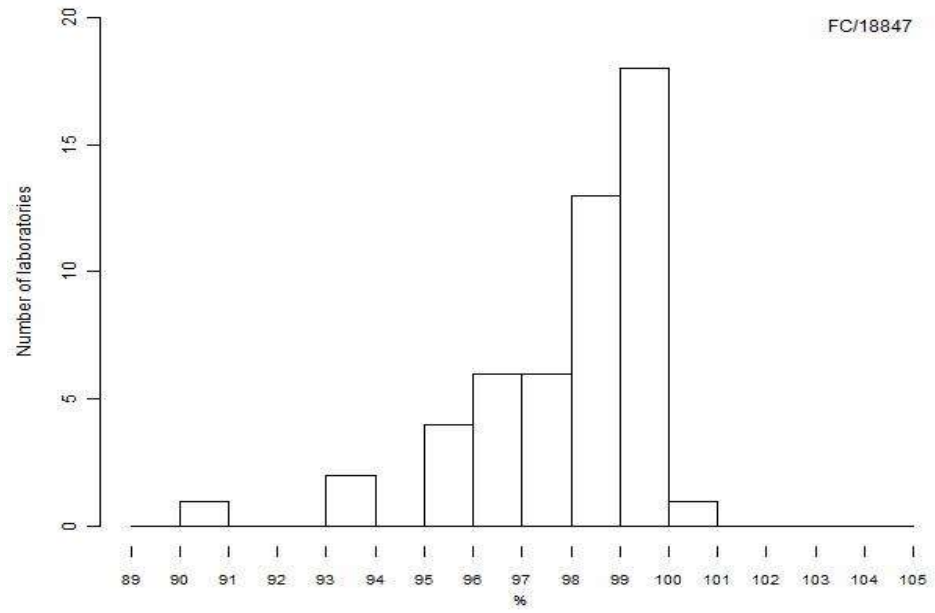
Results not represented on the graph

FC/18847 = 1.12
FC/18847 = 2.8
FC/18848 = 0.86

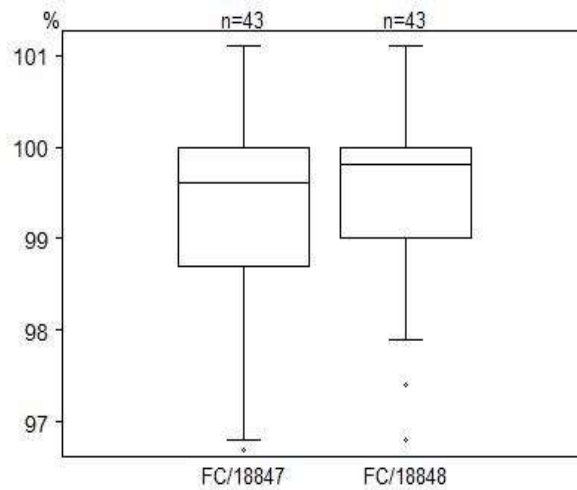
Sum K+L % B lymphocytes



Lymphosum %

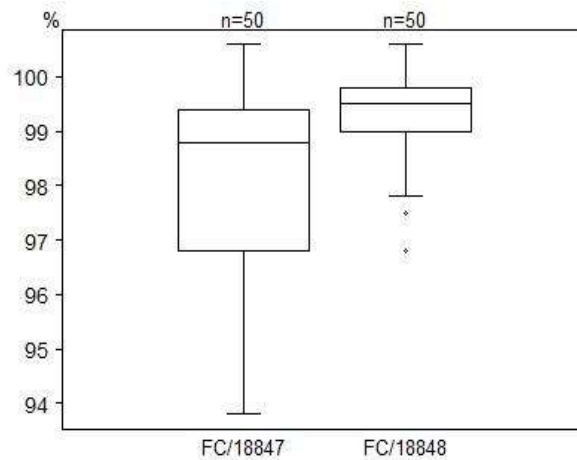


Sum K+L % B lymphocytes



Results not represented
on the graph
FC/18847 = 91.7 %
FC/18848 = 94 %

Lymphosum %



Results not represented
on the graph
FC/18847 = 90.2 %
FC/18848 = 103.7 %

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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