

**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 2023/3

Sciensano/Flow cytometry/87-E

Biological health risks
Quality of laboratories
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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 ($M \pm 2.7$ 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):

Klinische gezondheid | EKE klinische biologie | sciensano.be

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

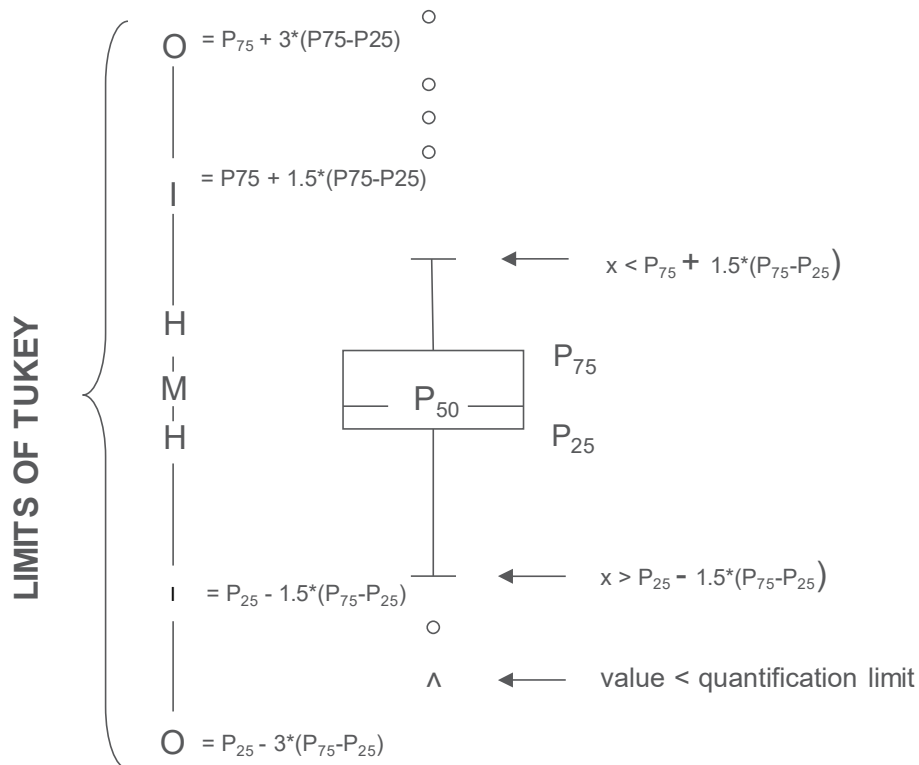
Santé clinique | EEQ biologie clinique | sciensano.be

- 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/20326 and FC/20327) 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/20326

	%	10⁹/L
Leukocytes		5.1
Lymphocytes	27.3	
CD3⁺ cells	80.2	1.12
CD4⁺CD3⁺ cells	56.2	0.78
CD8⁺CD3⁺ cells	22.9	0.32
CD19⁺ cells	8.20	0.11
NK cells	10.7	0.15
κ % B lymphocytes	52.6	
λ % B lymphocytes	47.1	
κ/λ ratio	1.12	

FC/20327

	%	10⁹/L
Leukocytes		9.3
Lymphocytes	32.4	
CD3⁺ cells	62.3	1.88
CD4⁺CD3⁺ cells	34.3	1.03
CD8⁺CD3⁺ cells	27.4	0.83
CD19⁺ cells	14.1	0.42
NK cells	23.2	0.70
κ % B lymphocytes	60.5	
λ % B lymphocytes	39.4	
κ/λ ratio	1.54	

PARTICIPATION

Fifty-one Belgian clinical laboratories participated in the survey 2023/3 (send-out of blood samples on 20 November 2023 (day 0)).

RESULTS

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

All the Belgian laboratories performed the analyses on day 1 or 2: 71% on day 1 and 29% on day 2.

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

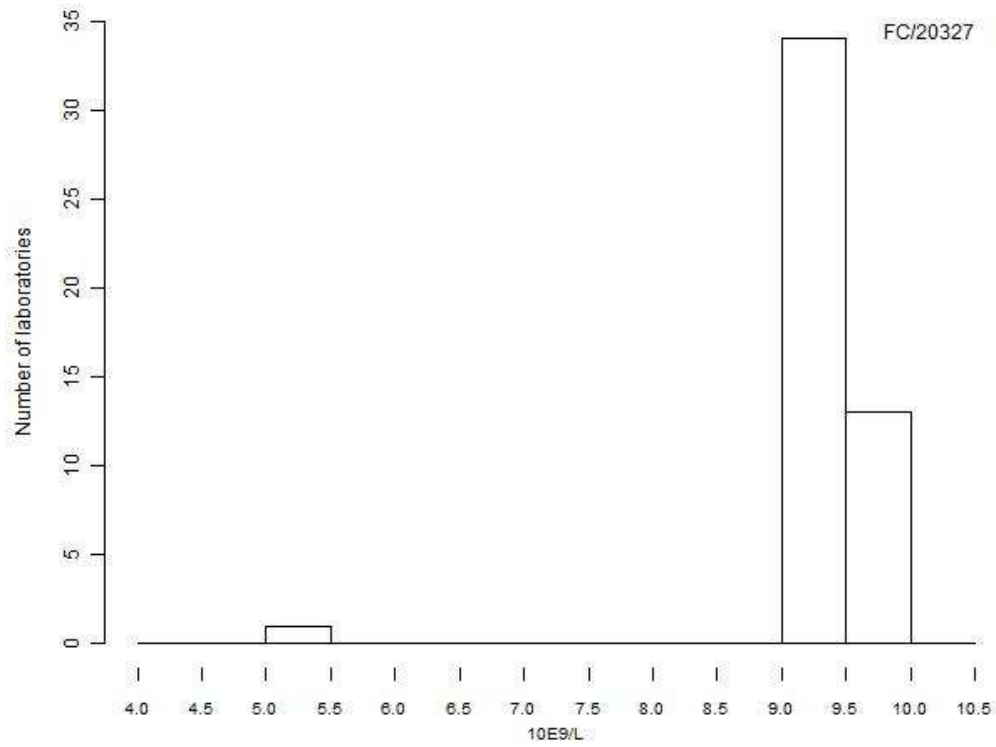
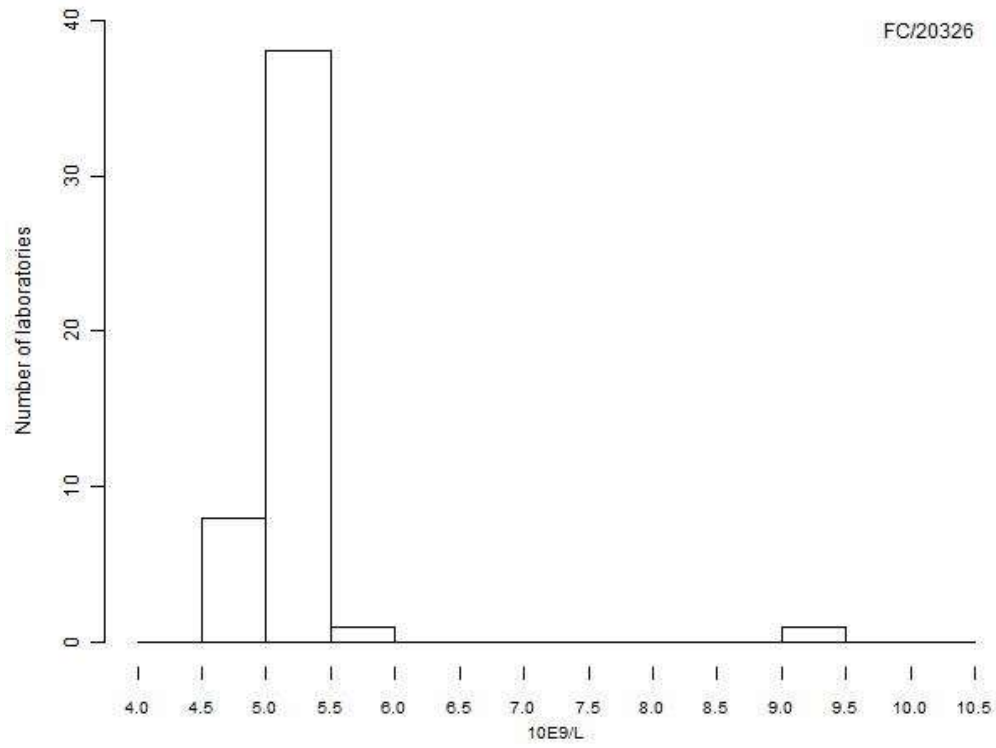
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=51). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the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

The following tables show the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/20326 and FC/20327:

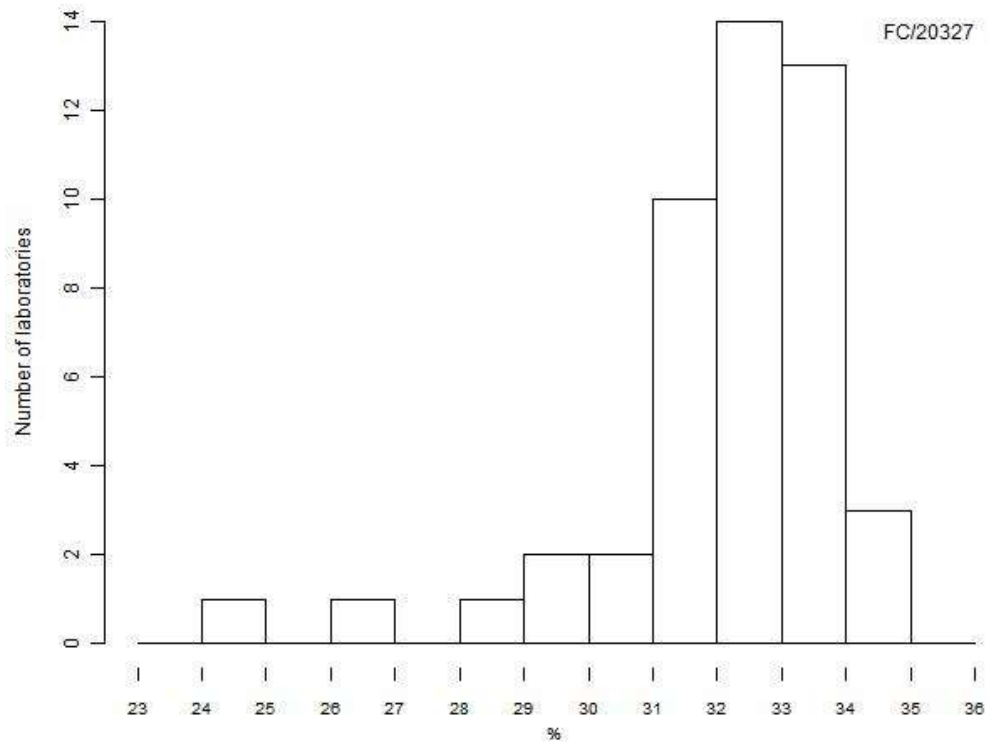
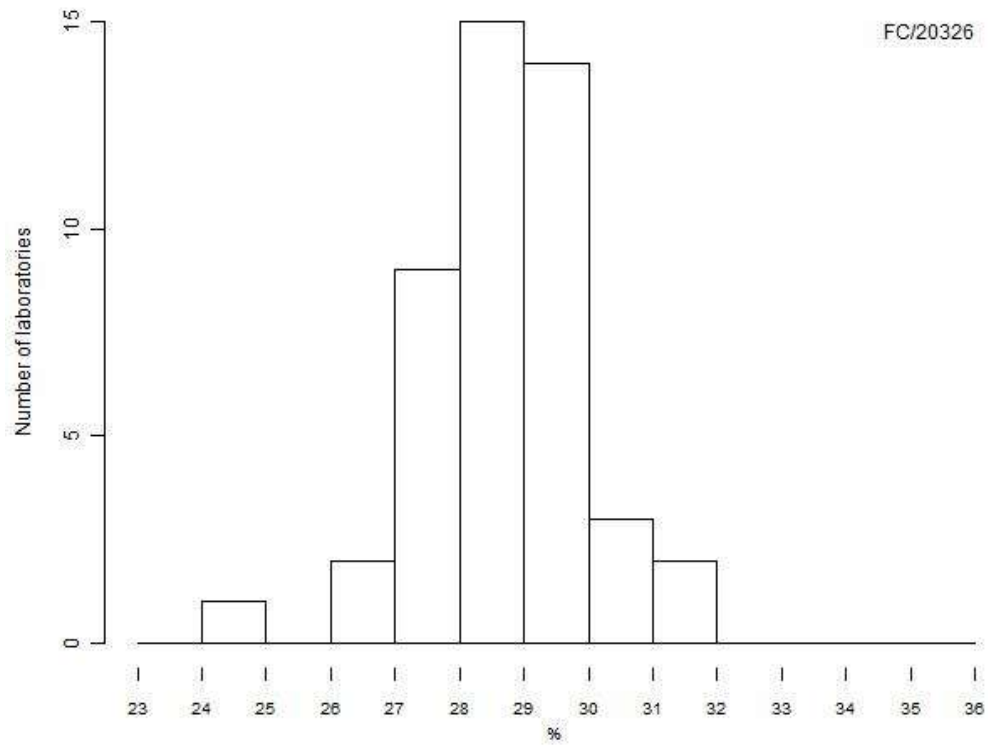
FC/20326	Median	SD	CV,%	N
WBC 10E9/L	5.10	0.11	2.2	49
Lympho% haematology analyser	28.8	1.0	3.3	46
Lympho% flow cytometer	27.8	2.1	7.7	45
CD3 %	79.4	1.8	2.2	50
CD3 10E9/L	1.158	0.075	6.5	49
CD4 %	56.2	1.4	2.5	50
CD4 10E9/L	0.811	0.058	7.2	49
CD8 %	21.2	1.2	5.6	50
CD8 10E9/L	0.312	0.033	10.4	49
CD19 %	7.4	0.8	11.0	50
CD19 10E9/L	0.108	0.010	8.9	49
NKcells %	12.2	1.5	12.2	50
NKcells 10E9/L	0.177	0.023	13.0	49
Kappa % B lymphocytes	54.0	2.1	3.8	41
Lambda % B lymphocytes	45.0	1.6	3.6	41
Kappa/lambda	1.20	0.08	6.8	41
Sum K+L % B lymphocytes	99.8	0.7	0.7	41
Lymphosum %	99.4	0.8	0.8	50

FC/20327	Median	SD	CV,%	N
WBC 10E9/L	9.43	0.20	2.1	50
Lympho% haematology analyser	32.6	1.2	3.6	47
Lympho% flow cytometer	31.9	2.0	6.3	46
CD3 %	62.4	2.0	3.3	51
CD3 10E9/L	1.902	0.123	6.5	50
CD4 %	33.5	2.7	8.2	51
CD4 10E9/L	1.020	0.087	8.5	50
CD8 %	27.3	1.5	5.4	51
CD8 10E9/L	0.825	0.067	8.2	50
CD19 %	13.0	1.2	9.4	51
CD19 10E9/L	0.394	0.043	10.9	50
NKcells %	22.8	3.4	14.8	51
NKcells 10E9/L	0.691	0.095	13.8	50
Kappa % B lymphocytes	63.0	3.4	5.4	42
Lambda % B lymphocytes	36.0	4.1	11.5	42
Kappa/lambda	1.72	0.25	14.6	42
Sum K+L % B lymphocytes	99.9	0.8	0.8	42
Lymphosum %	99.1	1.6	1.6	51

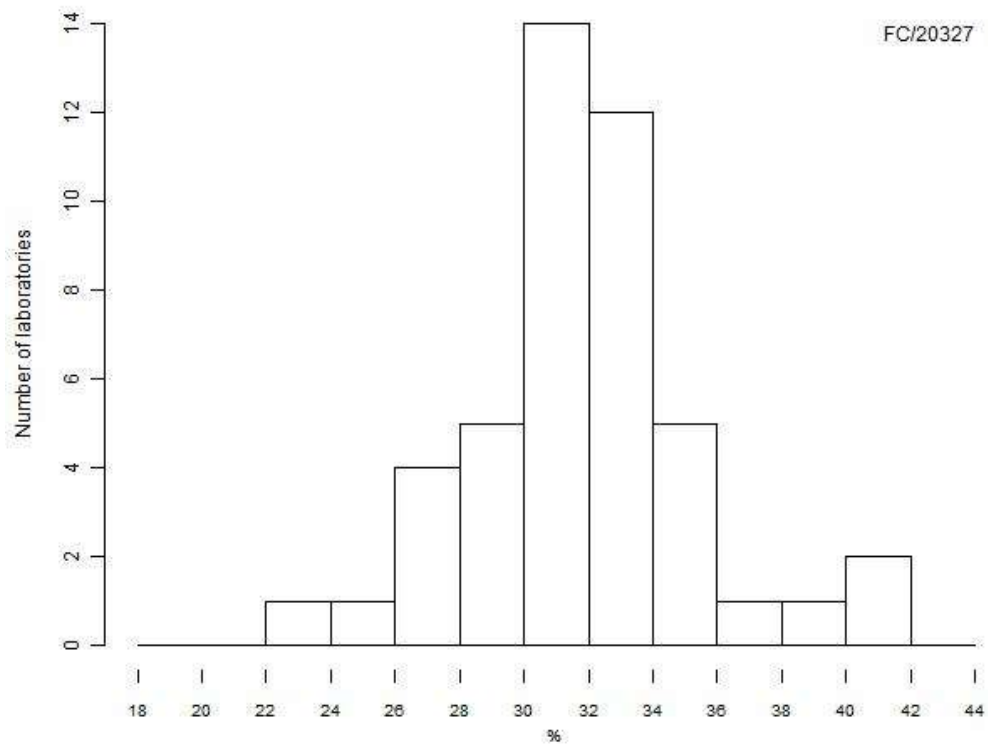
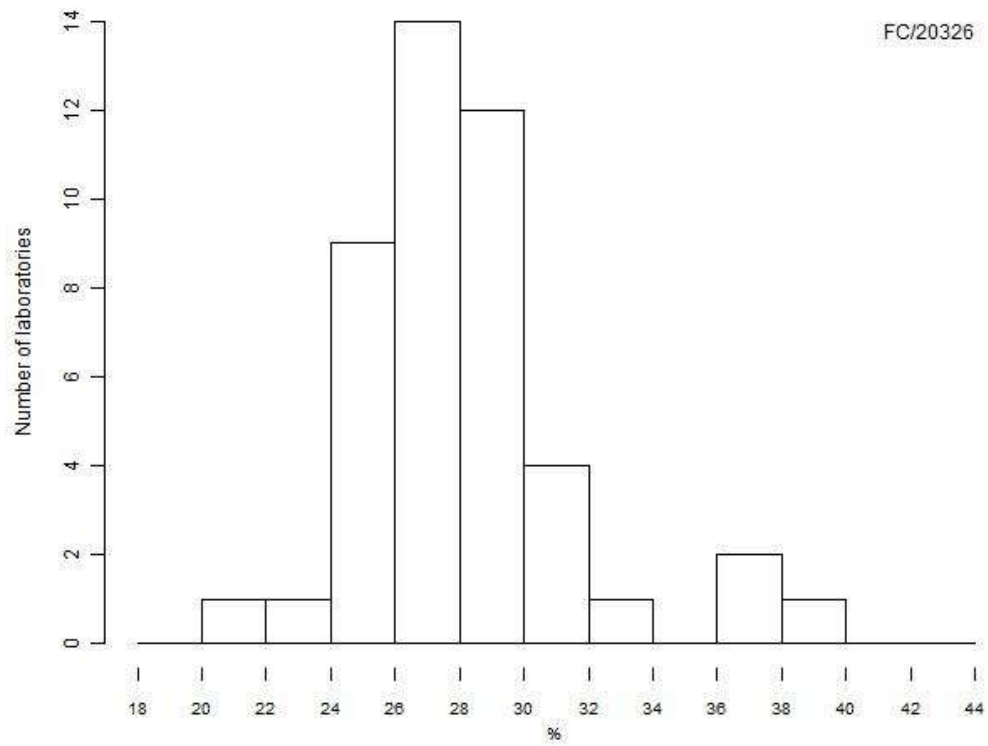
WBC 10E9/L



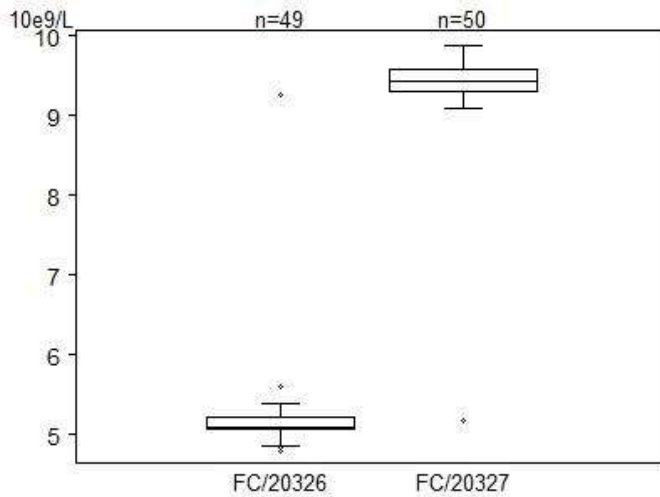
Lympho% haematology analyser



Lympho% flow cytometer



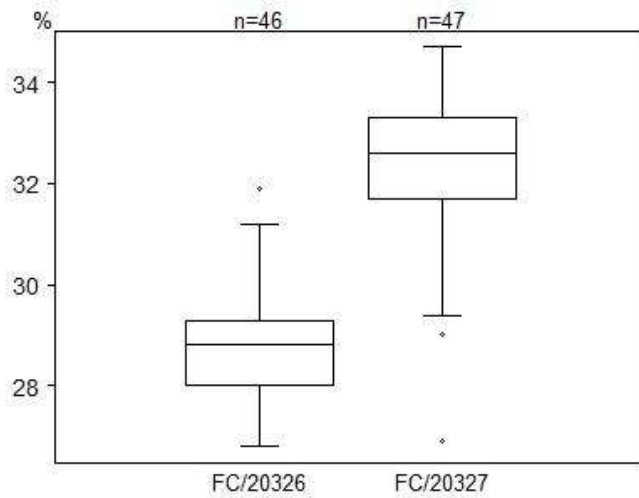
WBC 10E9/L



Results not represented on the graph

FC/20326 = 5140 10e9/
 FC/20327 = 10.2 10e9/l
 FC/20327 = 9440 10e9/

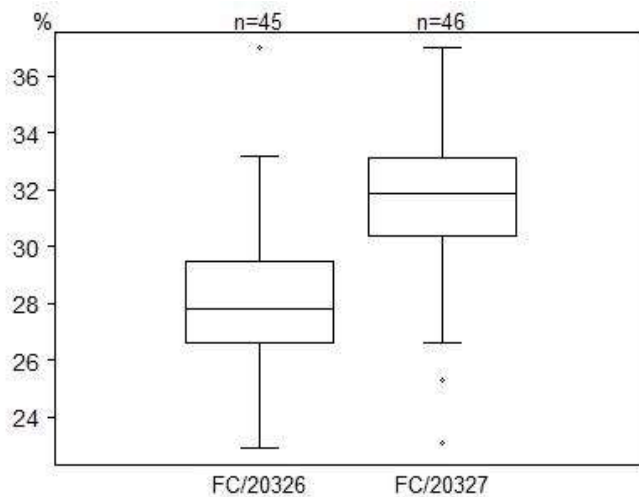
Lympho% haematology analyser



Results not represented on the graph

FC/20326 = 24.4 %
 FC/20327 = 24.7 %

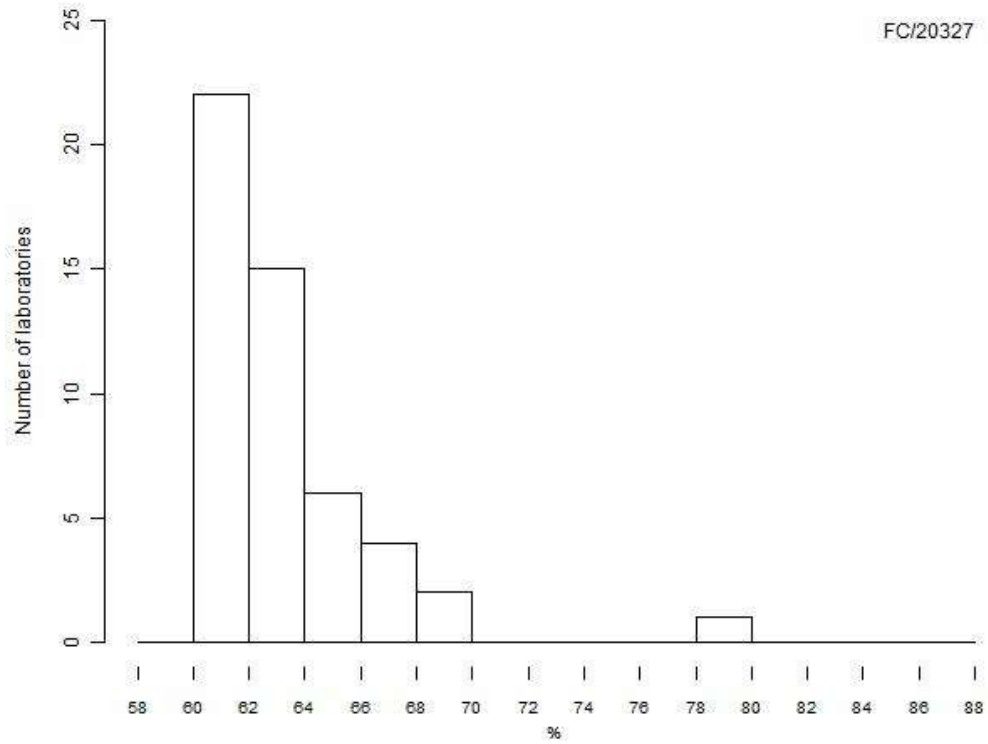
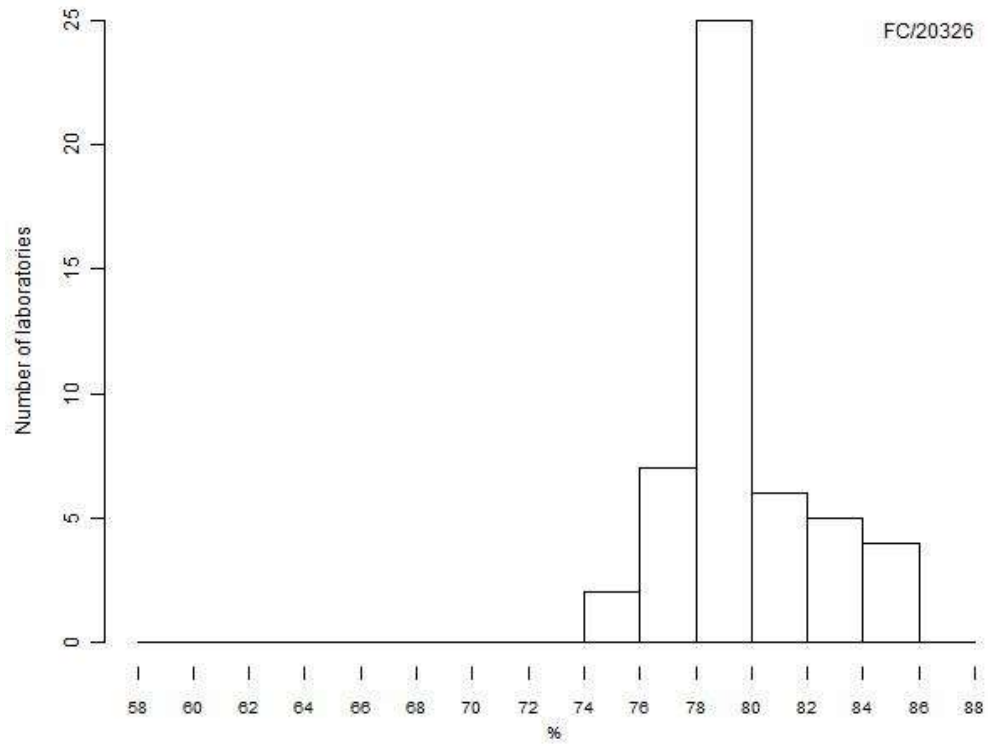
Lympho% flow cytometer



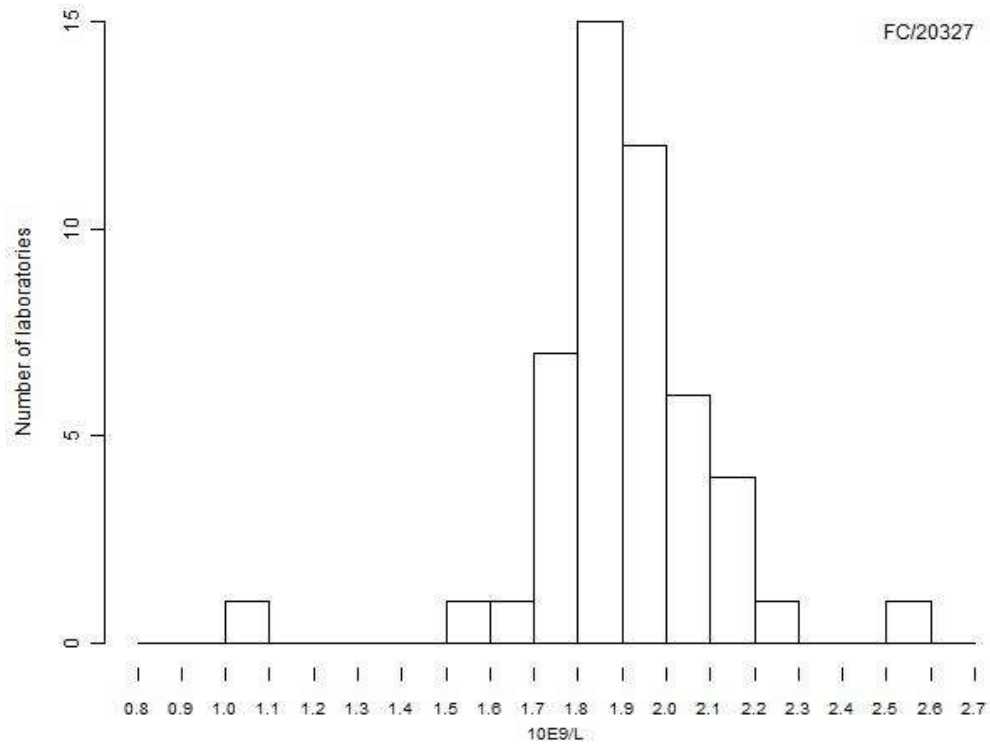
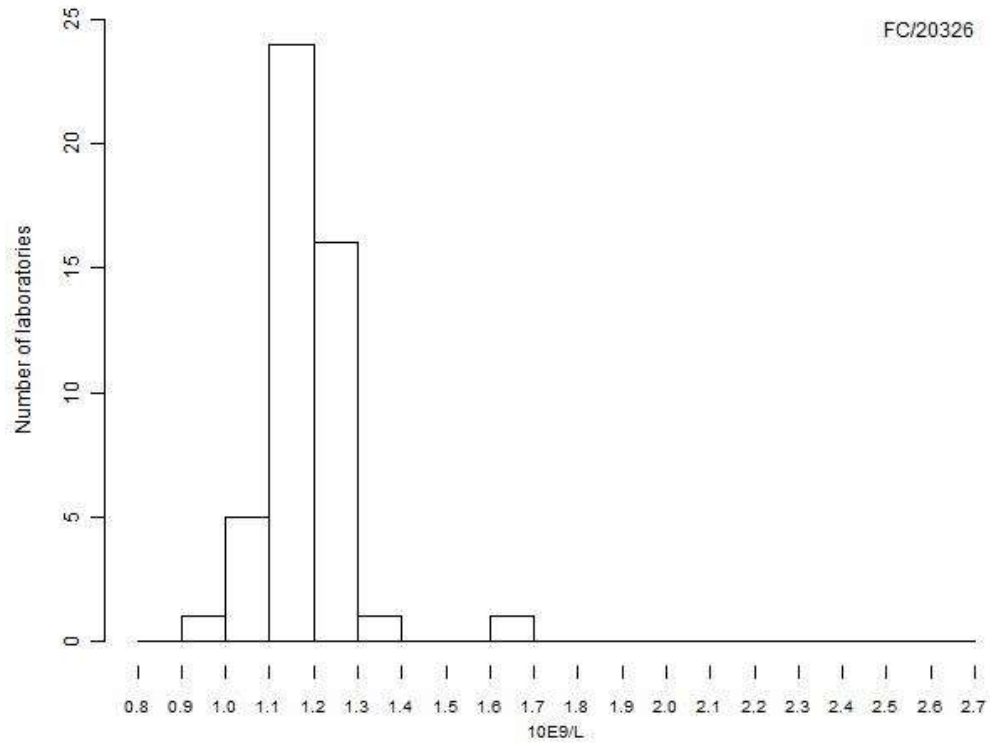
Results not represented on the graph

FC/20326 = 20.6 %
 FC/20326 = 38 %
 FC/20326 = 39 %
 FC/20327 = 38.8 %
 FC/20327 = 41.2 %
 FC/20327 = 41.4 %

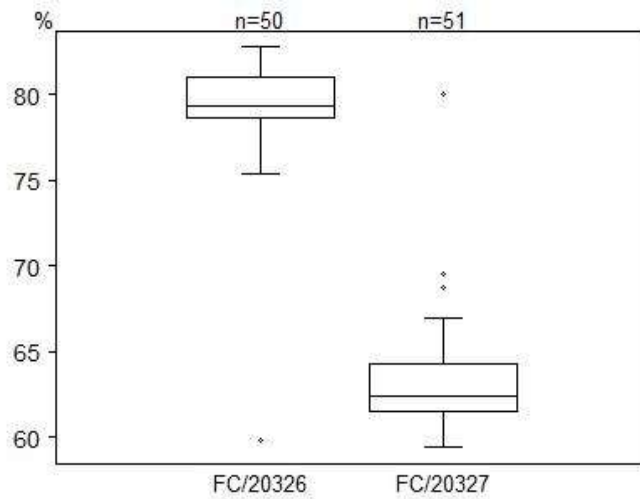
CD3 %



CD3 10E9/L



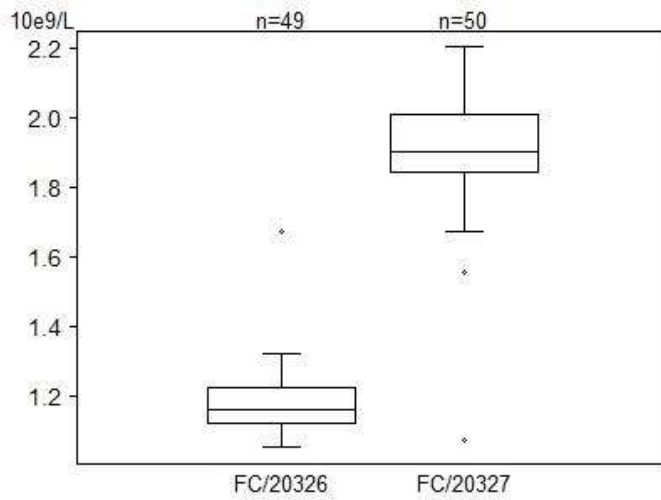
CD3 %



Results not represented
on the graph

FC/20326 = 84.7 %
FC/20326 = 84.9 %
FC/20326 = 85 %
FC/20326 = 85.3 %

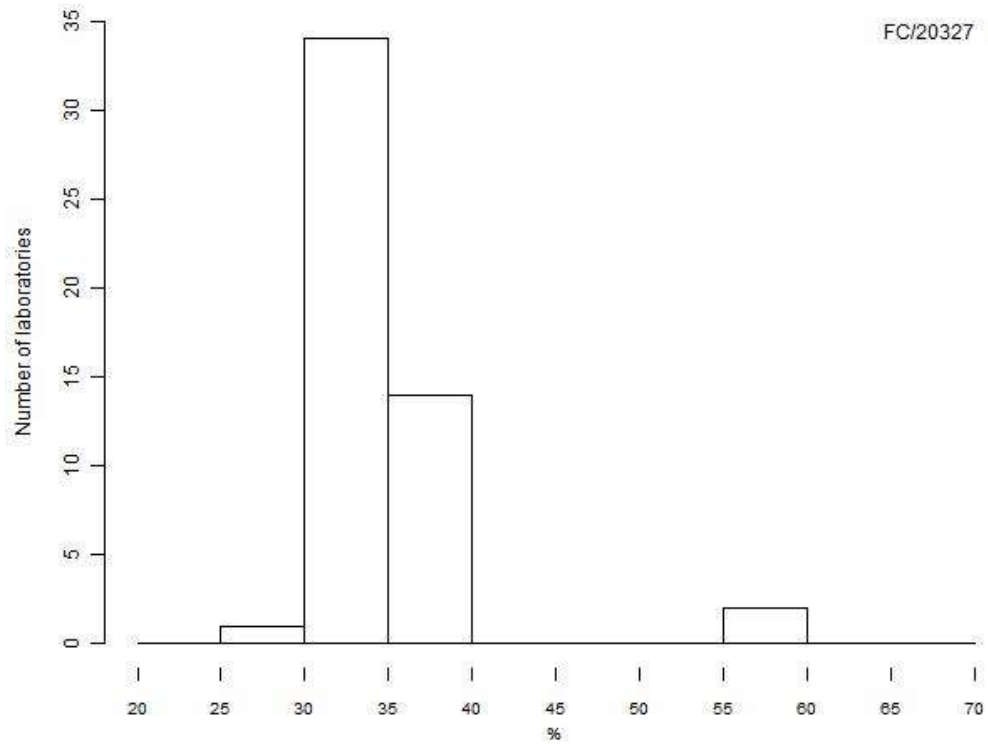
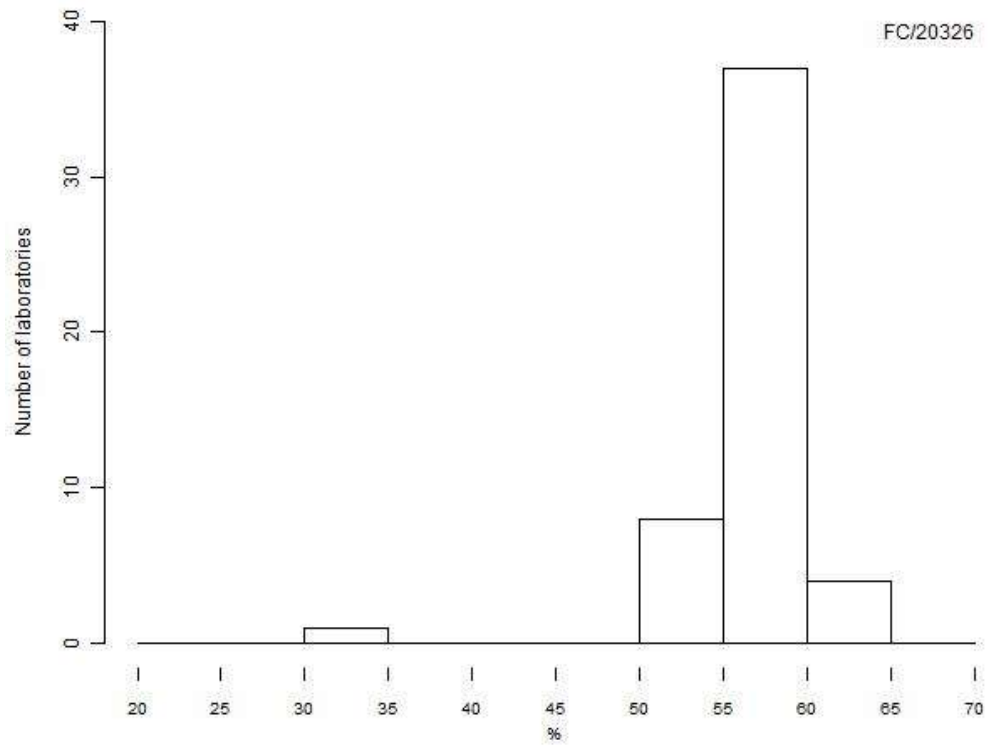
CD3 10E9/L



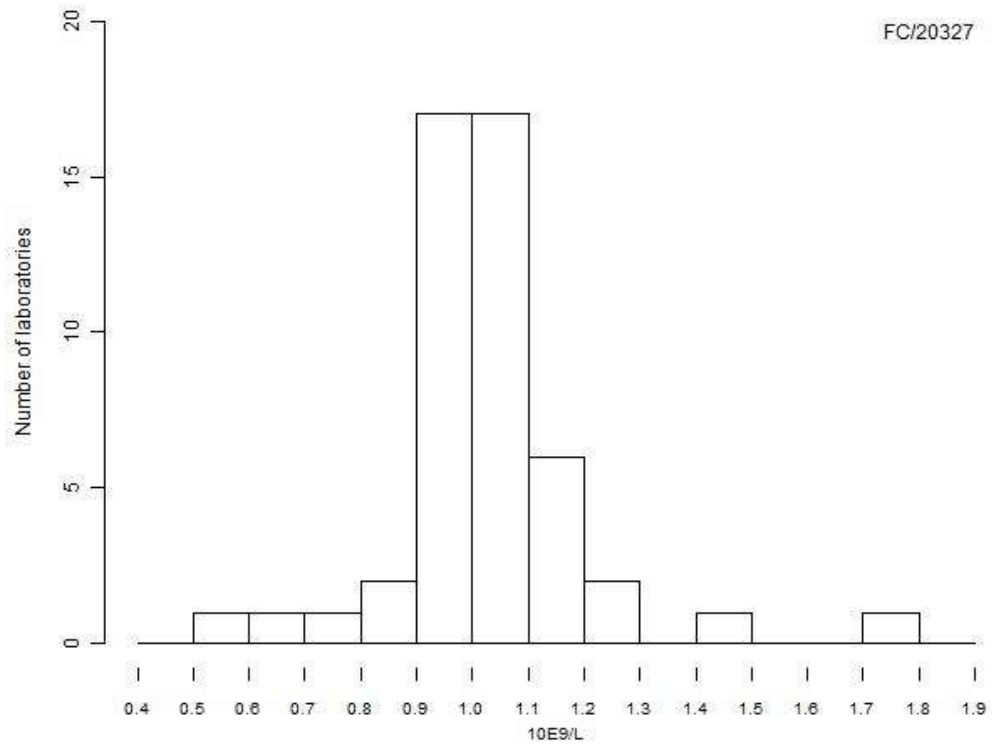
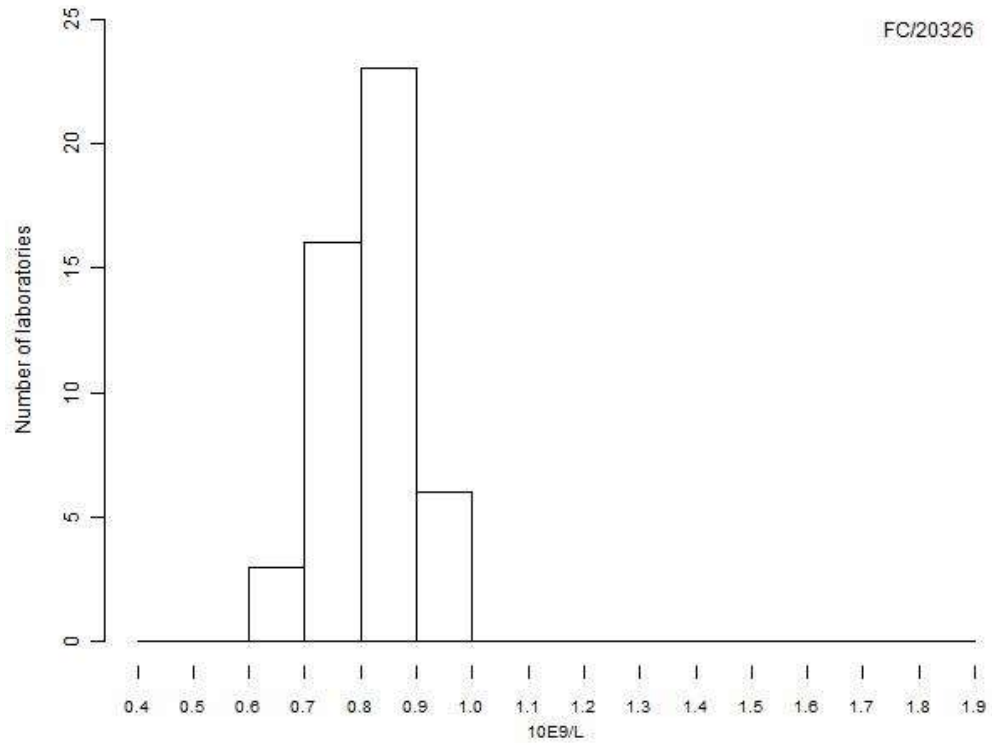
Results not represented
on the graph

FC/20326 = 0.912 10e9/
FC/20326 = 1171 10e9/
FC/20327 = 2.52 10e9/l
FC/20327 = 1897 10e9/

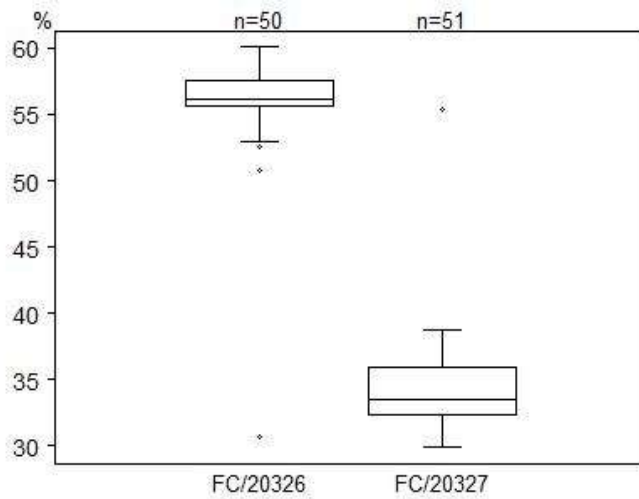
CD4 %



CD4 10E9/L



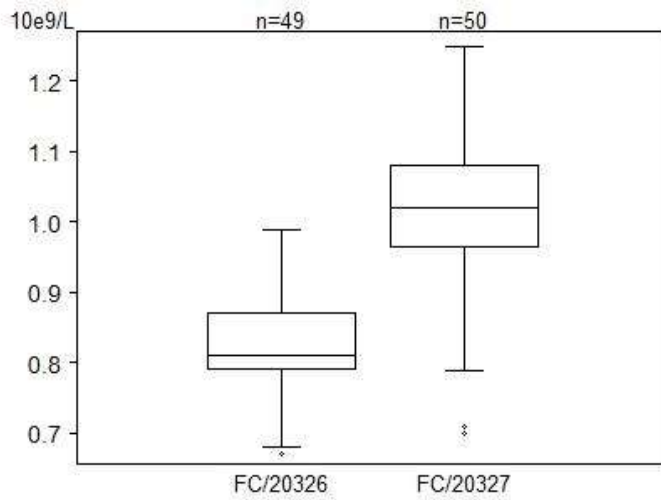
CD4 %



Results not represented
on the graph

FC/20326 = 61.9 %
FC/20326 = 62 %
FC/20326 = 62.8 %

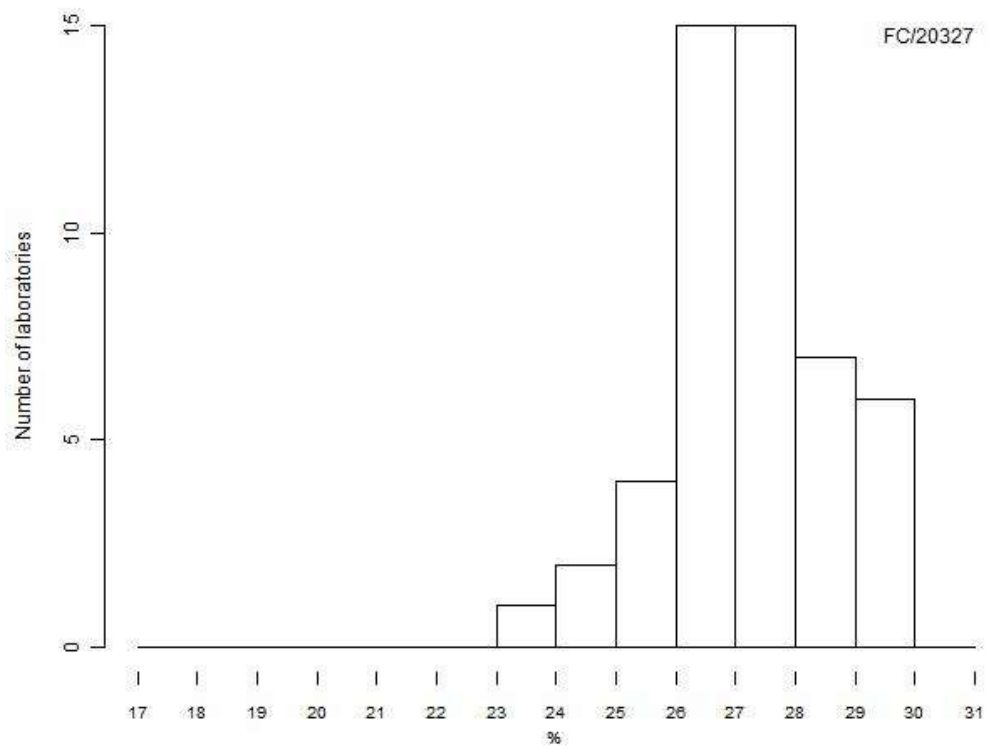
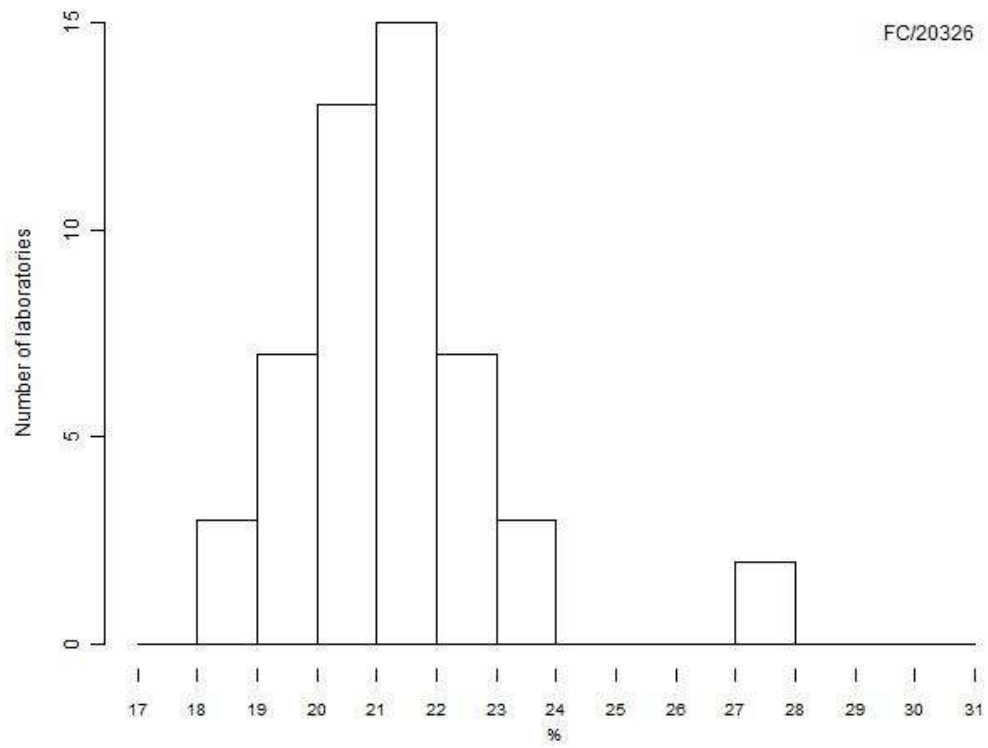
CD4 10E9/L



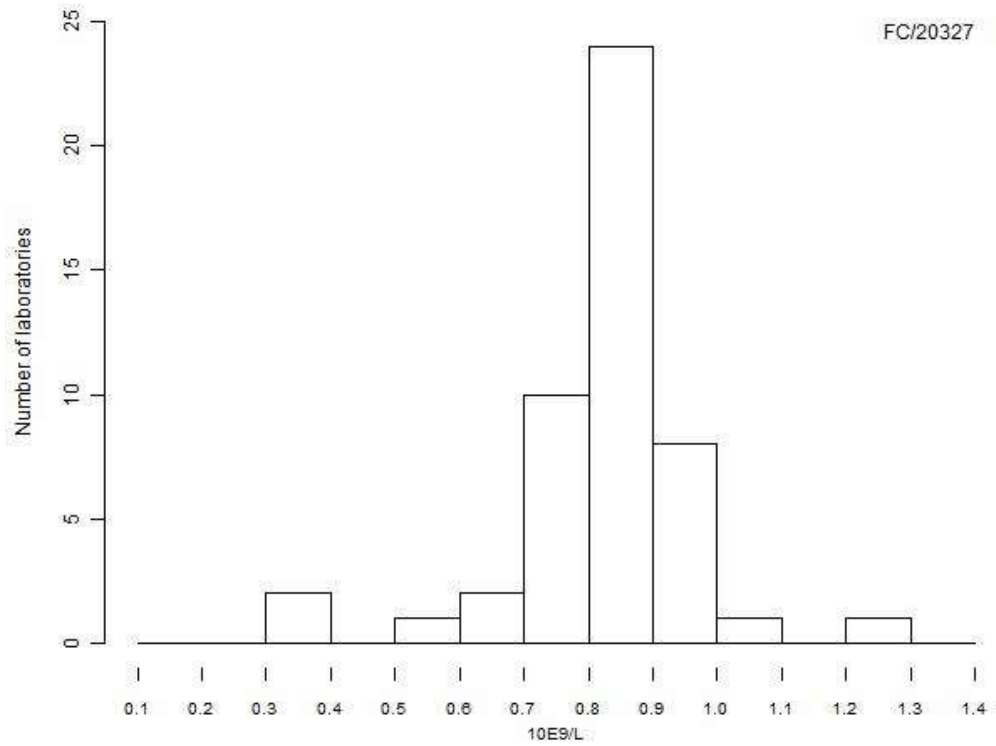
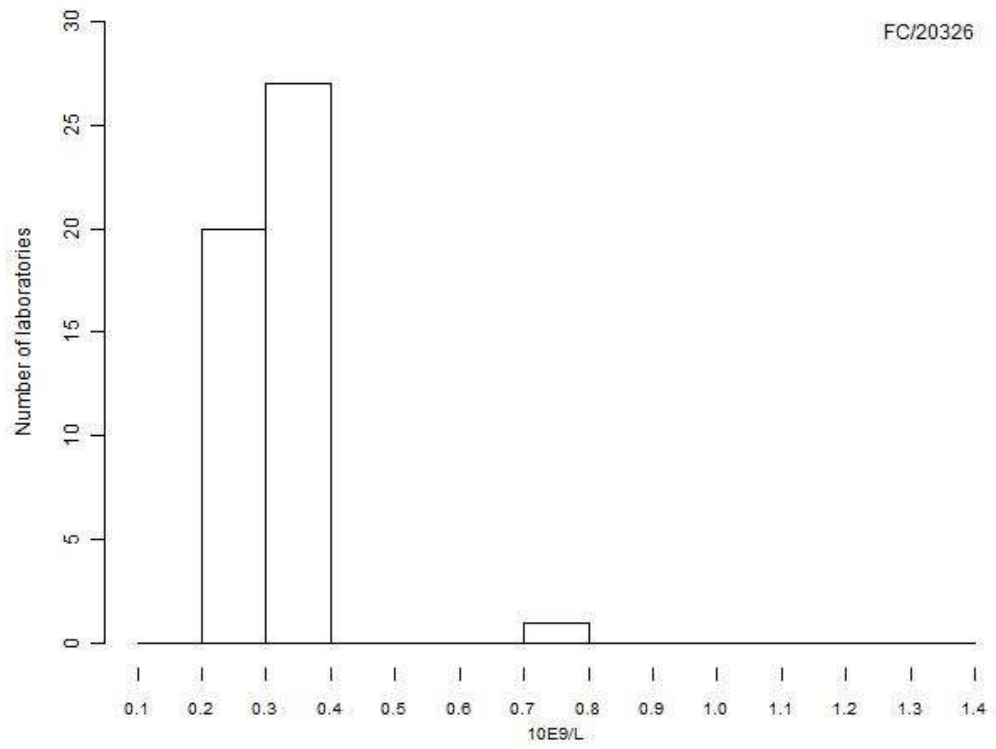
Results not represented
on the graph

FC/20326 = 824 10e9/L
FC/20327 = 0.54 10e9/L
FC/20327 = 1.447 10e9
FC/20327 = 1.718 10e9
FC/20327 = 995 10e9/L

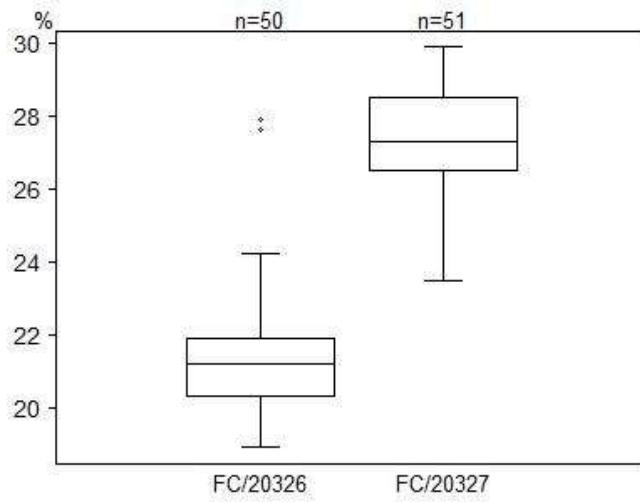
CD8 %



CD8 10E9/L

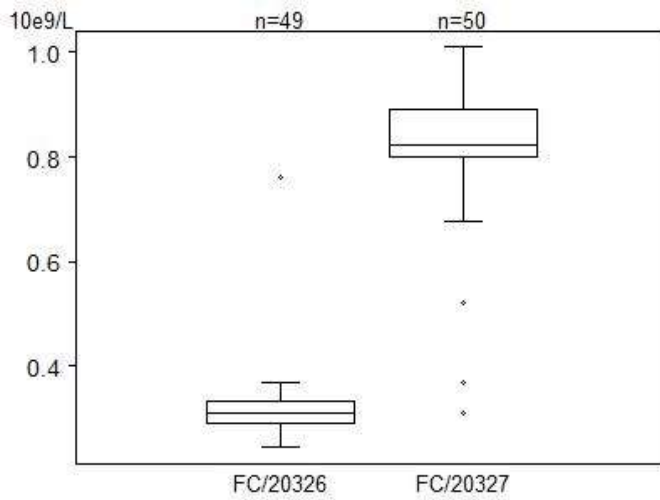


CD8 %



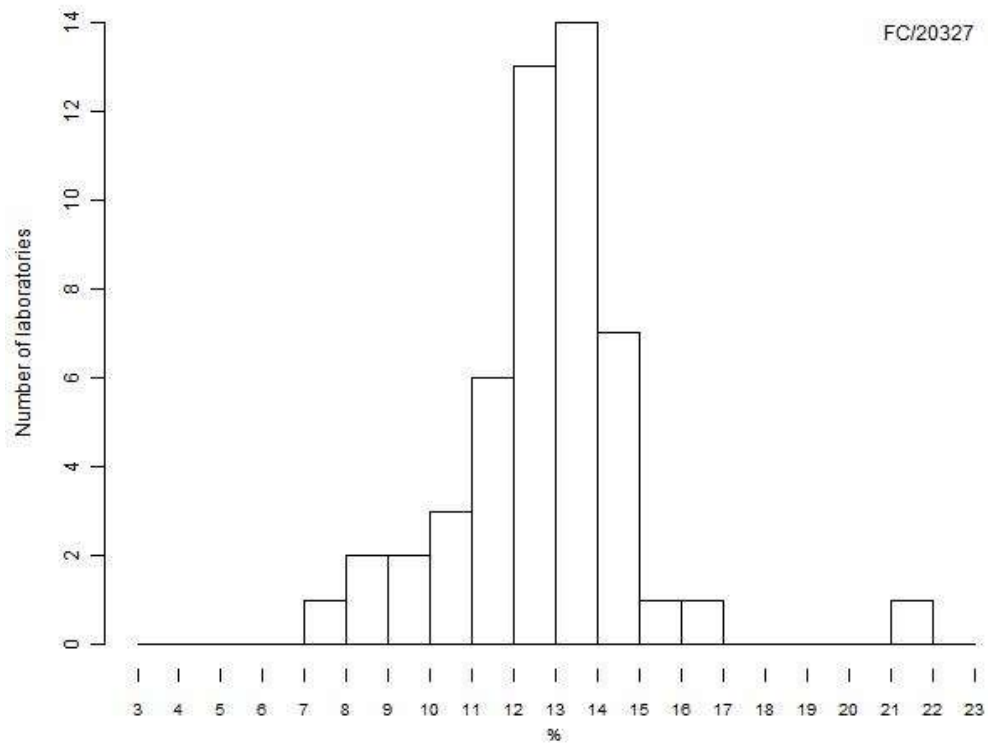
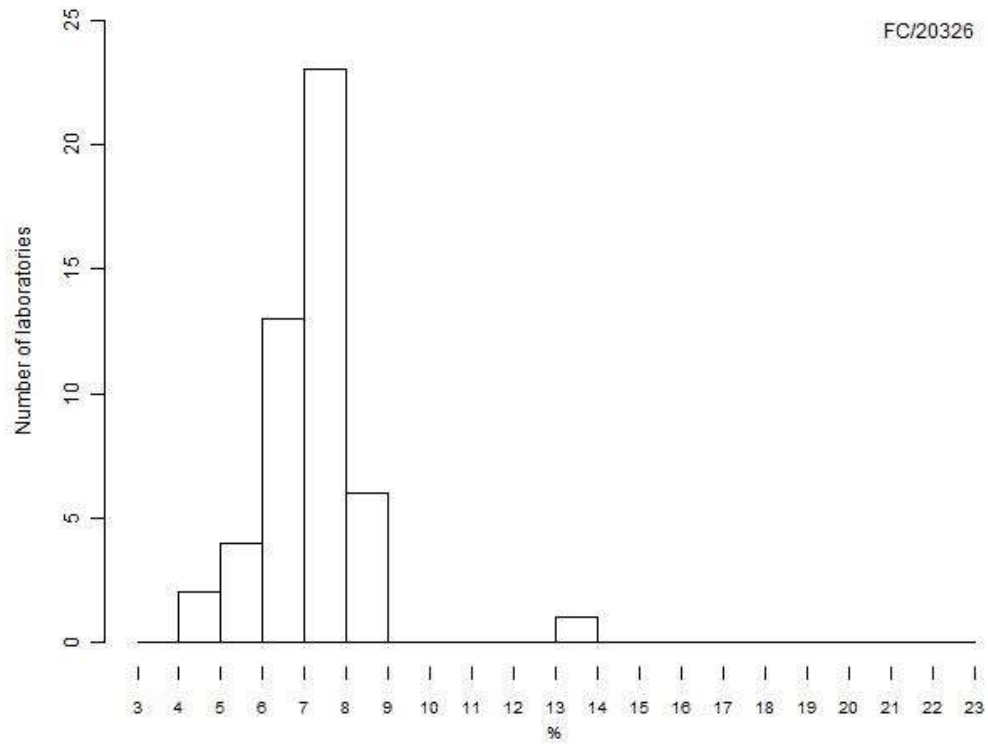
Results not represented
on the graph
FC/20327 = 41.2 %

CD8 10E9/L

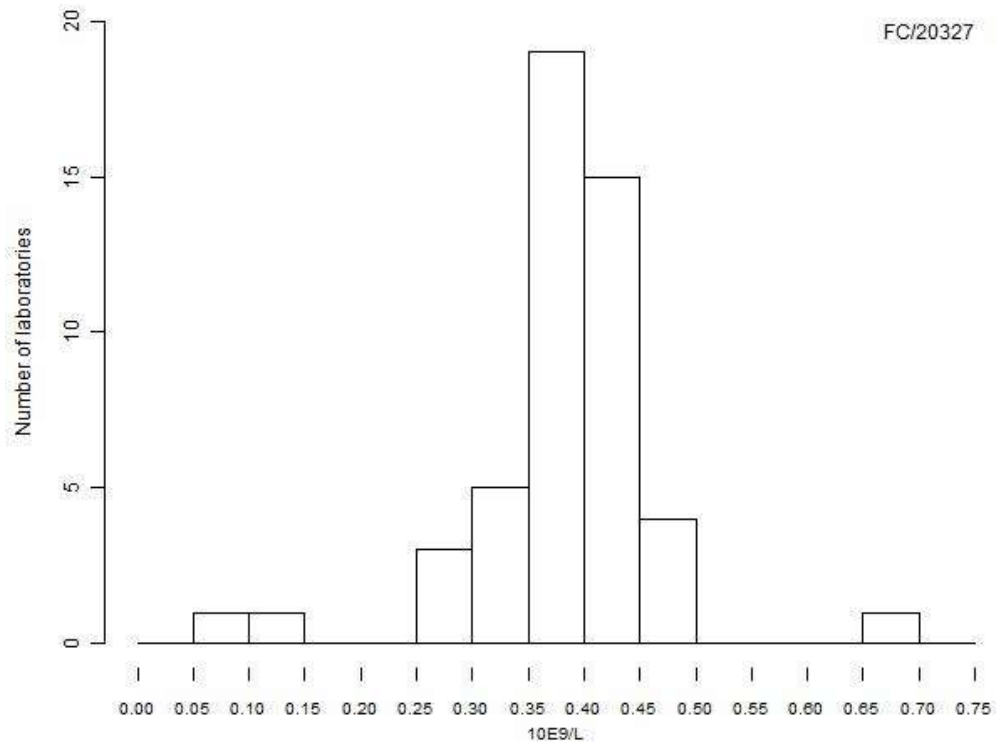
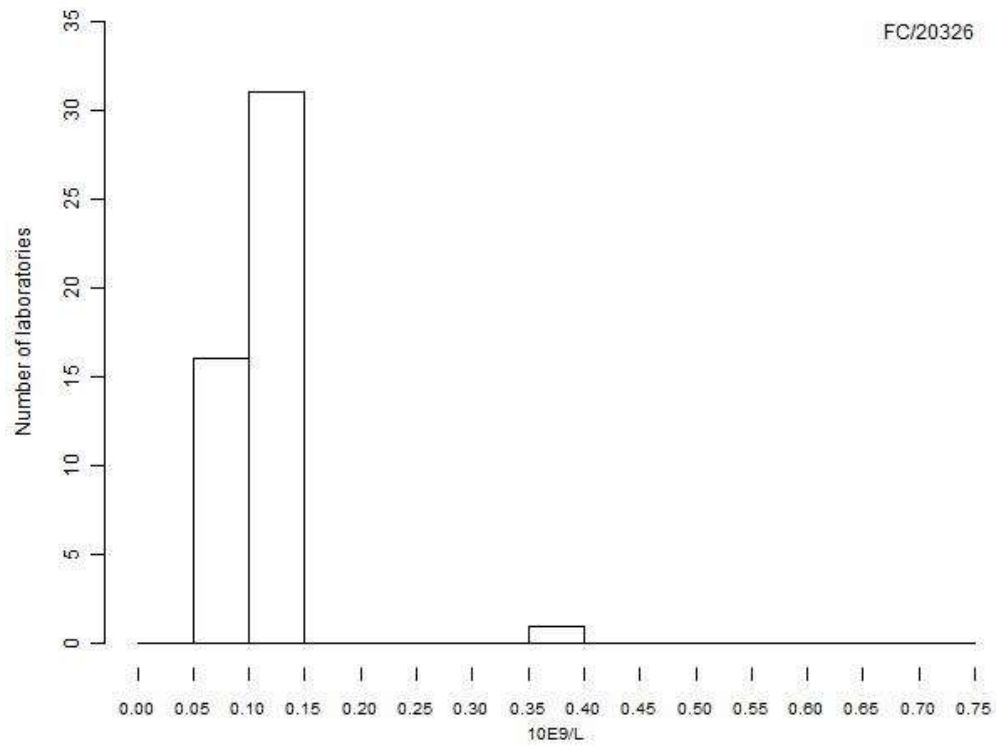


Results not represented
on the graph
FC/20326 = 329 10e9/L
FC/20327 = 1.278 10e9
FC/20327 = 898 10e9/L

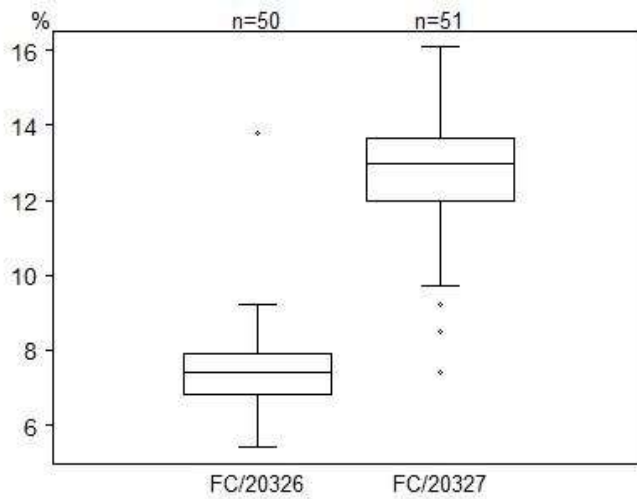
CD19 %



CD19 10E9/L



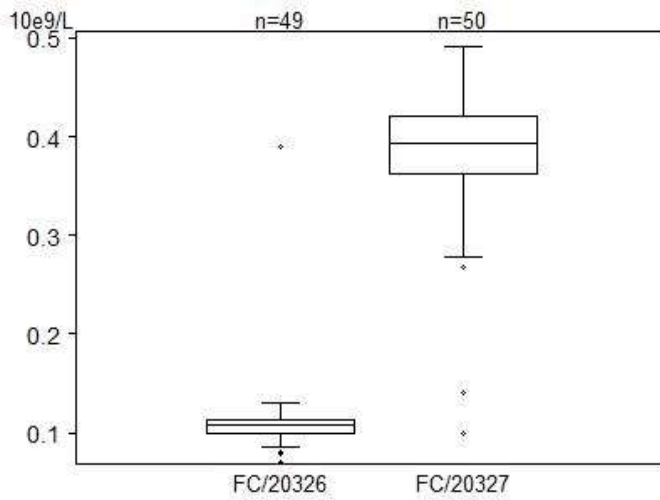
CD19 %



Results not represented
on the graph

FC/20326 = 4.6 %
FC/20326 = 4.7 %
FC/20326 = 70 %
FC/20327 = 21.7 %

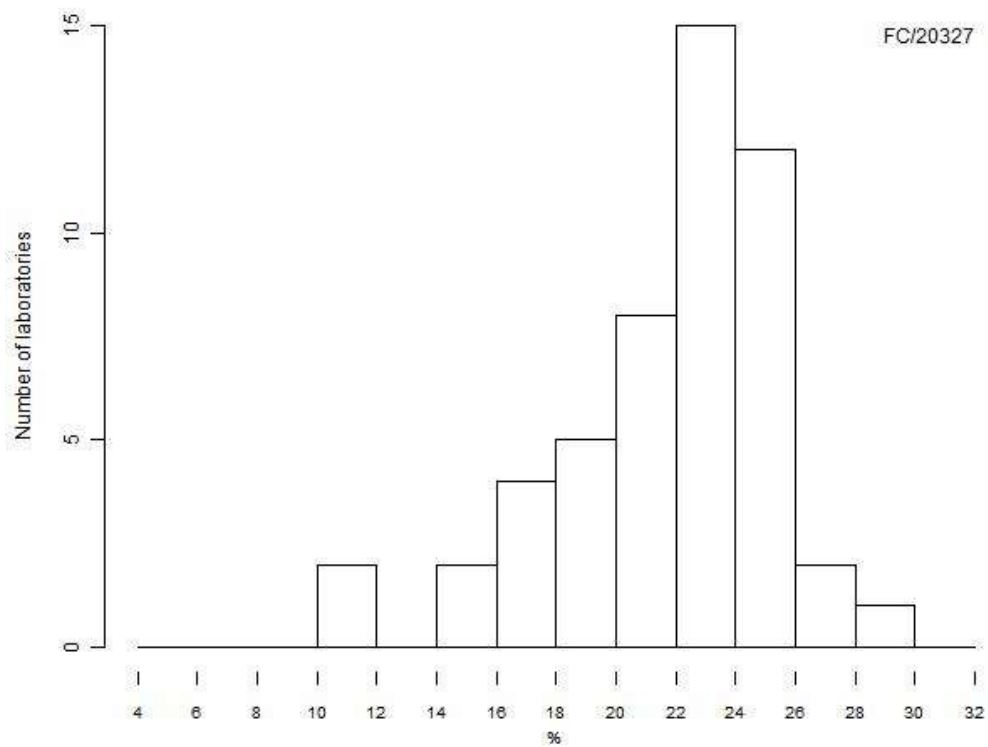
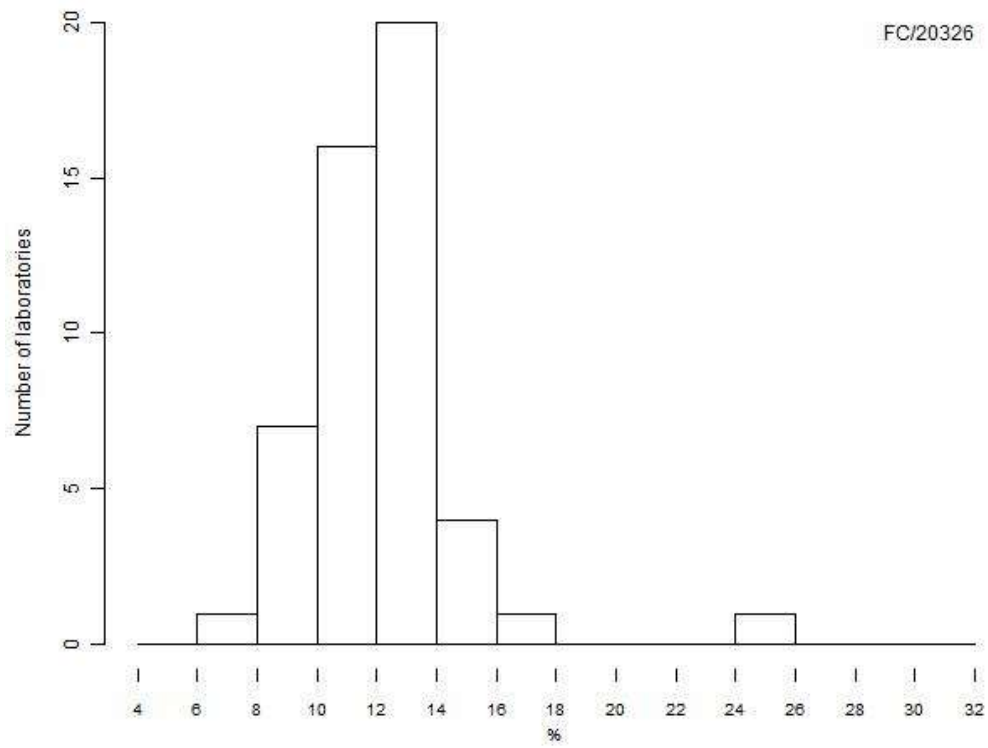
CD19 10E9/L



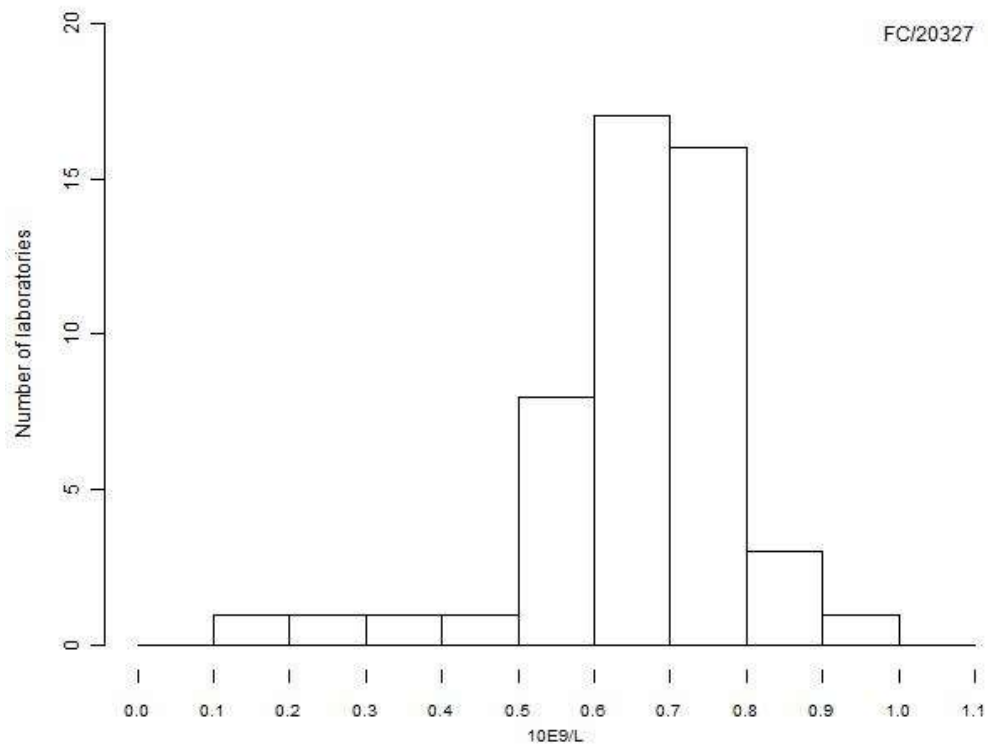
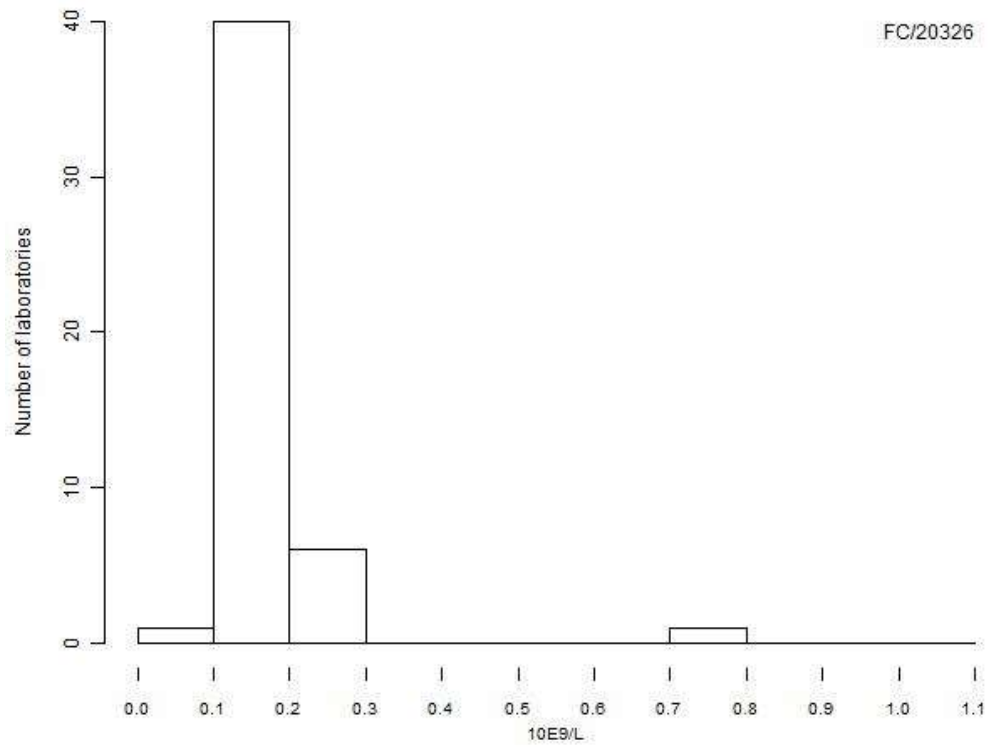
Results not represented
on the graph

FC/20326 = 110 10e9/L
FC/20327 = 0.673 10e9
FC/20327 = 427 10e9/L

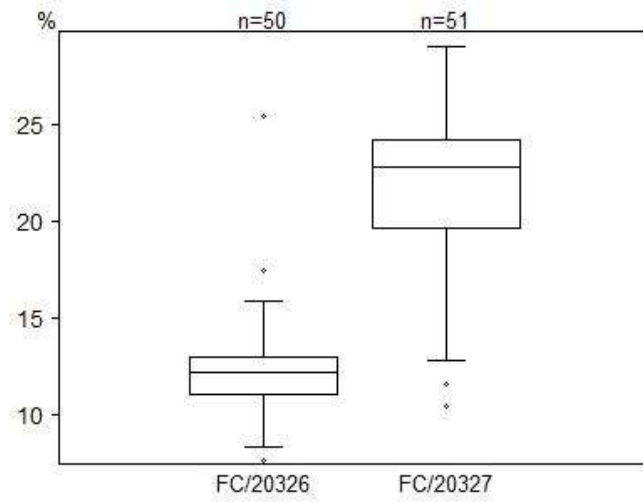
NKcells %



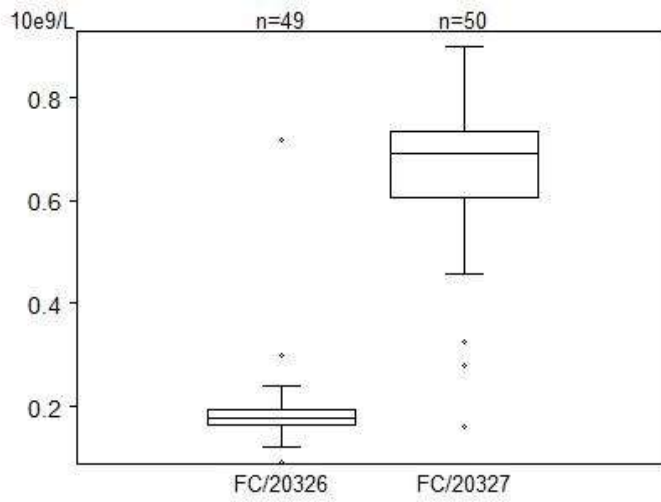
NKcells 10E9/L



NKcells %



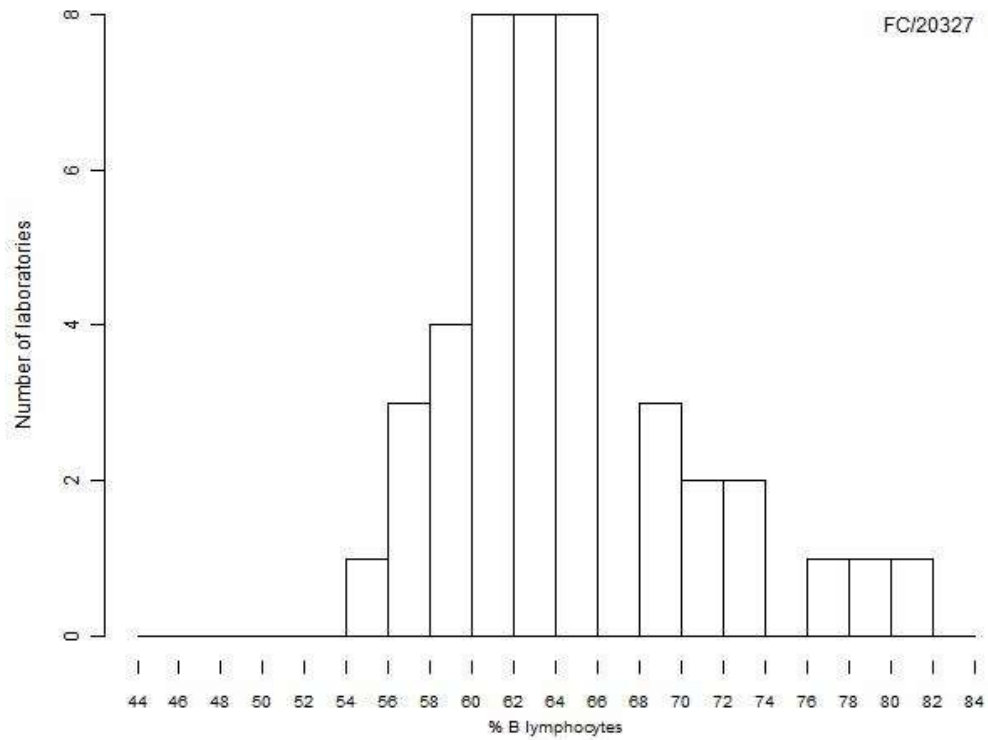
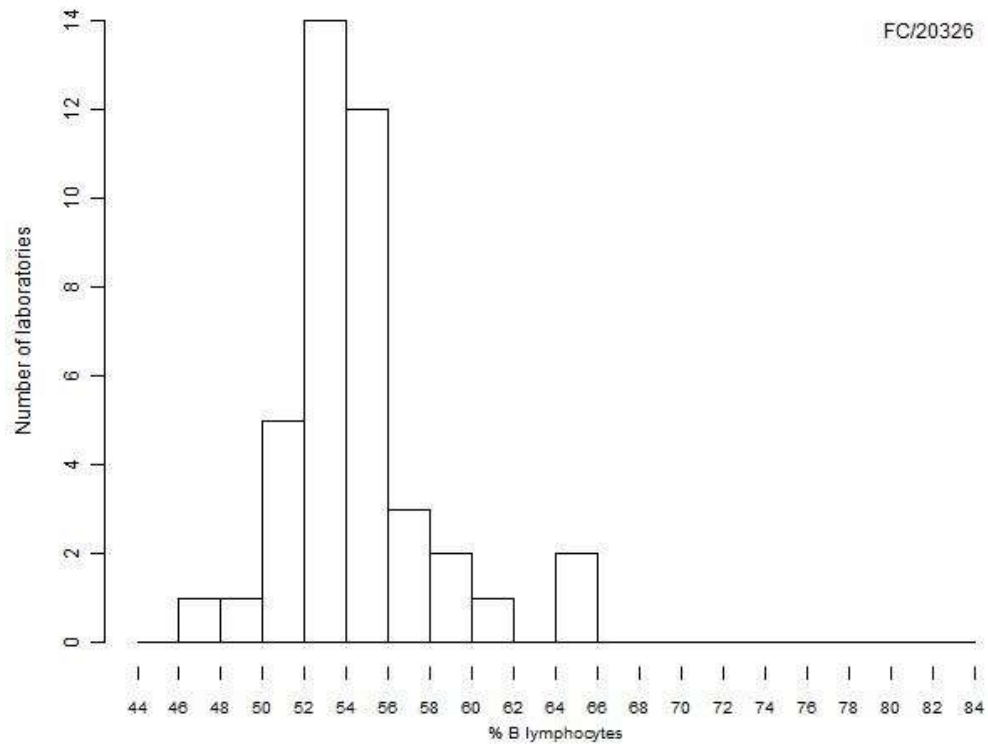
NKcells 10E9/L



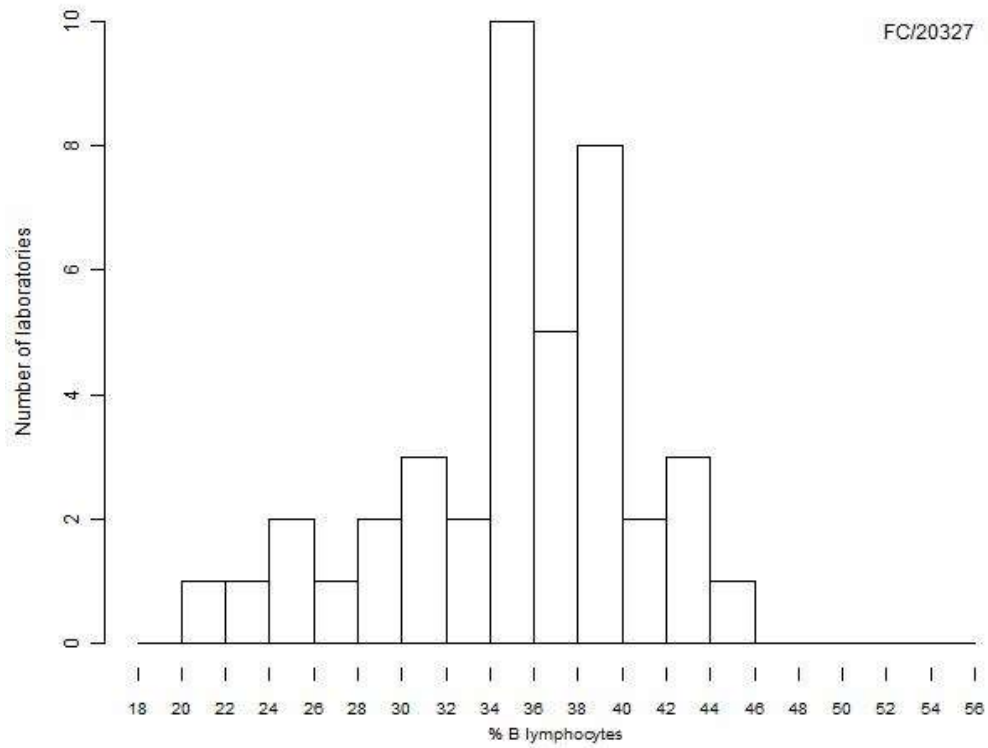
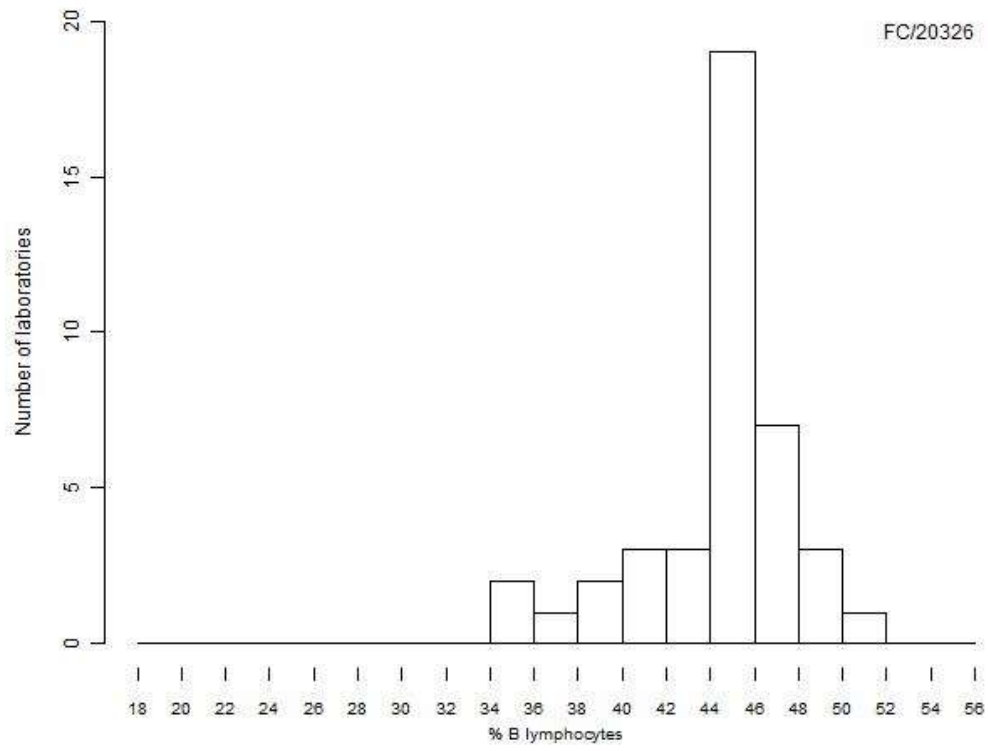
Results not represented on the graph

FC/20326 = 193 10e9/L
FC/20327 = 0.947 10e9
FC/20327 = 749 10e9/L

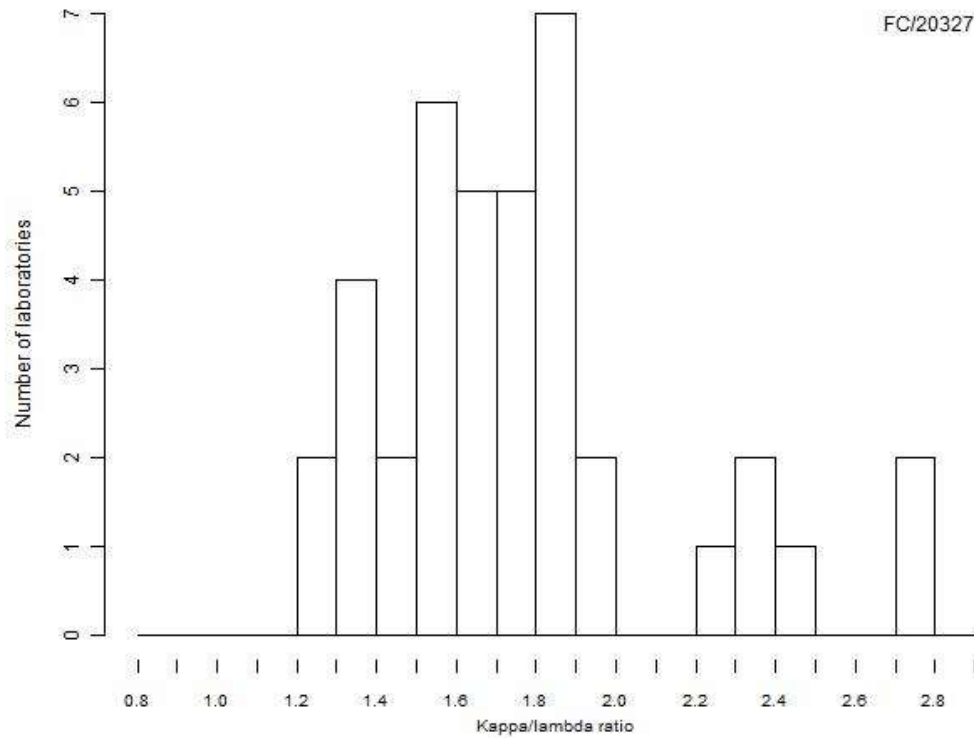
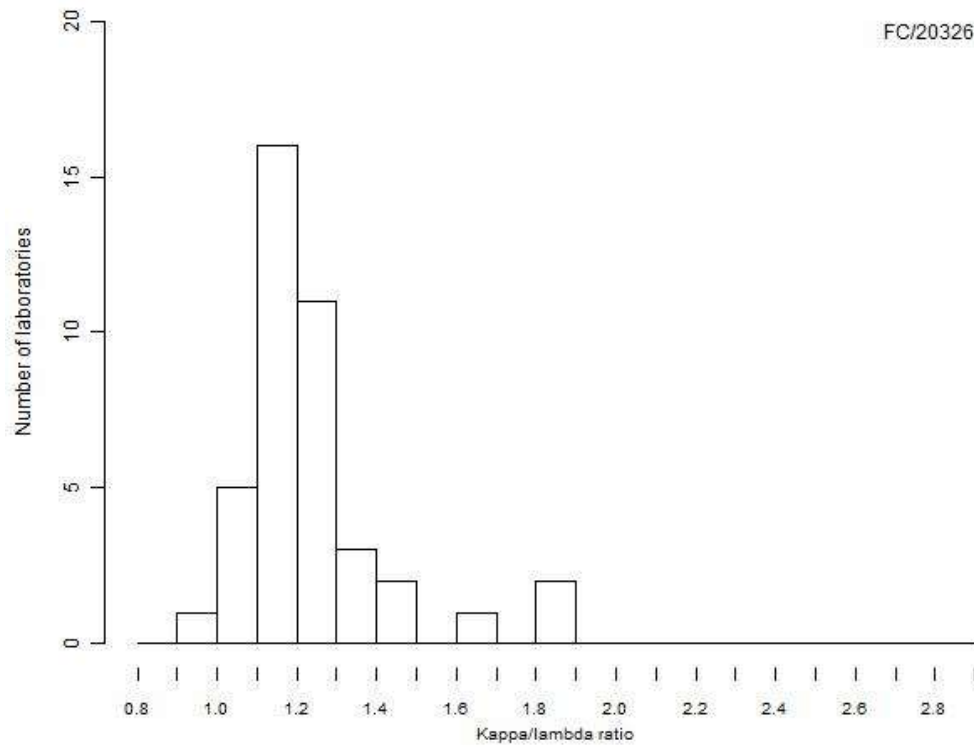
Kappa % B lymphocytes



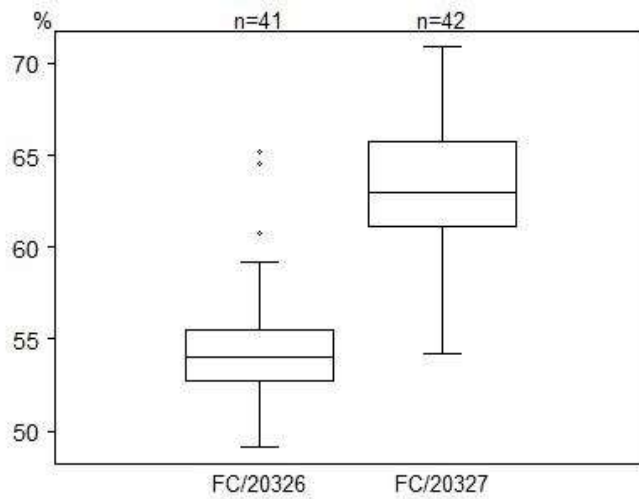
Lambda % B lymphocytes



Kappa/lambda



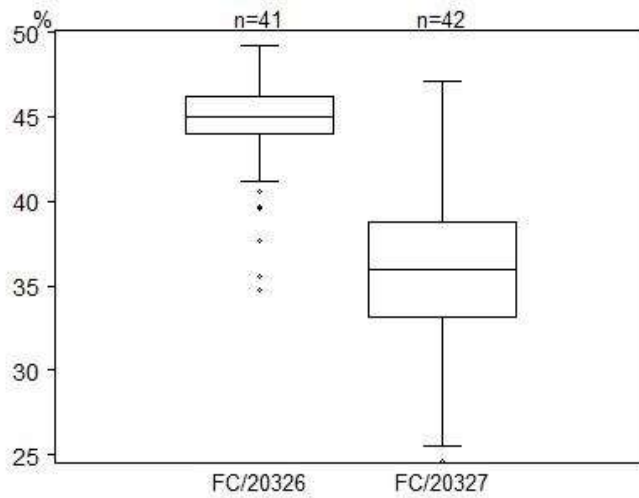
Kappa % B lymphocytes



Results not represented on the graph

- FC/20326 = 48 %
- FC/20327 = 73.4 %
- FC/20327 = 73.9 %
- FC/20327 = 77.1 %
- FC/20327 = 78.9 %
- FC/20327 = 81.6 %

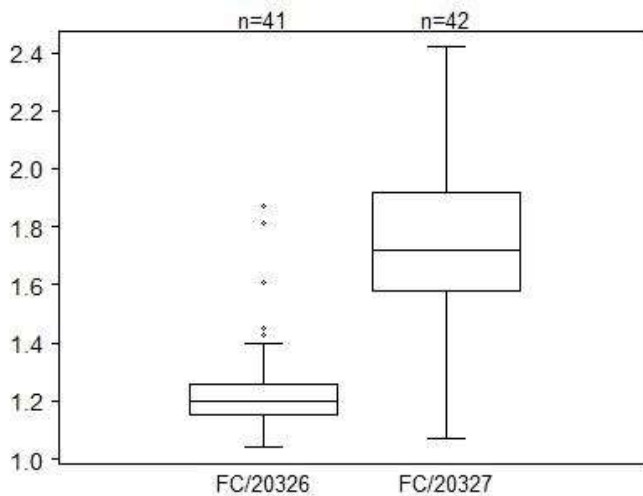
Lambda % B lymphocytes



Results not represented on the graph

- FC/20326 = 52 %
- FC/20327 = 20.8 %
- FC/20327 = 22.8 %
- FC/20327 = 62.4 %

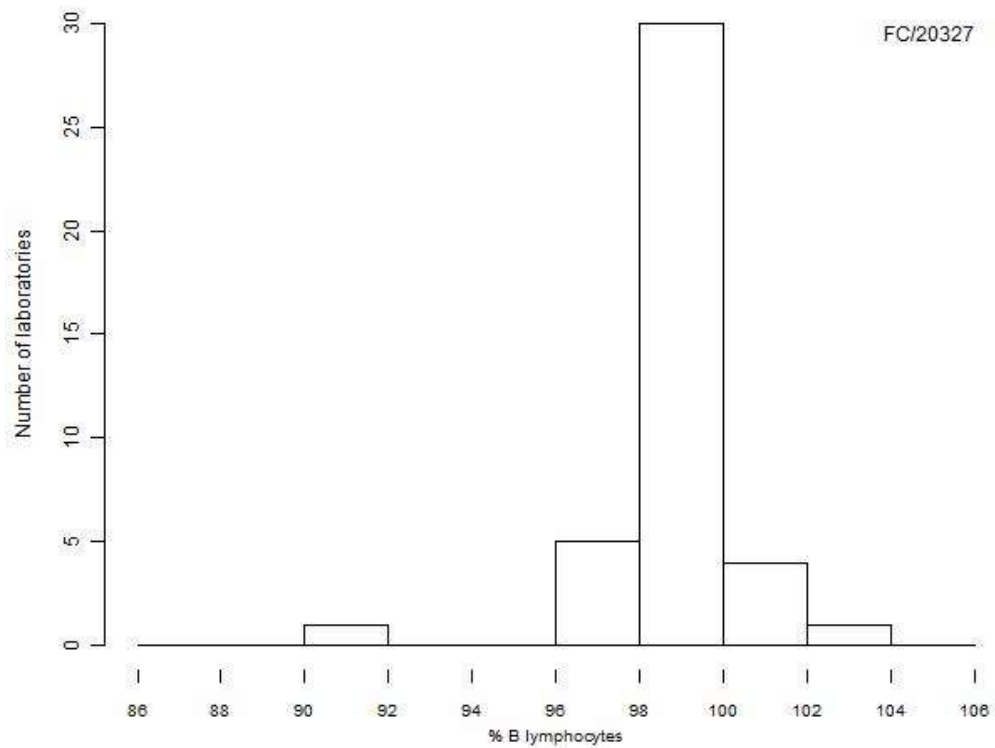
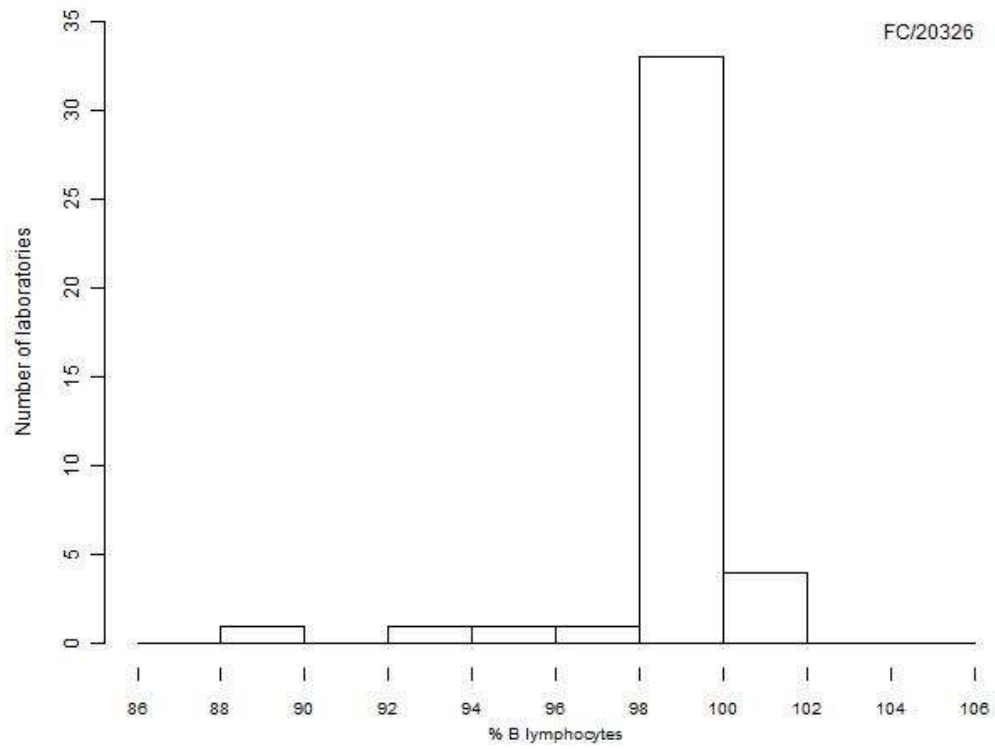
Kappa/lambda



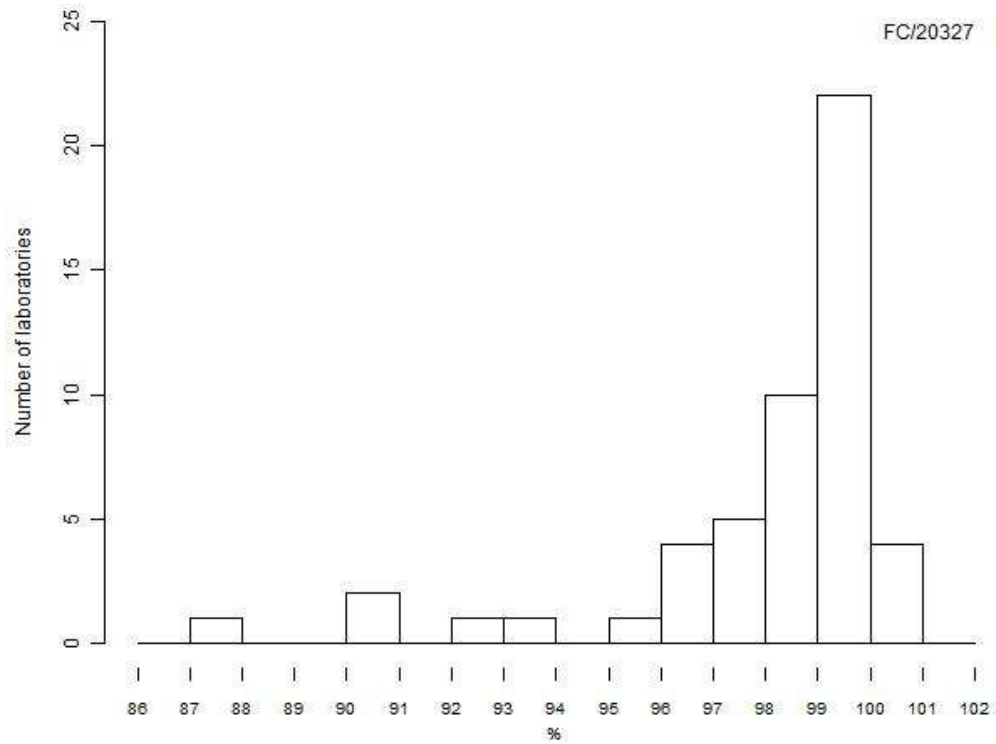
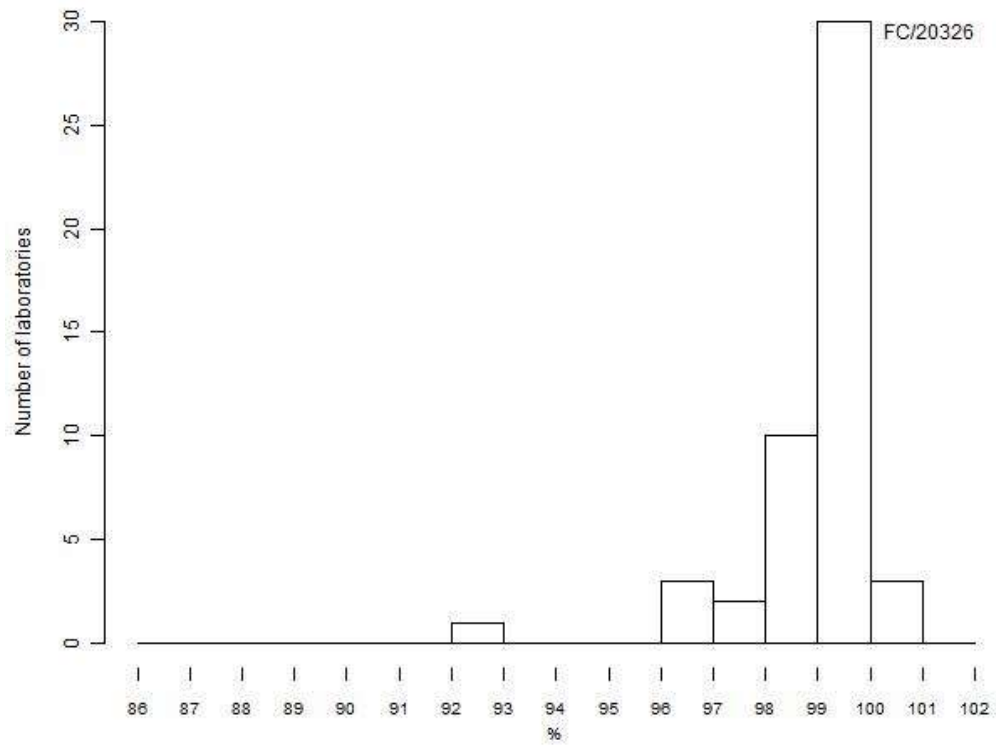
Results not represented on the graph

- FC/20326 = 0.92
- FC/20327 = 2.77
- FC/20327 = 2.78
- FC/20327 = 3
- FC/20327 = 3.38
- FC/20327 = 3.79

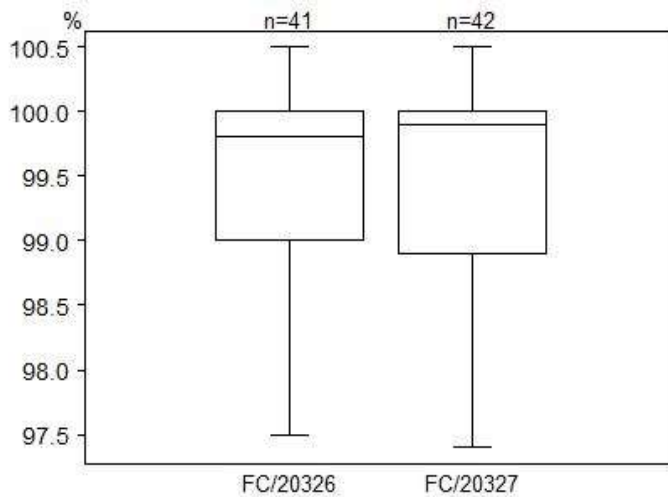
Sum K+L % B lymphocytes



Lymphosum %



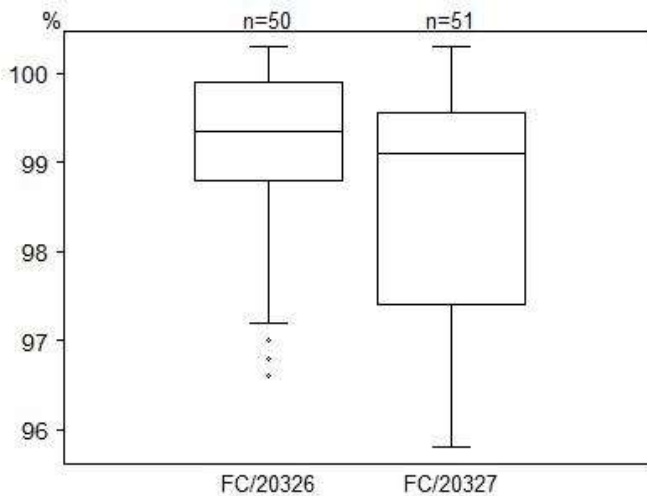
Sum K+L % B lymphocytes



Results not represented on the graph

FC/20326 = 88.7 %
FC/20326 = 92.4 %
FC/20326 = 95.9 %
FC/20327 = 91.1 %
FC/20327 = 96.4 %
FC/20327 = 102 %
FC/20327 = 102.6 %
FC/20327 = 144 %

Lymphosum %



Results not represented on the graph

FC/20326 = 93 %
FC/20326 = 162 %
FC/20327 = 87.8 %
FC/20327 = 90.4 %
FC/20327 = 90.9 %
FC/20327 = 92.3 %
FC/20327 = 94 %

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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