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 2020/3

Sciensano/Flow cytometry/75-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: 19/01/2021

Authorization to release the report: By Lobna Bouacida, scheme coordinator, on 18/02/2021.

Digitally signed by Lobna Bouacida
Date: 2021.02.18 11:10:48 +01'00'

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

TABLE OF CONTENTS

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 ($M_{M/G}$).

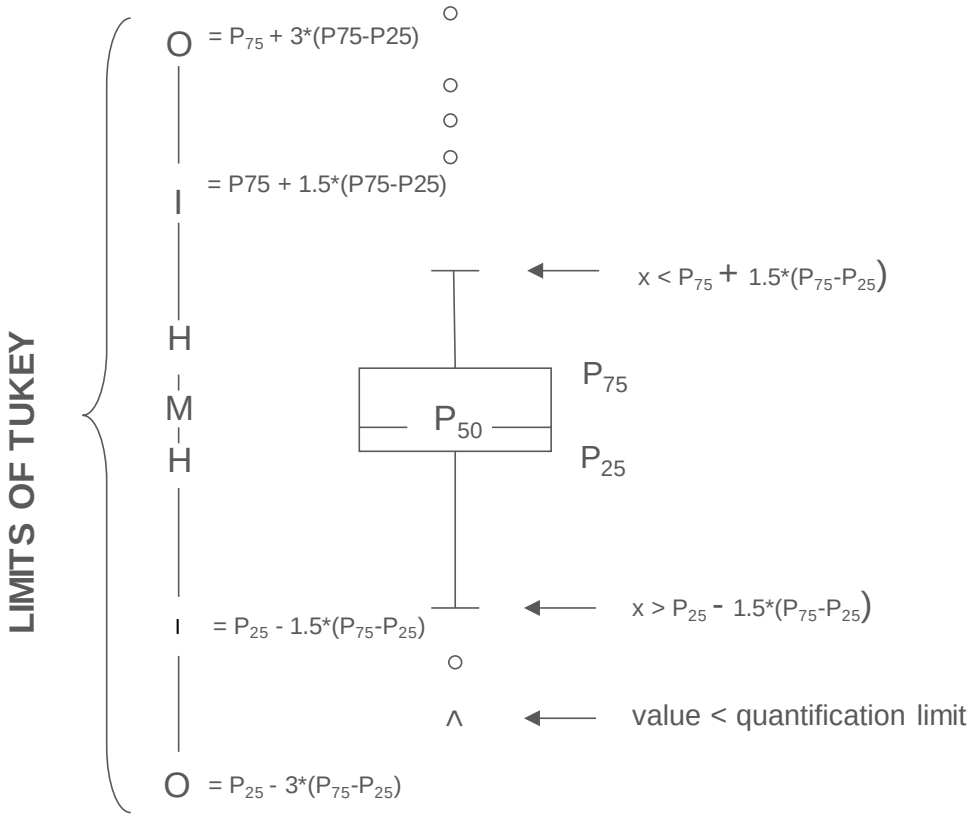
More information can be found in 3 brochures available on our website (only in Dutch and French):
https://www.wiv-isp.be/QML/index_nl.htm
https://www.wiv-isp.be/QML/index_fr.htm
(Choose “brochures” in the menu)
or directly on the following webpage (only in Dutch and French):
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_nl/brochures.htm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

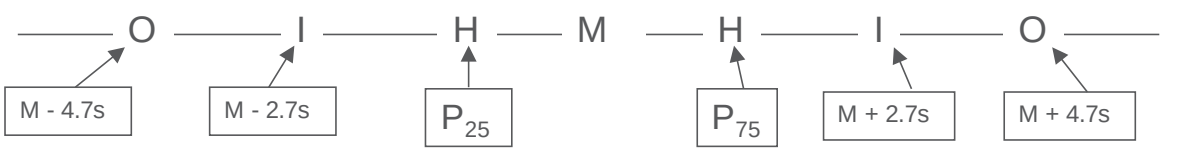
1. Informatiebrochure over de externe kwaliteitsevaluatieprogramma's voor klinische laboratoria (Algemene informatiebrochure over de externe evaluatie)/
   https://www.wiv-isp.be/QML/Informatiebrochure_EKE.pdf
   Brochure d’information sur les programmes d’évaluation externe de la qualité pour les laboratoires 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/17584 and FC/17585) 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, hepatitis C and syphilis. Homogeneity was confirmed based on white blood cells determination.

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

FC17584

	%	10^9/L
Leukocytes		7.0
Lymphocytes	33.4	
$CD3^+$ cells	63.6	1.66
$CD4^+CD3^+$ cells	36.4	0.95
$CD8^+CD3^+$ cells	26.3	0.68
$CD19^+$ cells	12.1	0.32
NK cells	21.7	0.57
κ % B lymphocytes	64.2	
λ % B lymphocytes	35.7	
κ/λ ratio	1.8	

FC17585

	%	10^9/L
Leukocytes		10.7
Lymphocytes	30.8	
$CD3^+$ cells	74.6	2.70
$CD4^+CD3^+$ cells	57.4	2.08
$CD8^+CD3^+$ cells	16.5	0.60
$CD19^+$ cells	16.5	0.60
NK cells	6.9	0.25
κ % B lymphocytes	62.9	
λ % B lymphocytes	37.1	
κ/λ ratio	1.7	

PARTICIPATION

Fifty-two laboratories (1 Canadian and 51 Belgian clinical laboratories) participated in the survey 2020/3 (send-out of blood samples on November 23, 2020 (day 0)).

RESULTS

98% of the Belgian laboratories received the samples on day 1 or 2. 45 laboratories (88%) received the samples on day 1 and five (10%) received them on day 2. One laboratory received them on day 3.

75% of the Belgian laboratories (n=38) performed the analyses on day 1 and 23% on day 2 (n=12). The laboratory that received the samples on day 3 performed the analyses the same day.

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

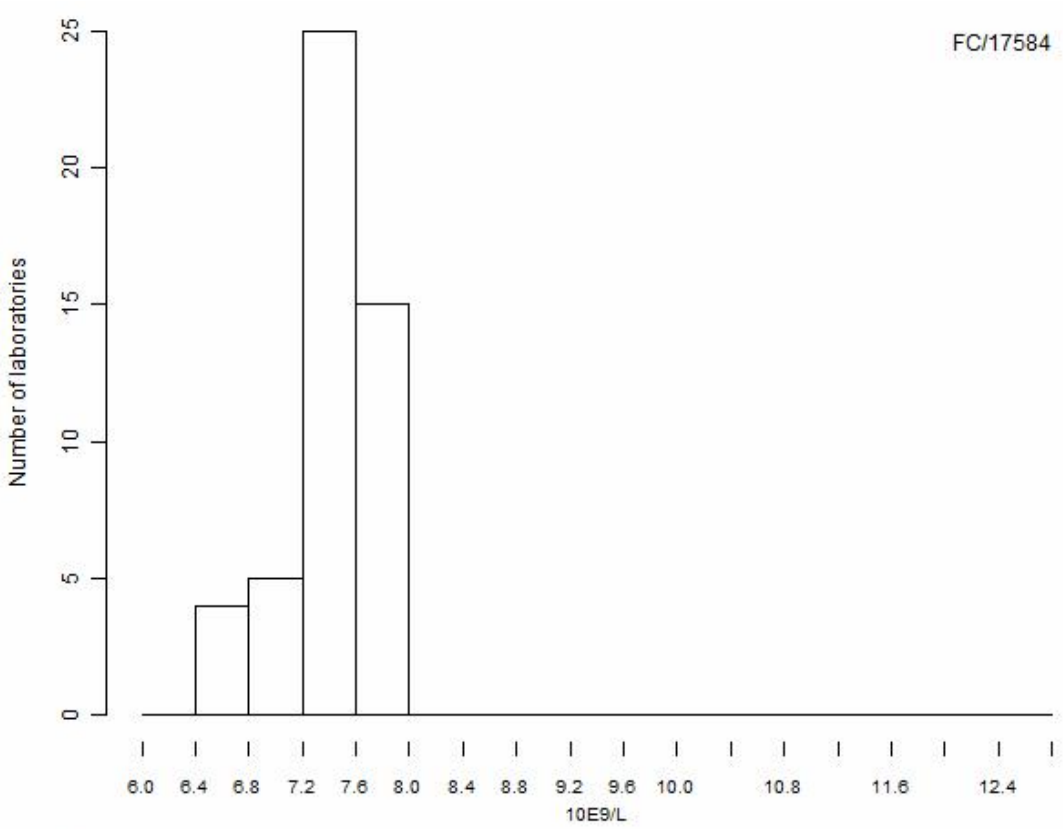
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=51). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the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

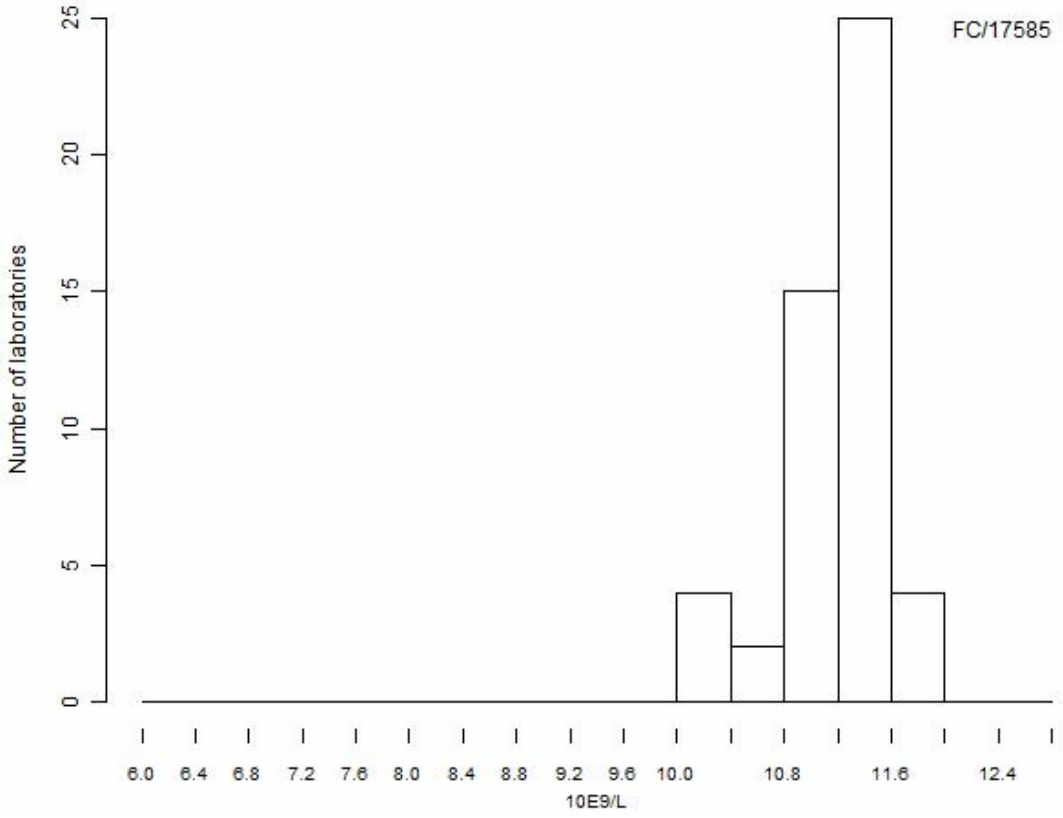
The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/17584 and FC/17585:

FC/17584	Median	SD	CV,%	N
WBC 10E9/L	7.51	0.23	3.1	48
Lympho% haematology analyser	36.8	1.0	2.8	48
Lympho% flow cytometer	35.8	2.6	7.3	45
CD3 %	65.3	2.0	3.1	50
CD3 10E9/L	1.824	0.126	6.9	49
CD4 %	36.8	1.7	4.6	50
CD4 10E9/L	1.010	0.092	9.1	49
CD8 %	26.8	1.5	5.5	50
CD8 10E9/L	0.722	0.046	6.4	49
CD19 %	11.0	0.9	8.1	50
CD19 10E9/L	0.300	0.037	12.3	49
NKcells %	22.4	2.5	11.1	50
NKcells 10E9/L	0.610	0.121	19.8	49
Kappa % B lymphocytes	62.2	3.4	5.4	43
Lambda % B lymphocytes	37.0	3.0	8.2	43
Kappa/lambda	1.70	0.24	14.4	43
Sum K+L % B lymphocytes	99.9	0.4	0.4	43
Lymphosum %	98.9	1.2	1.2	50

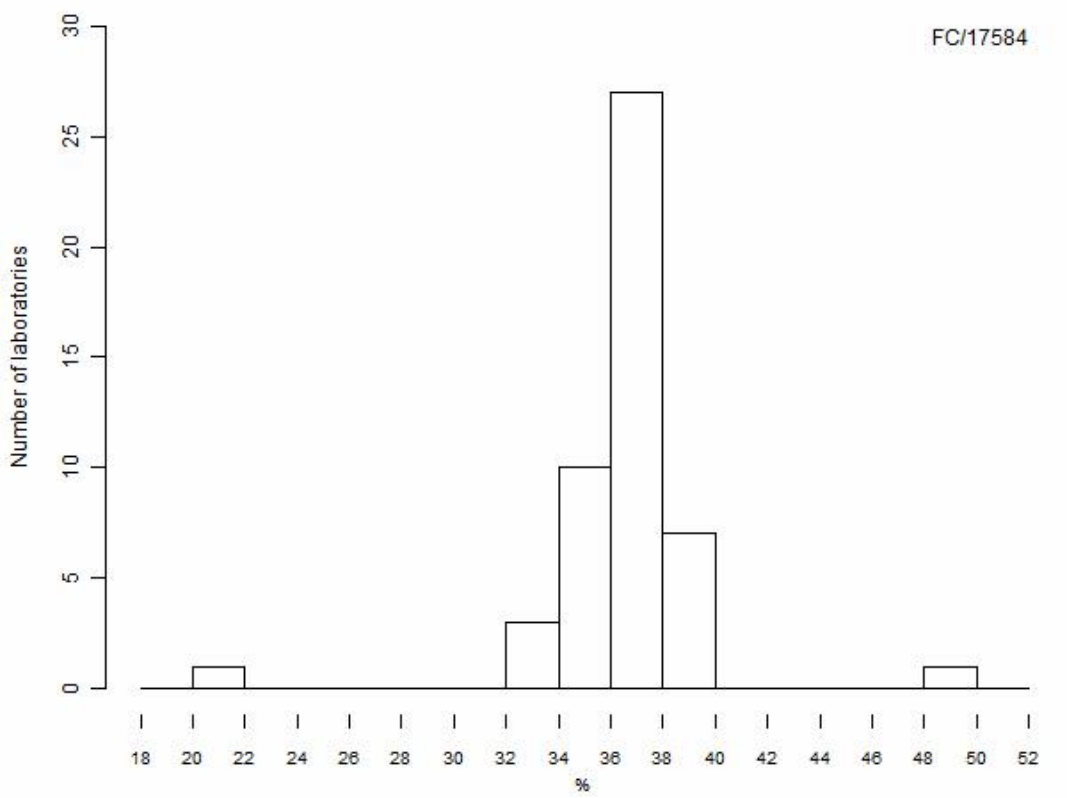
FC/17585	Median	SD	CV,%	N
WBC 10E9/L	11.34	0.32	2.8	49
Lympho% haematology analyser	32.3	0.9	2.7	49
Lympho% flow cytometer	31.3	1.7	5.4	46
CD3 %	78.5	2.2	2.7	51
CD3 10E9/L	2.844	0.258	9.1	50
CD4 %	60.0	1.9	3.2	51
CD4 10E9/L	2.189	0.216	9.9	50
CD8 %	17.4	1.0	6.0	51
CD8 10E9/L	0.617	0.052	8.4	50
CD19 %	13.7	2.1	15.2	51
CD19 10E9/L	0.480	0.095	19.7	50
NKcells %	7.0	1.2	16.5	51
NKcells 10E9/L	0.250	0.058	23.1	50
Kappa % B lymphocytes	65.1	2.4	3.8	44
Lambda % B lymphocytes	34.1	2.0	5.8	44
Kappa/lambda	1.89	0.17	9.0	44
Sum K+L % B lymphocytes	99.8	1.0	1.0	44
Lymphosum %	99.5	0.7	0.7	51

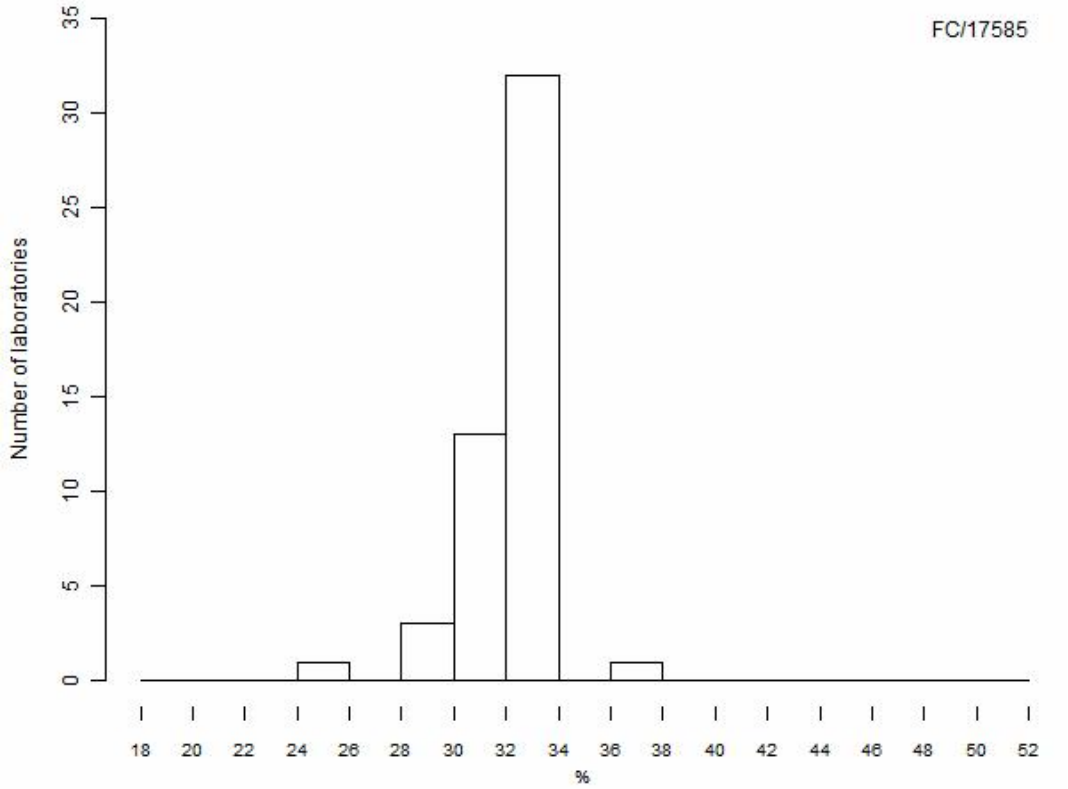
WBC 10E9/L


FC/17584
Number of laboratories
0
5
10
15
20
25
6.0
6.4
6.8
7.2
7.6
8.0
8.4
8.8
9.2
9.6
10.0
10.8
11.6
12.4
10E9/L

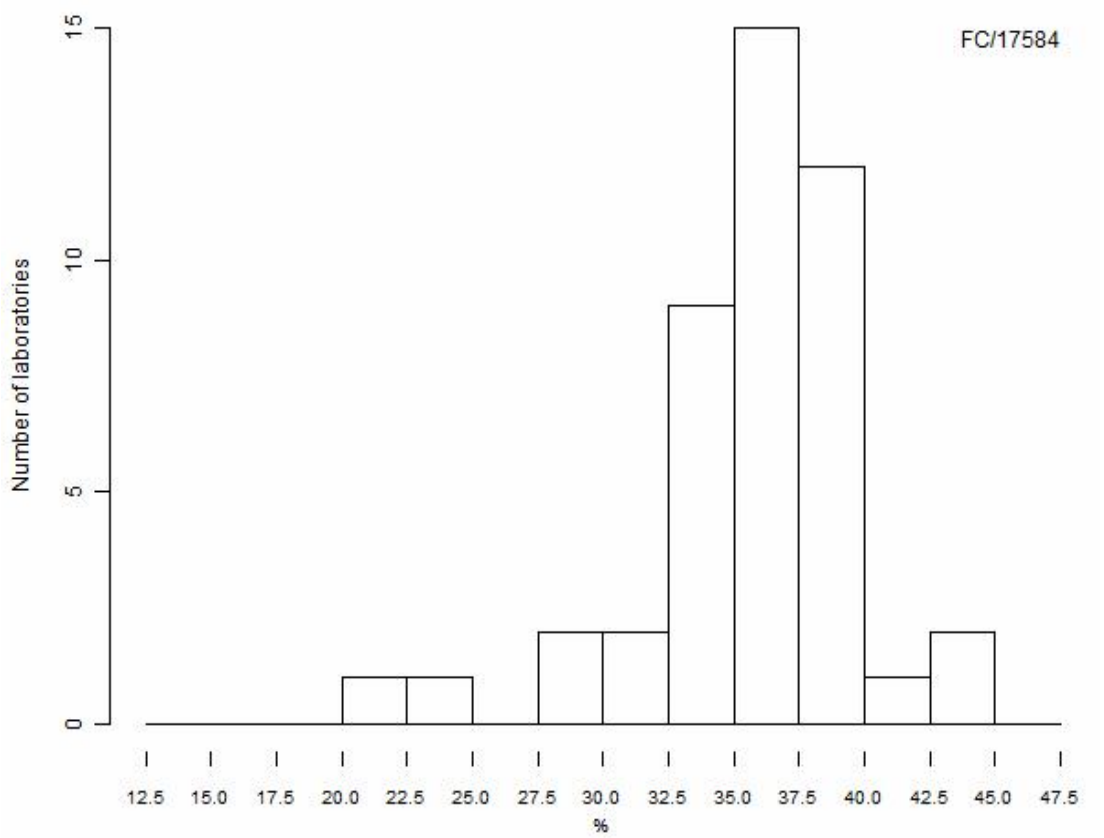

FC/17585
Number of laboratories
0
5
10
15
20
25
6.0
6.4
6.8
7.2
7.6
8.0
8.4
8.8
9.2
9.6
10.0
10.8
11.6
12.4
10E9/L

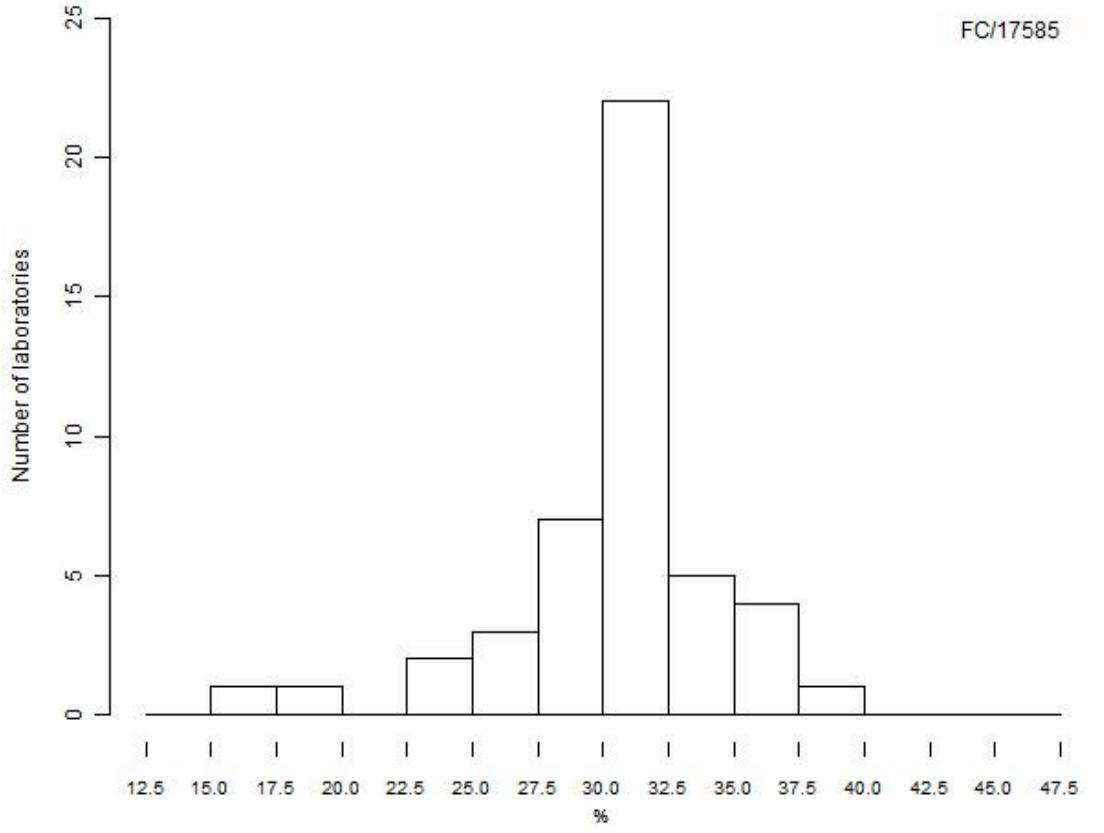
Lympho% haematology analyser



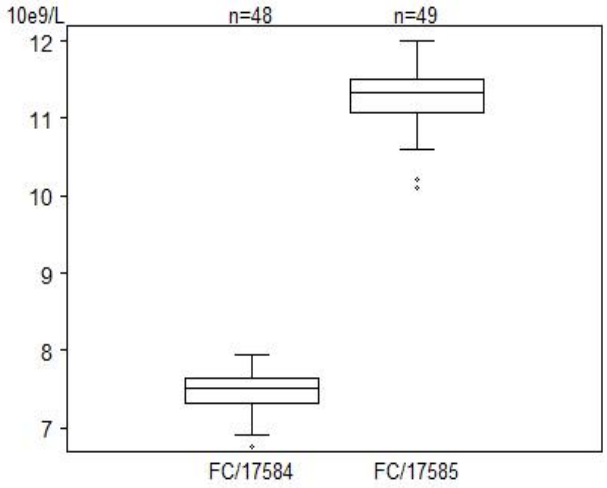


Lympho% flow cytometer





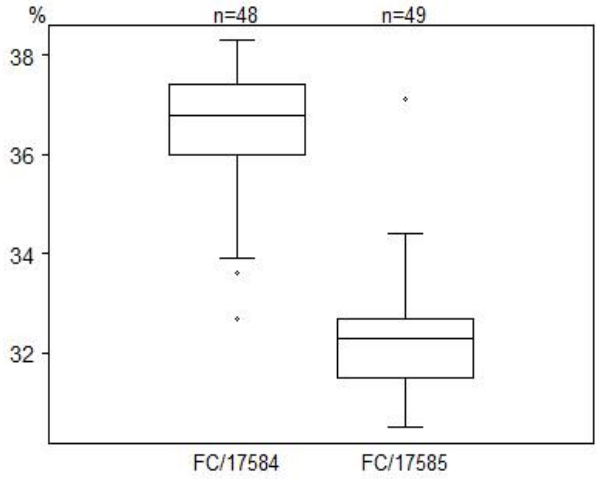
WBC 10E9/L



Results not represented on the graph

FC/17584 = 6.59 10e9/l

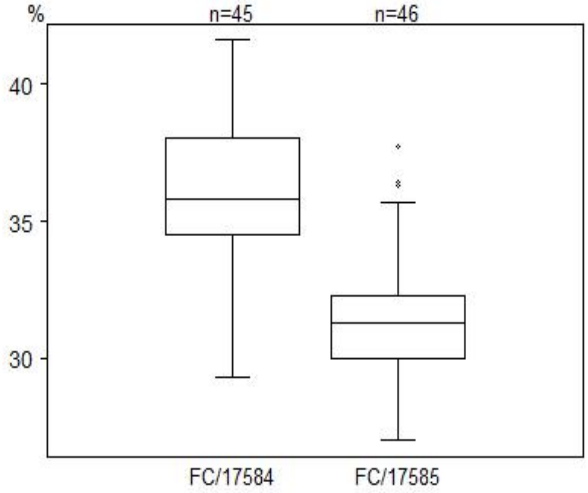
Lympho% haematology analyser



Results not represented on the graph

FC/17584 = 20.7 %
FC/17584 = 40 %
FC/17584 = 48.2 %
FC/17585 = 24.8 %
FC/17585 = 28.5 %
FC/17585 = 28.8 %
FC/17585 = 29.2 %

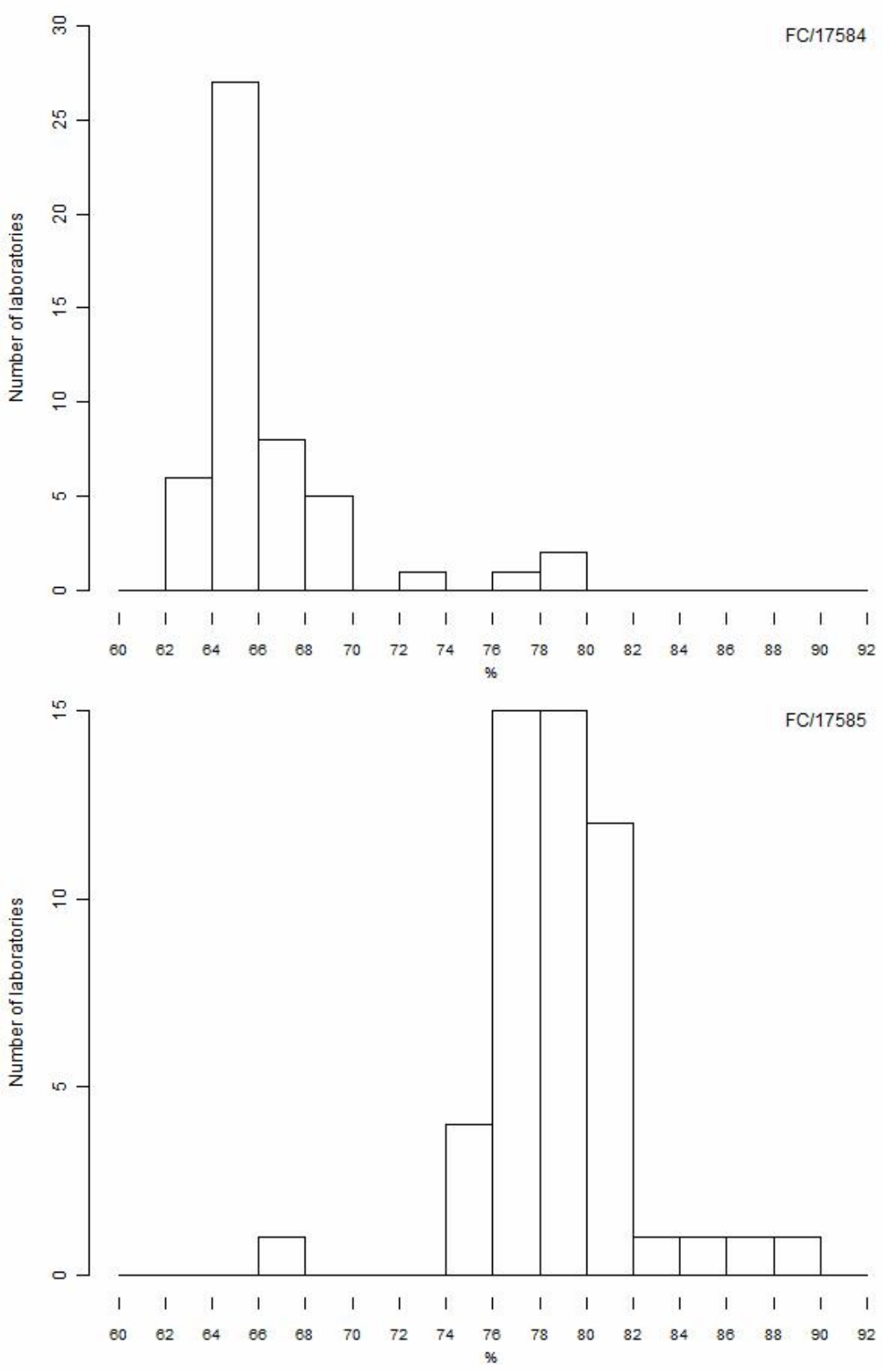
Lympho% flow cytometer



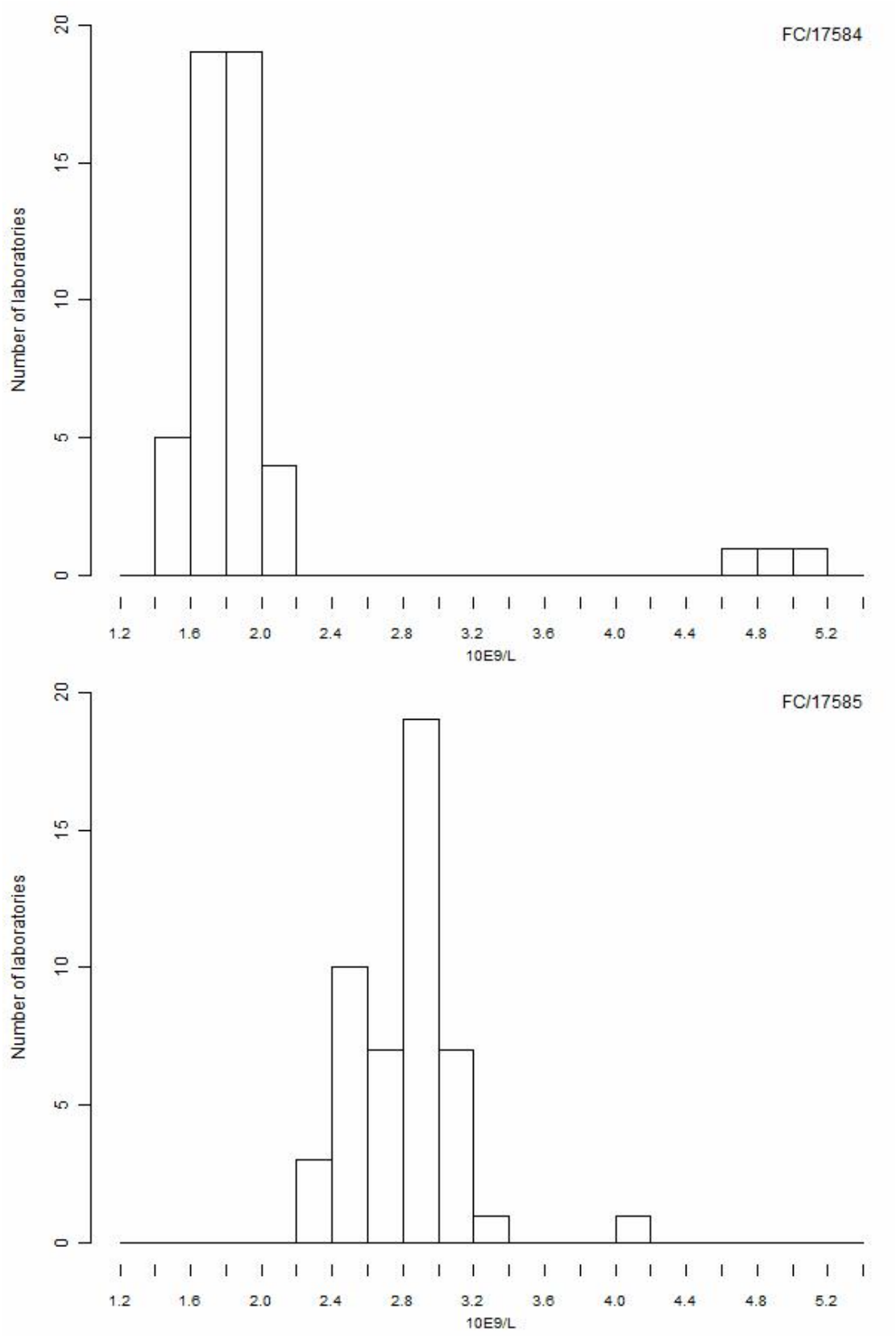
Results not represented on the graph

FC/17584 = 22.1 %
FC/17584 = 25 %
FC/17584 = 44.7 %
FC/17584 = 45 %
FC/17585 = 15.4 %
FC/17585 = 18 %
FC/17585 = 22.9 %
FC/17585 = 24.3 %
FC/17585 = 26.1 %

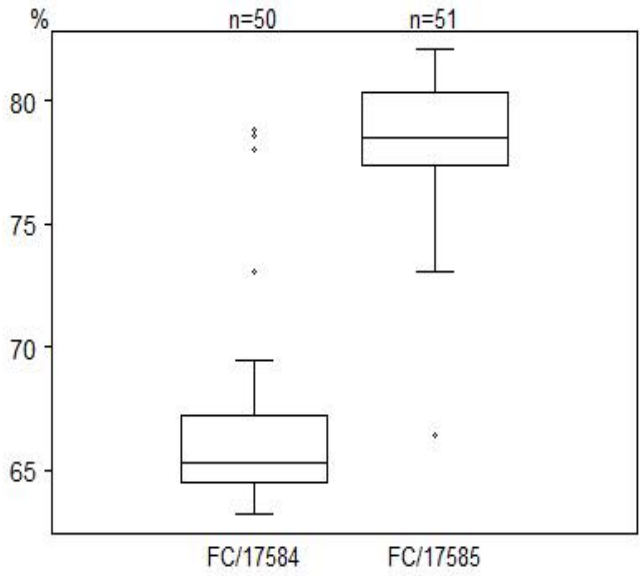
CD3 %


FC/17584
Number of laboratories
0
5
10
15
20
25
30
60
62
64
66
68
70
72
74
76
78
80
82
84
86
88
90
92
%
FC/17585
Number of laboratories
0
5
10
15
60
62
64
66
68
70
72
74
76
78
80
82
84
86
88
90
92
%

CD3 10E9/L


FC/17584
Number of laboratories
0
5
10
15
20
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
5.2
10E9/L
FC/17585
Number of laboratories
0
5
10
15
20
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
5.2
10E9/L

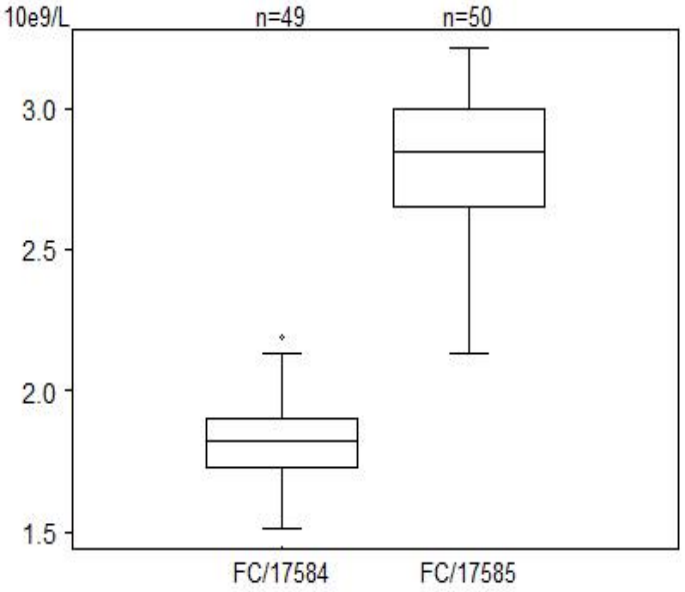
CD3 %



Results not represented on the graph

FC/17585 = 85.3 %
FC/17585 = 86.8 %
FC/17585 = 89.1 %

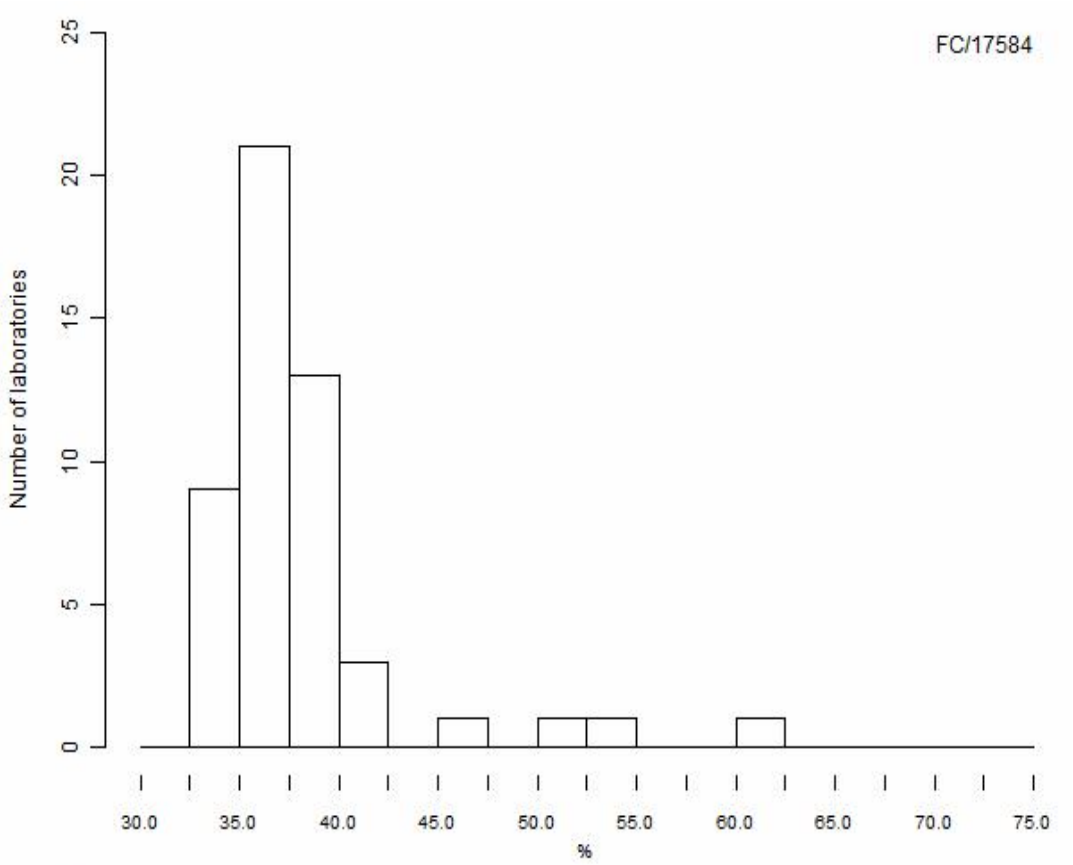
CD3 10E9/L

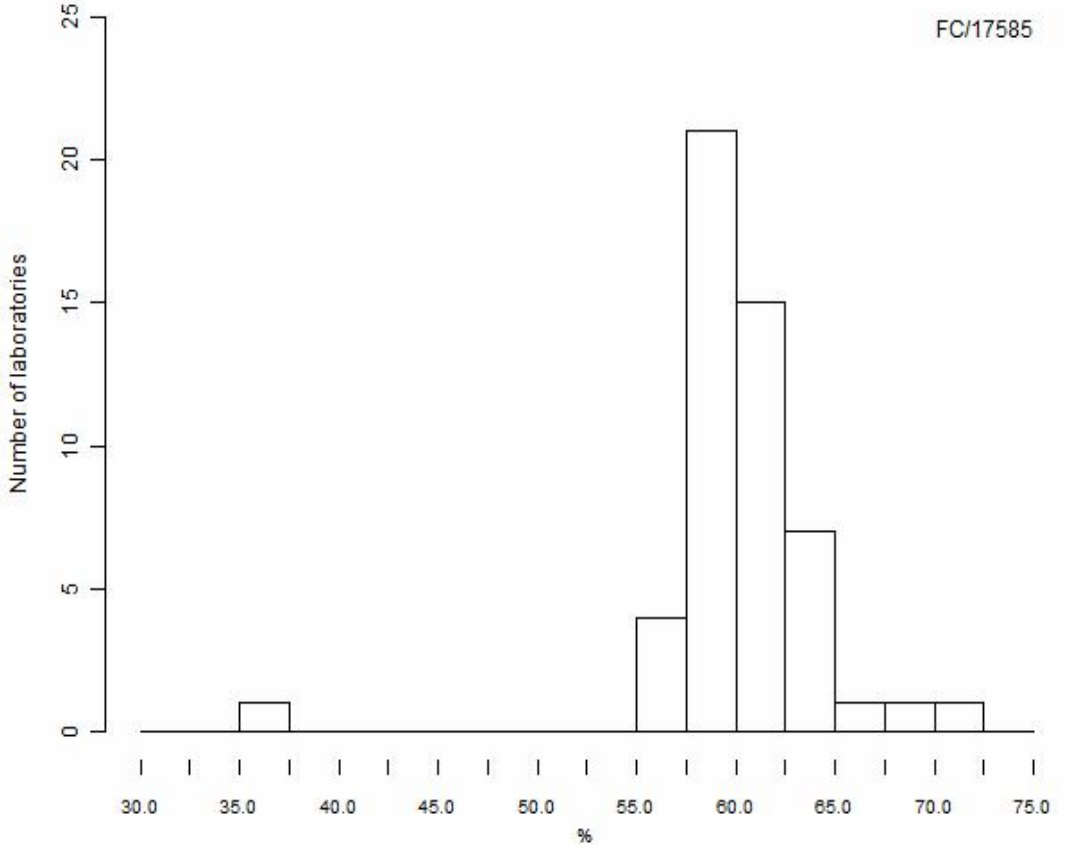


Results not represented on the graph

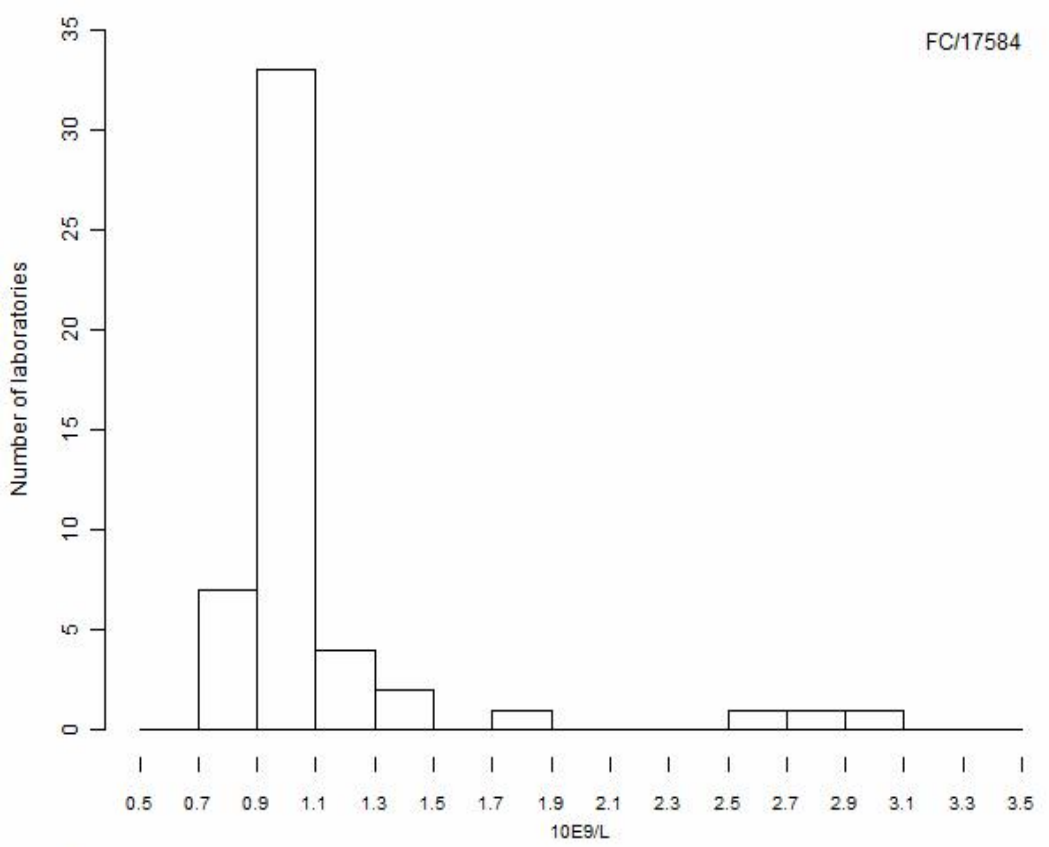
FC/17584 = 4.71 10e9/l
FC/17584 = 4.938 10e9
FC/17584 = 5.18 10e9/l
FC/17585 = 4.085 10e9
FC/17585 = 8.855 10e9
FC/17585 = 9.04 10e9/l
FC/17585 = 9.07 10e9/l

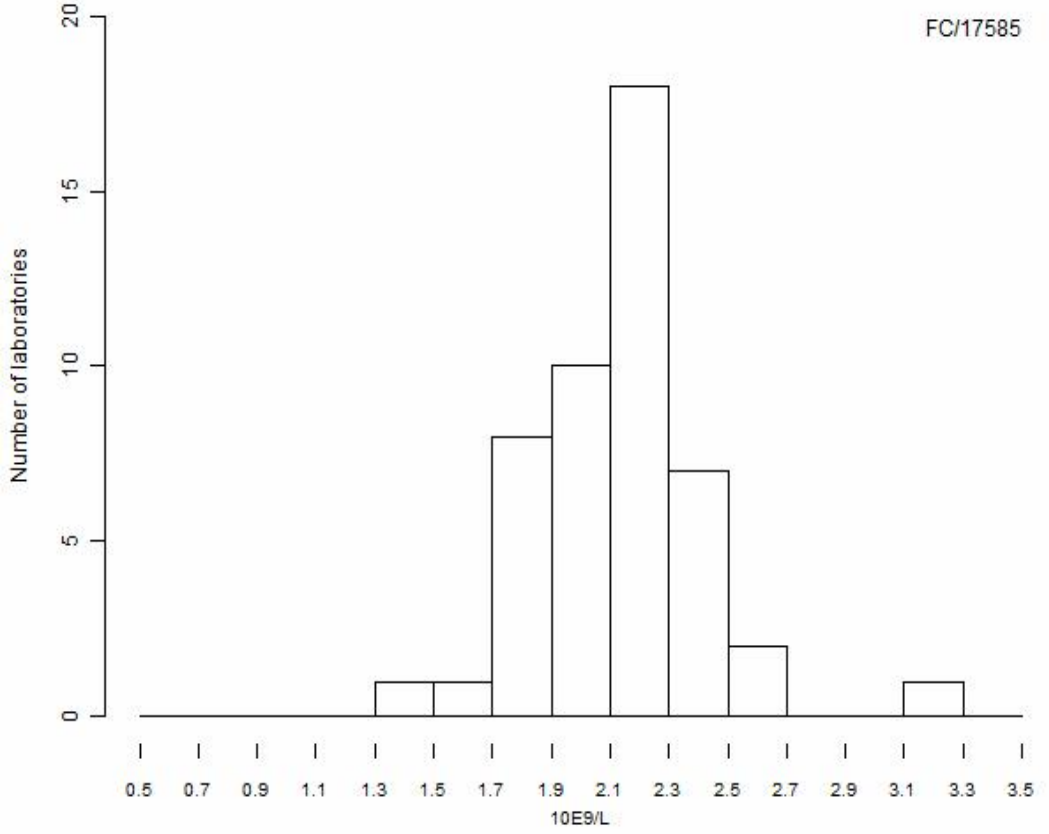
CD4 %


FC/17584
Number of laboratories
0
5
10
15
20
25
30.0
35.0
40.0
45.0
50.0
55.0
60.0
65.0
70.0
75.0
%

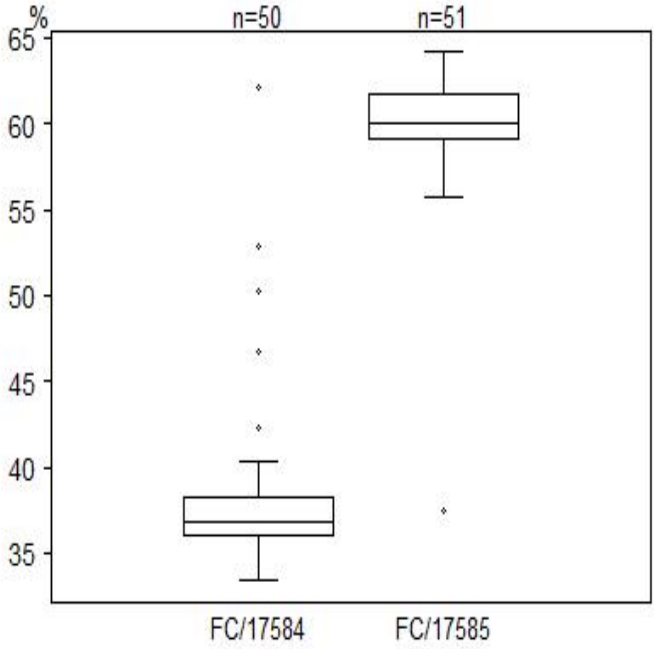

FC/17585
Number of laboratories
0
5
10
15
20
25
30.0
35.0
40.0
45.0
50.0
55.0
60.0
65.0
70.0
75.0
%

CD4 10E9/L


FC/17584
Number of laboratories
0
5
10
15
20
25
30
35
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
3.1
3.3
3.5
10E9/L


FC/17585
Number of laboratories
0
5
10
15
20
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
3.1
3.3
3.5
10E9/L

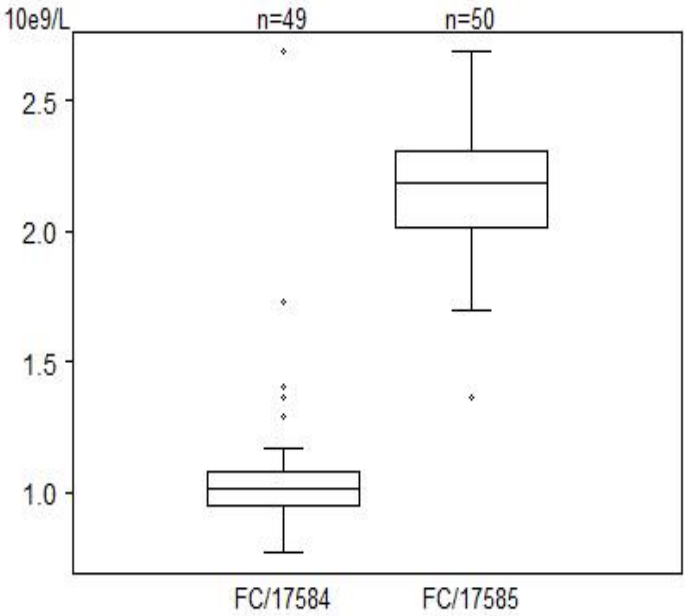
CD4 %



Results not represented on the graph

FC/17585 = 66.6 %
FC/17585 = 68.2 %
FC/17585 = 70.8 %

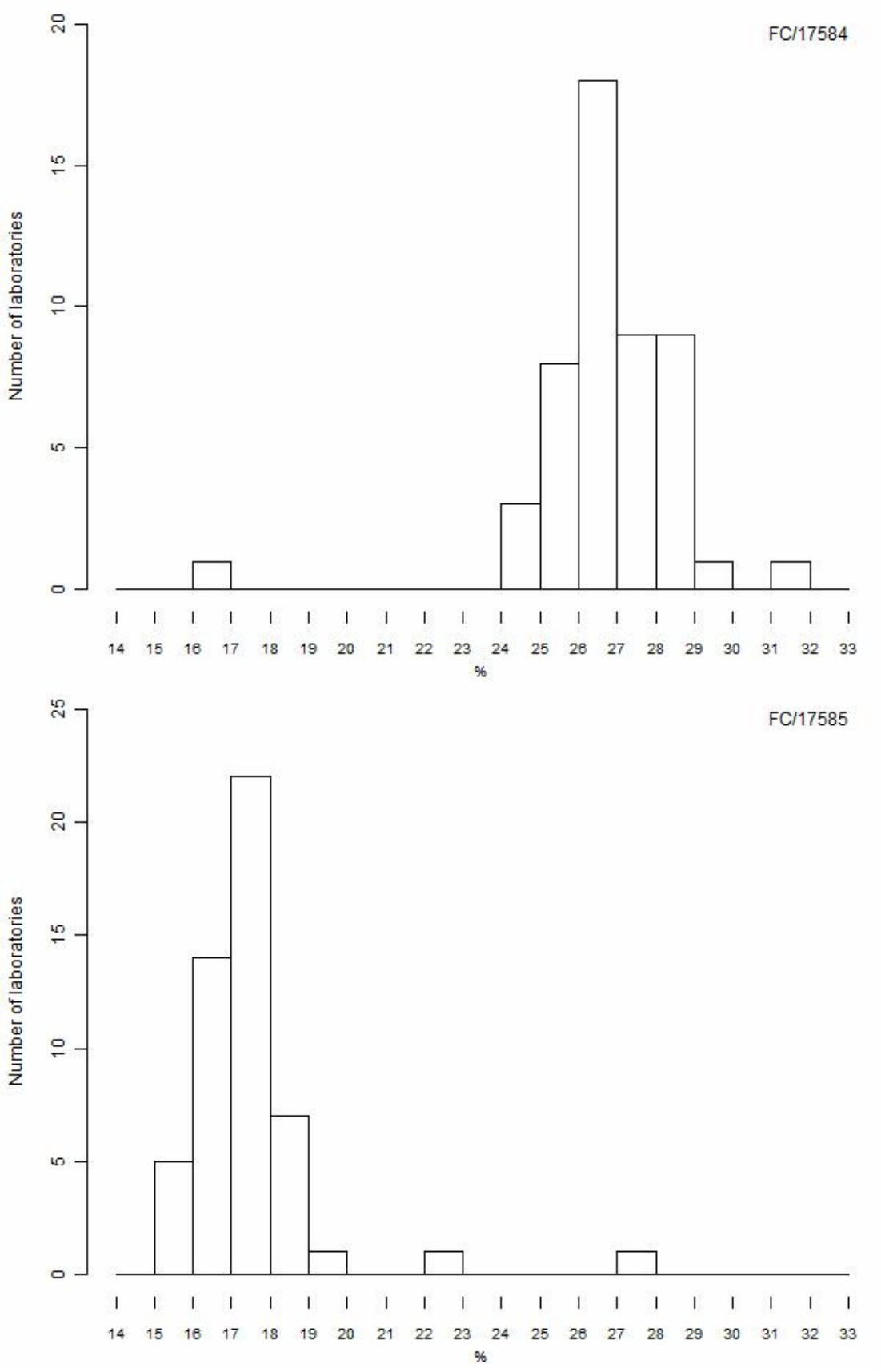
CD4 10E9/L



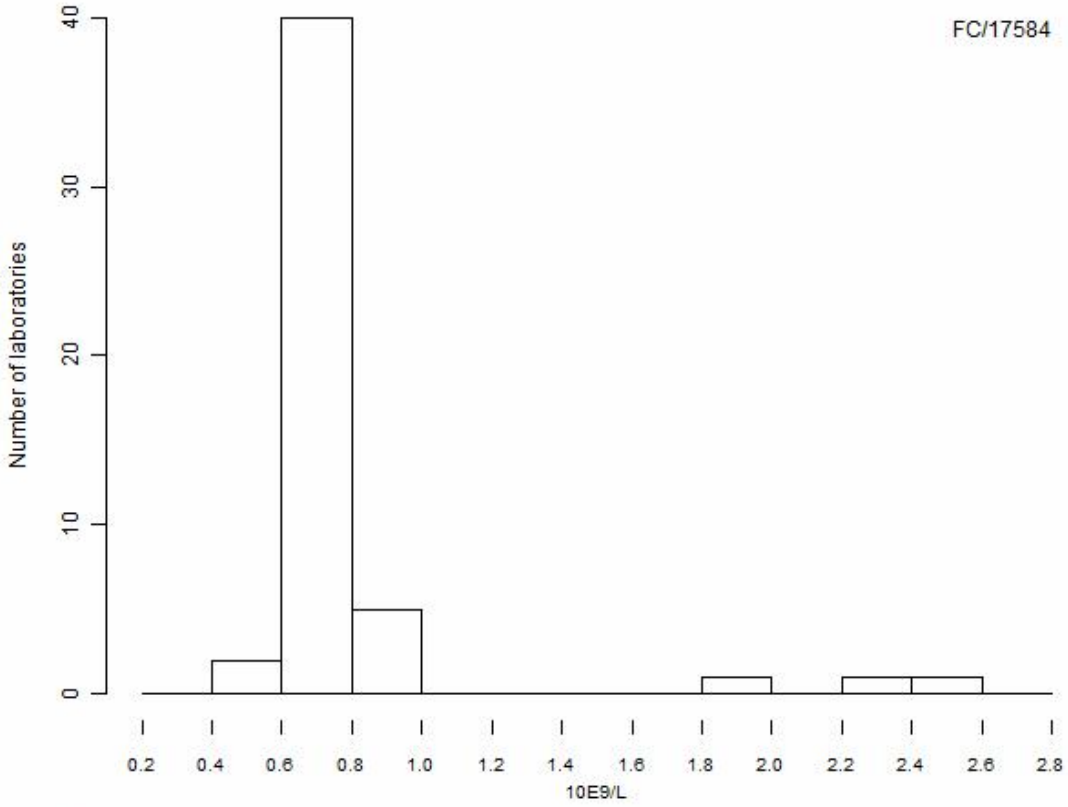
Results not represented on the graph

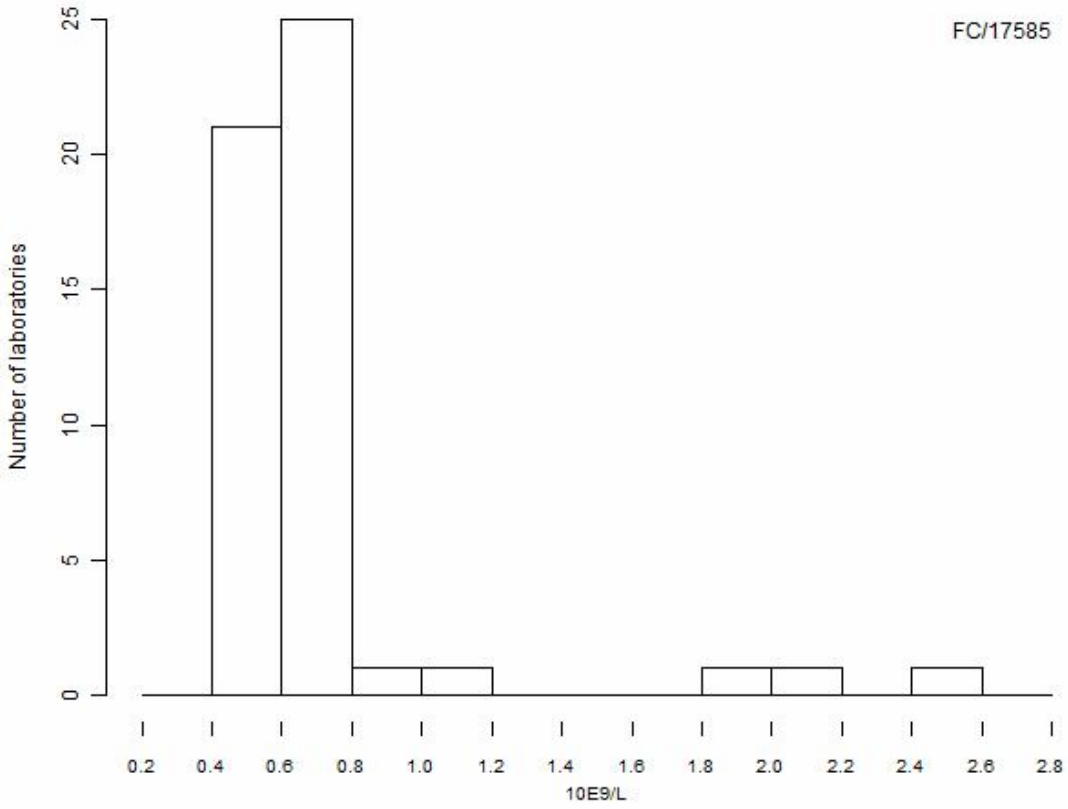
FC/17584 = 2.81 10e9/l
FC/17584 = 3.014 10e9
FC/17585 = 3.195 10e9
FC/17585 = 6.85 10e9/l
FC/17585 = 6.941 10e9
FC/17585 = 6.95 10e9/l

CD8 %

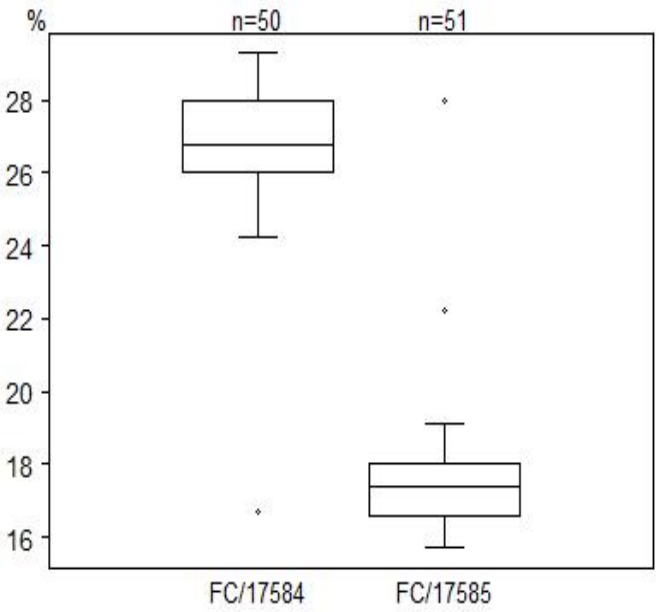

FC/17584
Number of laboratories
0
5
10
15
20
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
%
FC/17585
Number of laboratories
0
5
10
15
20
25
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
%

CD8 10E9/L


FC/17584
Number of laboratories
0
10
20
30
40
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
10E9/L


FC/17585
Number of laboratories
0
5
10
15
20
25
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
10E9/L

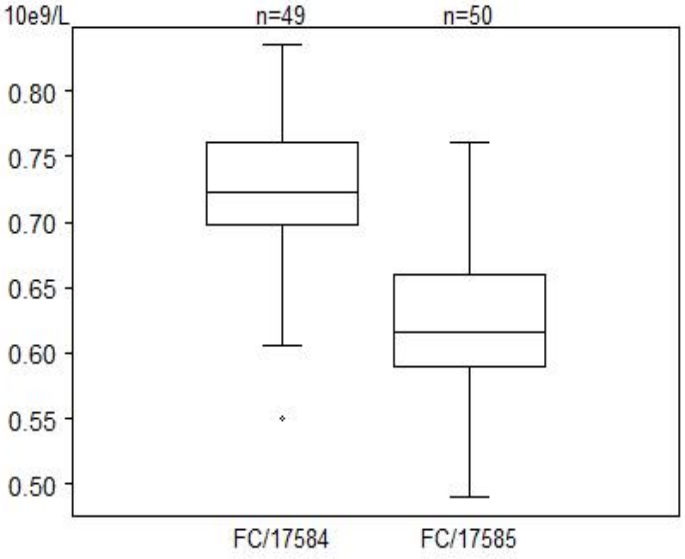
CD8 %



Results not represented on the graph

FC/17584 = 31.8 %

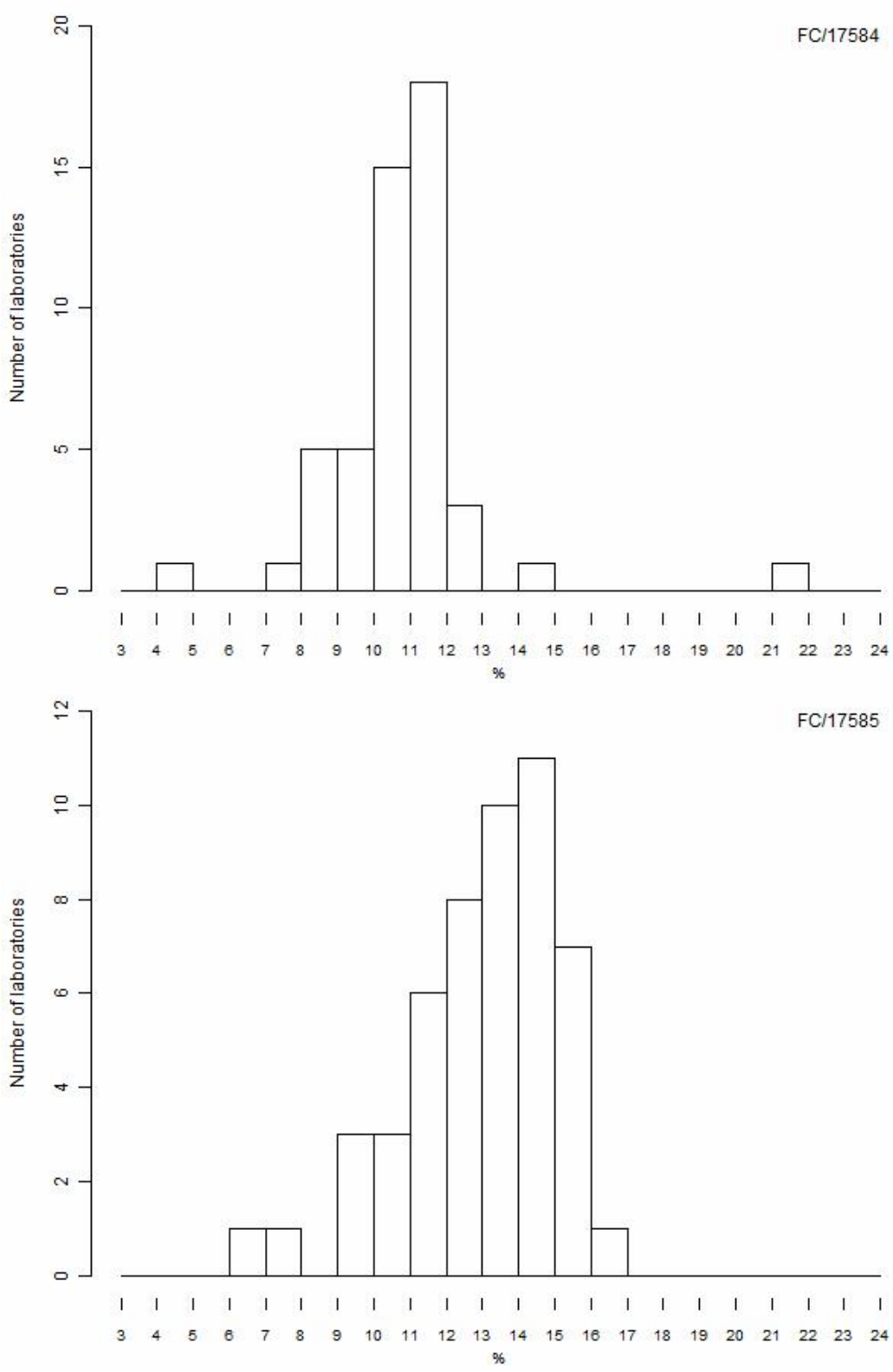
CD8 10E9/L



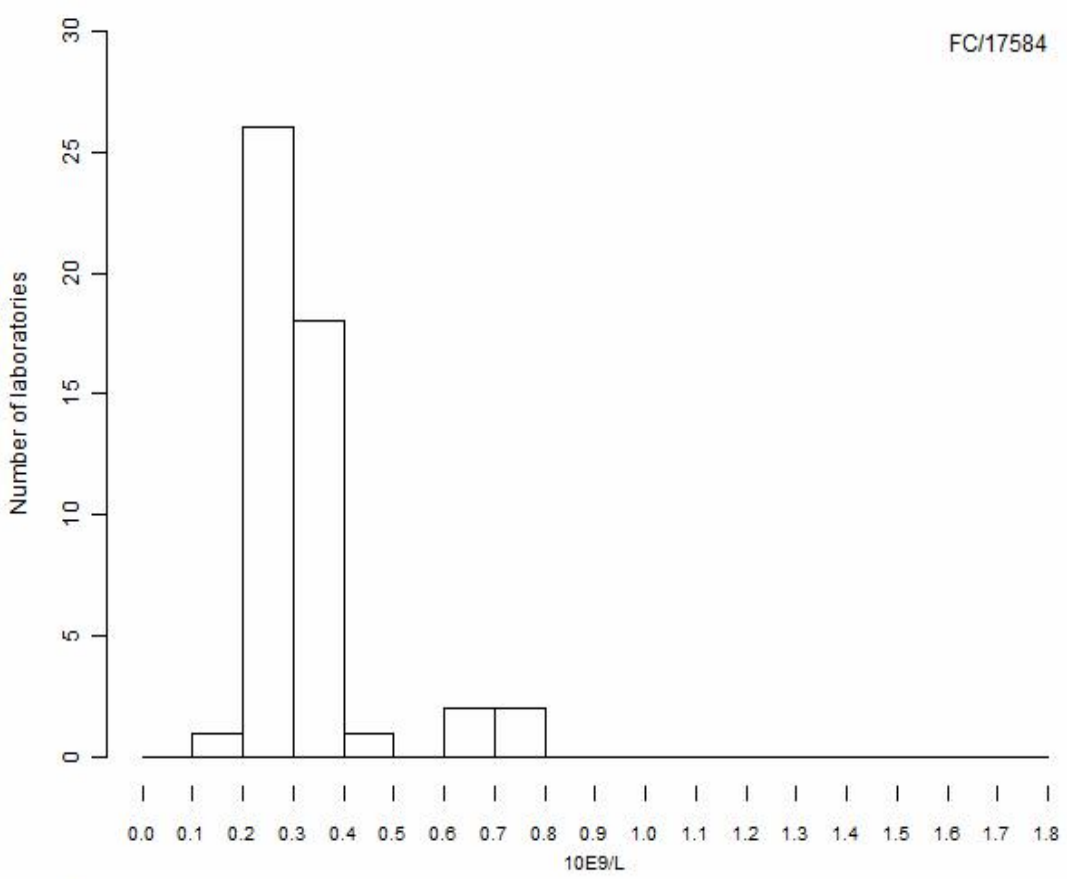
Results not represented on the graph

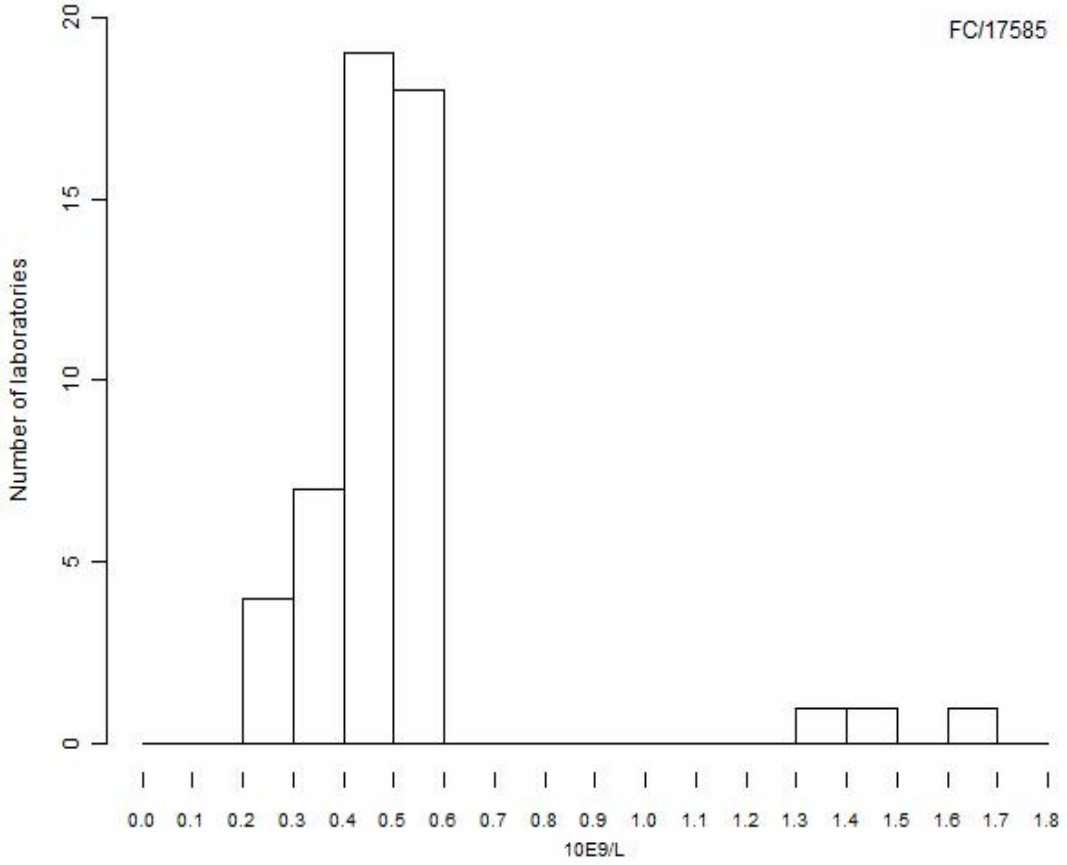
FC/17584 = 0.464 10e9
FC/17584 = 1.98 10e9/l
FC/17584 = 2.27 10e9/l
FC/17584 = 2.42 10e9/l
FC/17585 = 0.86 10e9/l
FC/17585 = 1.019 10e9
FC/17585 = 1.94 10e9/l
FC/17585 = 2.15 10e9/l
FC/17585 = 2.56 10e9/l

CD19 %

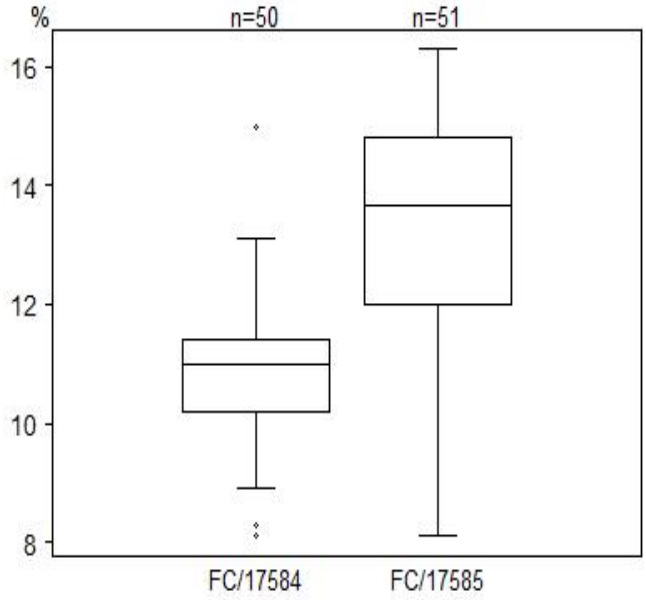

FC/17584
Number of laboratories
0
5
10
15
20
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
%
FC/17585
Number of laboratories
0
2
4
6
8
10
12
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
%

CD19 10E9/L


FC/17584
Number of laboratories
0
5
10
15
20
25
30
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8
10E9/L


FC/17585
Number of laboratories
0
5
10
15
20
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8
10E9/L

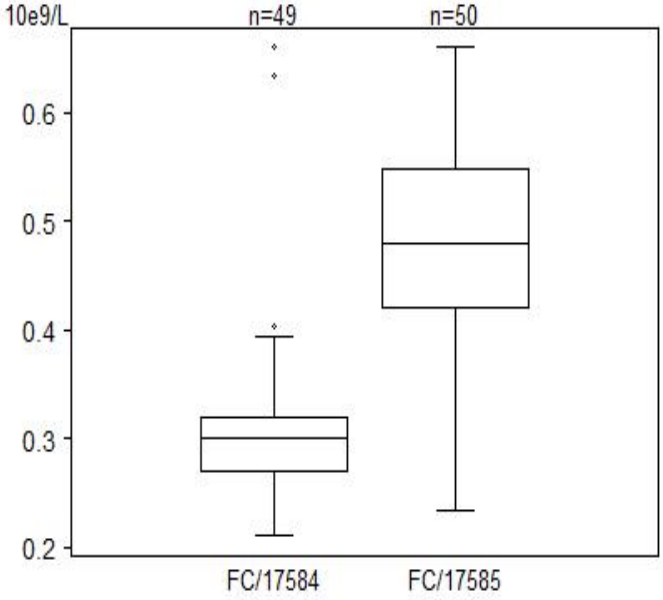
CD19 %



Results not represented on the graph

FC/17584 = 4.8 %
FC/17584 = 7.4 %
FC/17584 = 22 %
FC/17585 = 7 %
FC/17585 = 7.4 %

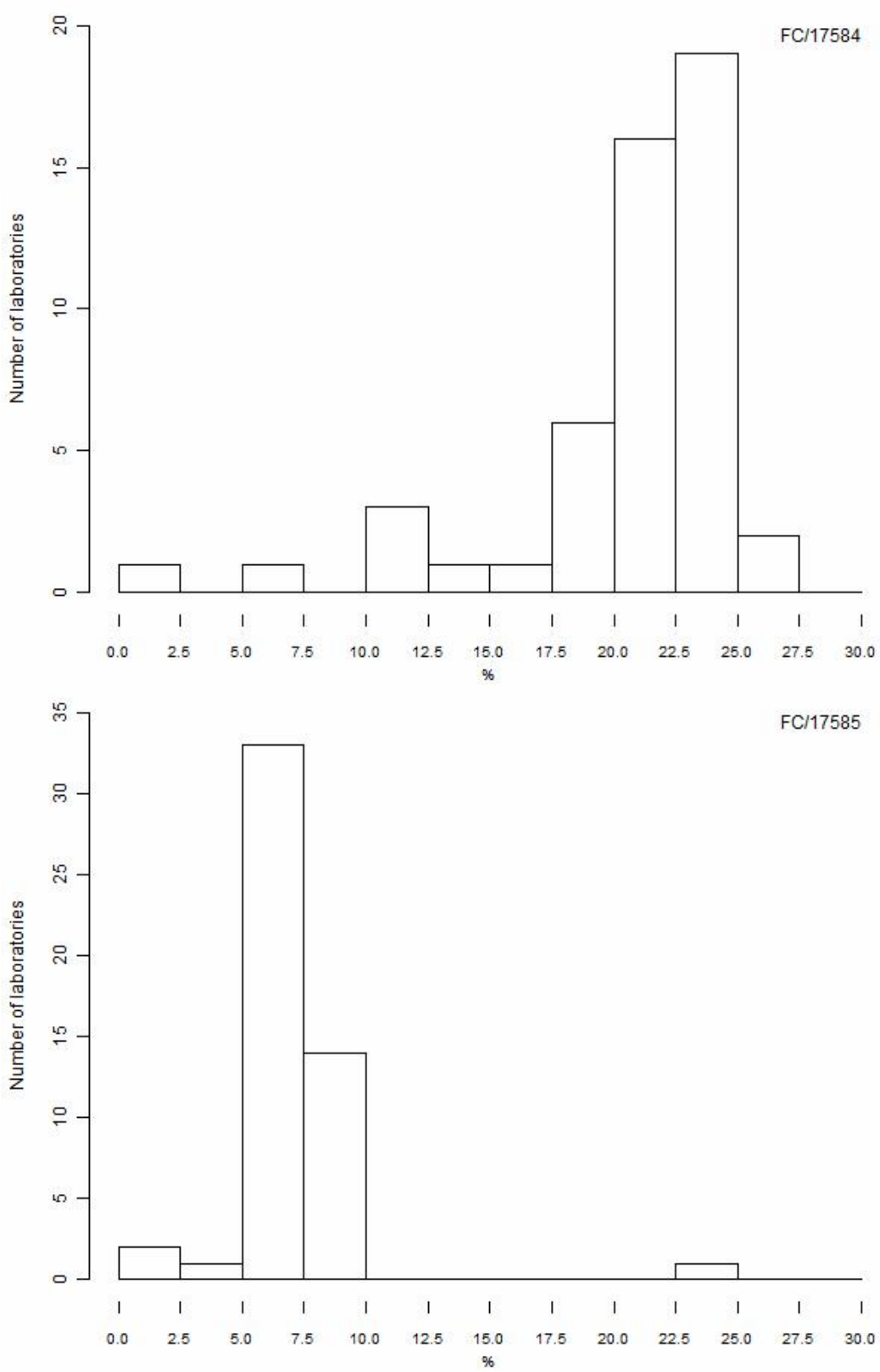
CD19 10E9/L



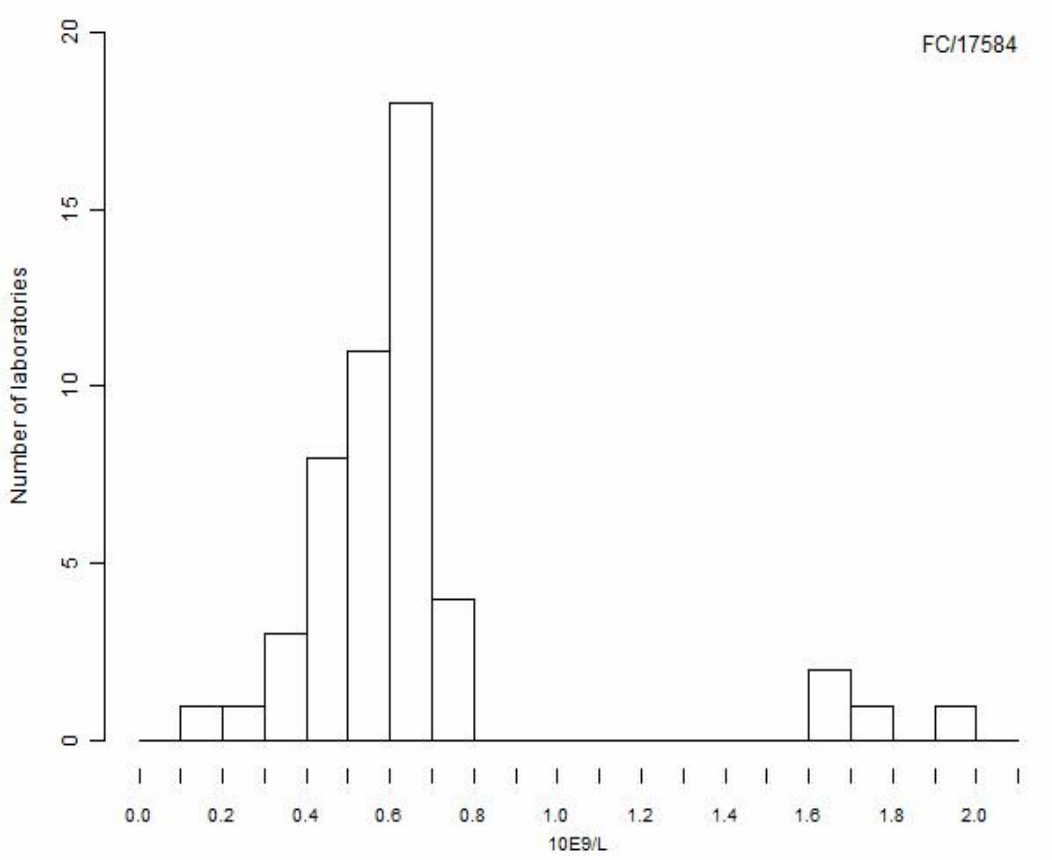
Results not represented on the graph

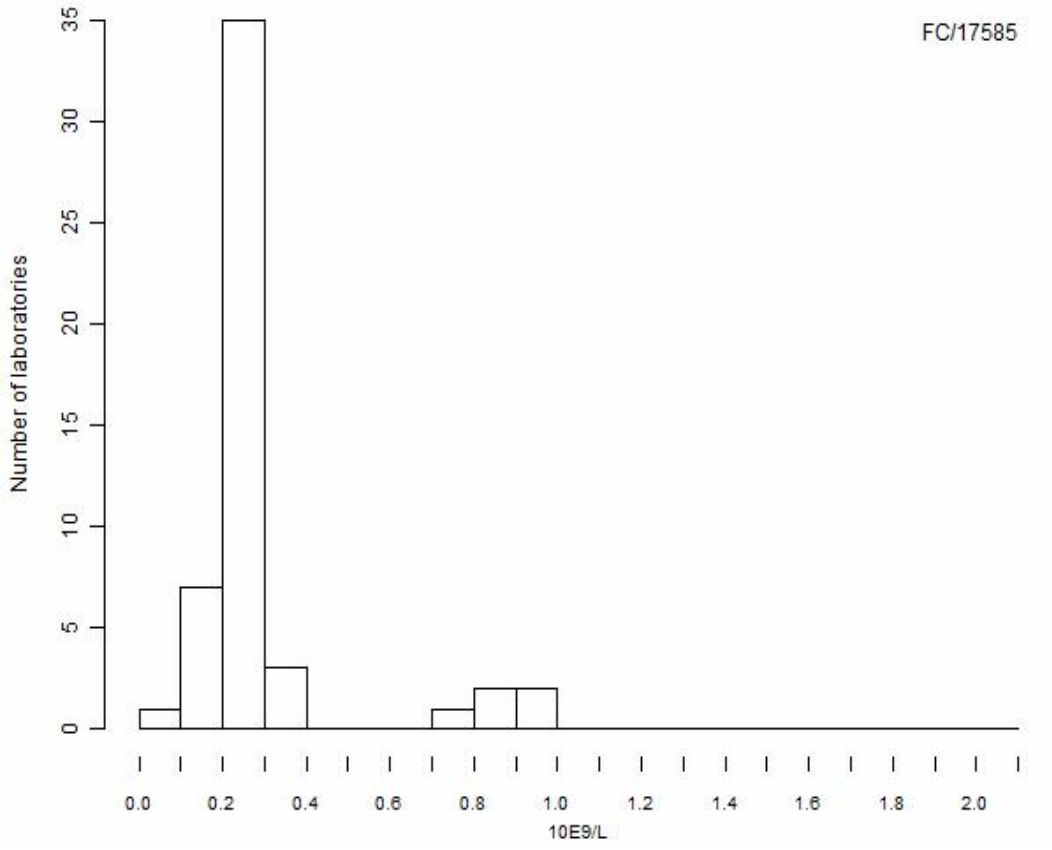
FC/17584 = 0.13 10e9/l
FC/17584 = 0.754 10e9
FC/17584 = 0.8 10e9/L
FC/17585 = 1.33 10e9/l
FC/17585 = 1.43 10e9/l
FC/17585 = 1.61 10e9/l

NKcells %

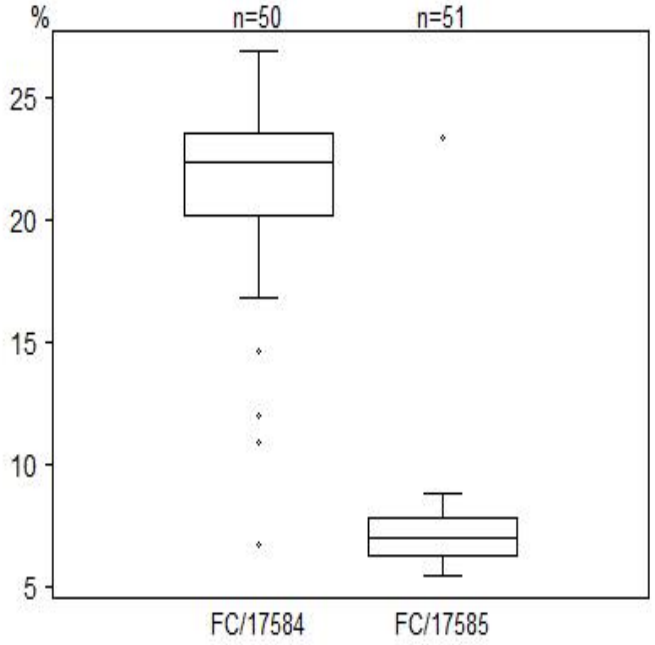

FC/17584
Number of laboratories
0
5
10
15
20
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
%
FC/17585
Number of laboratories
0
5
10
15
20
25
30
35
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
%

NKcells 10E9/L





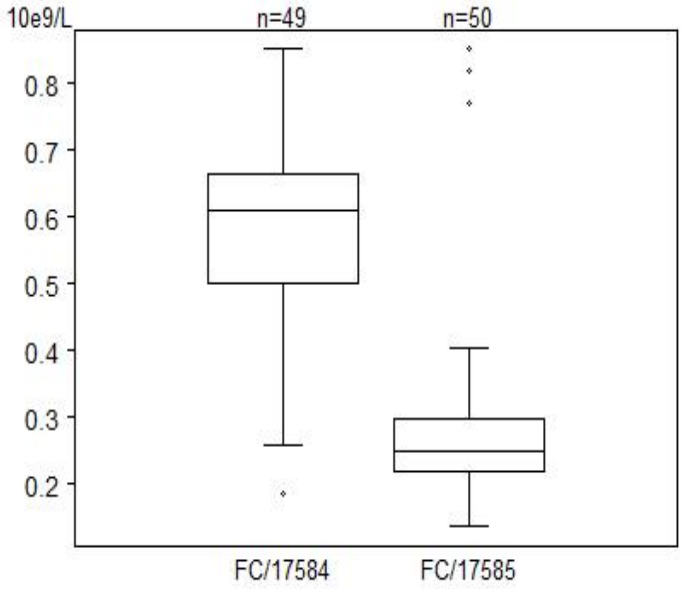
NKcells %



Results not represented on the graph

FC/17584 = 0.4 %
FC/17585 = 0.2 %
FC/17585 = 2.1 %
FC/17585 = 3.7 %

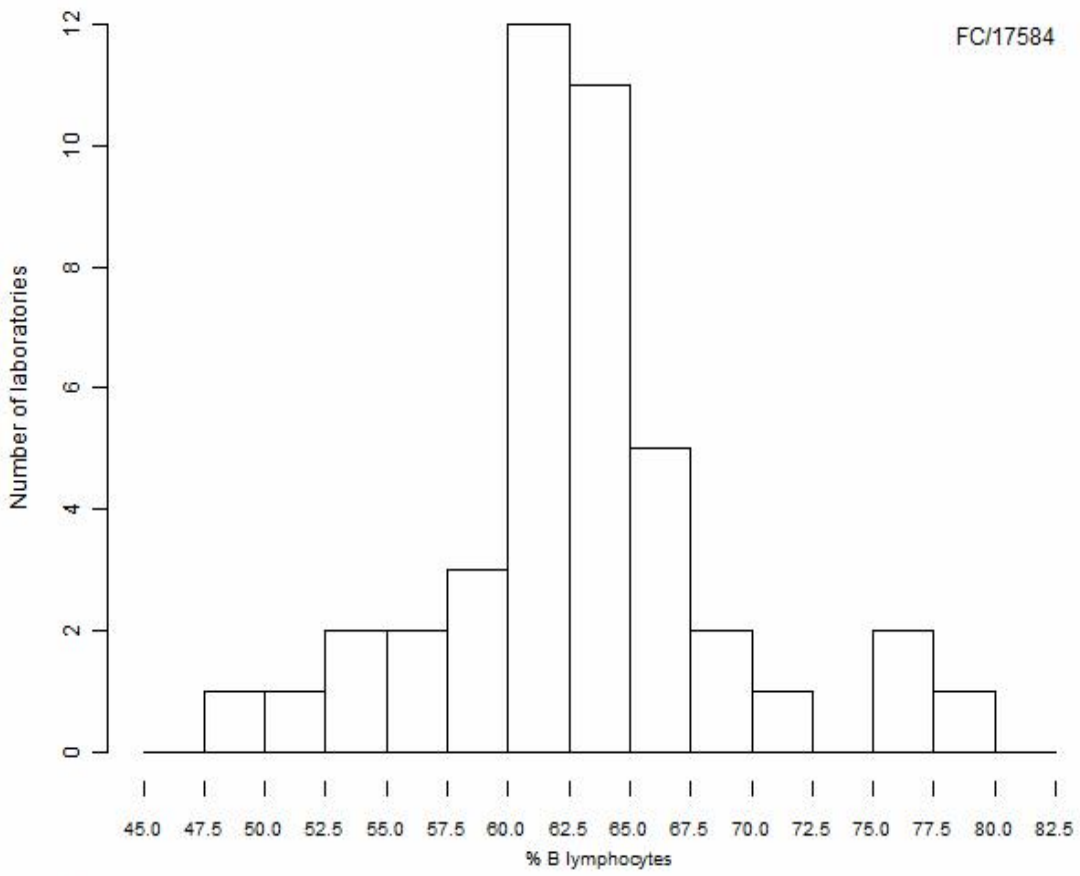
NKcells 10E9/L

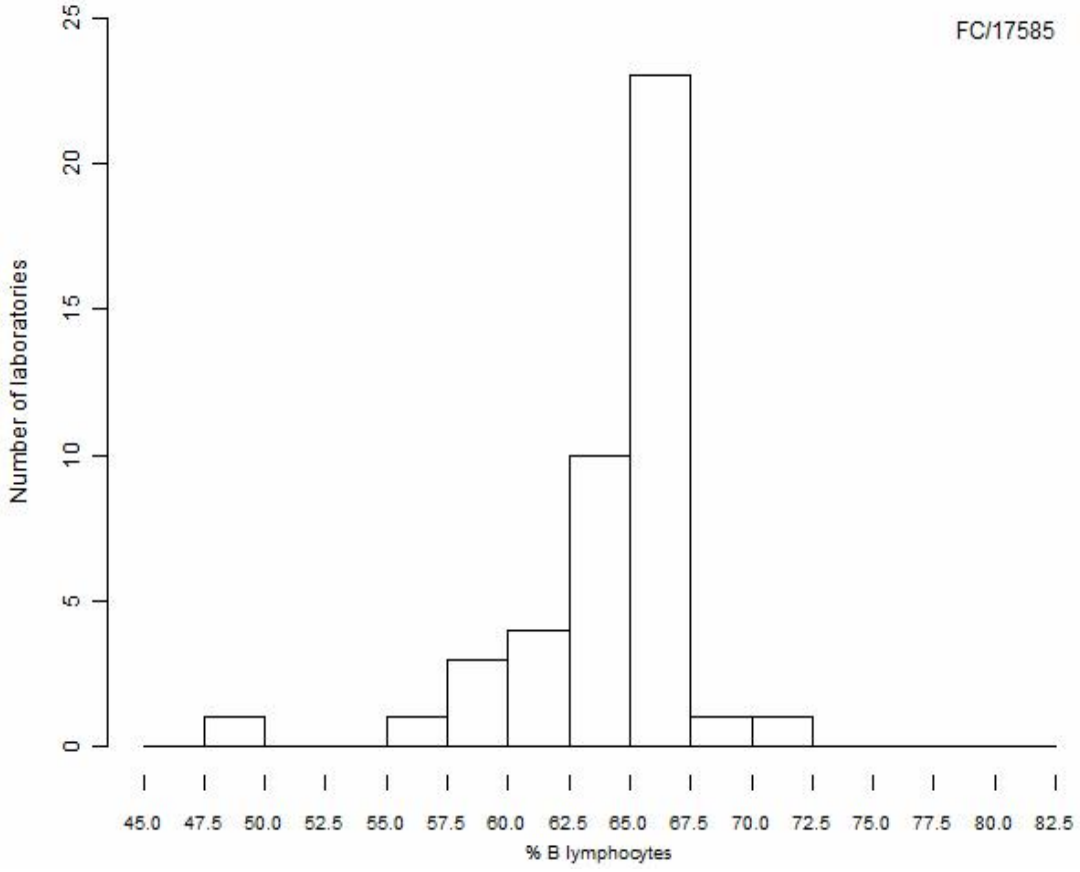


Results not represented on the graph

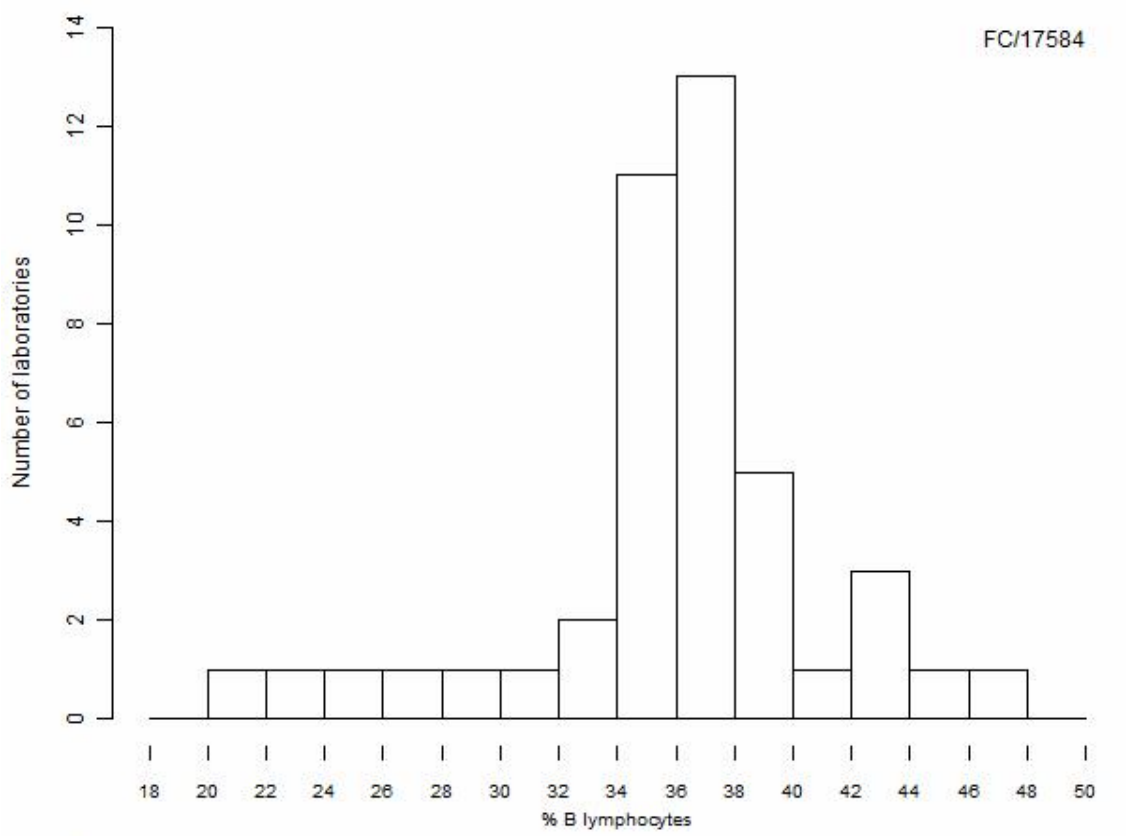
FC/17584 = 1.61 10e9/l
FC/17584 = 1.613 10e9
FC/17584 = 1.74 10e9/l
FC/17584 = 1.98 10e9/l
FC/17585 = 0.076 10e9
FC/17585 = 0.91 10e9/l
FC/17585 = 0.945 10e9

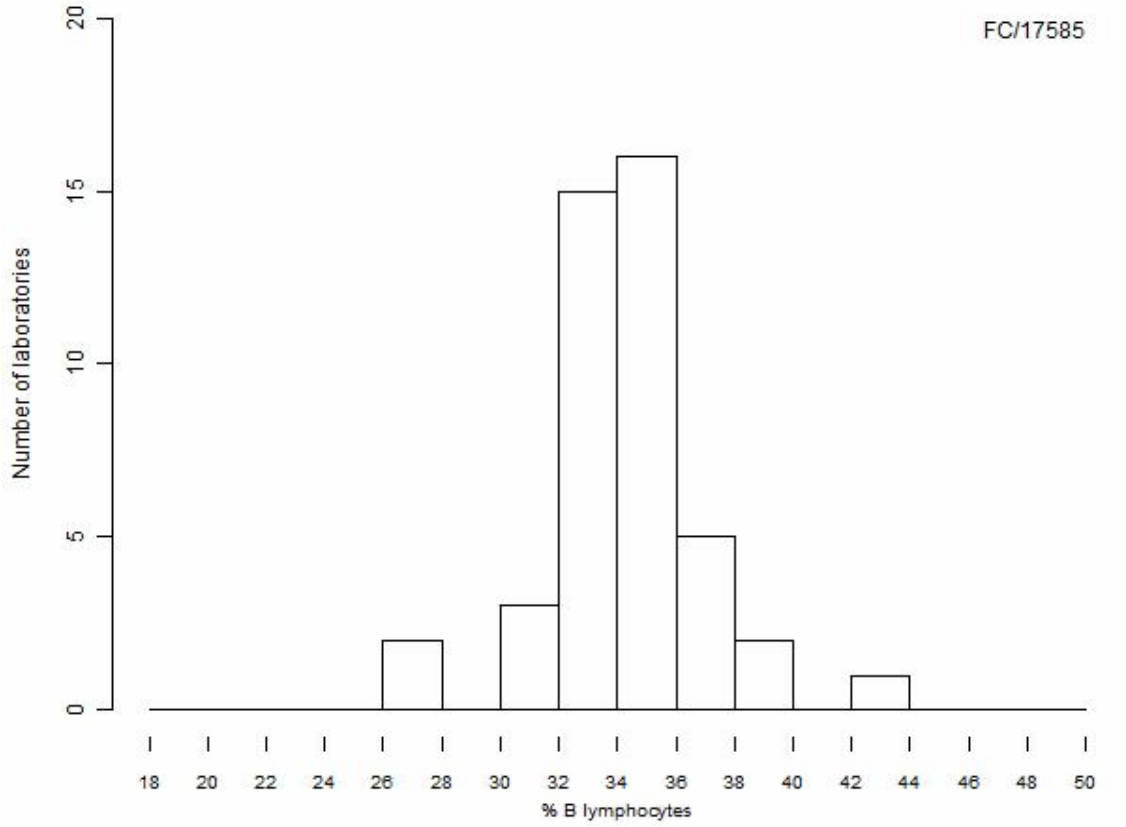
Kappa % B lymphocytes



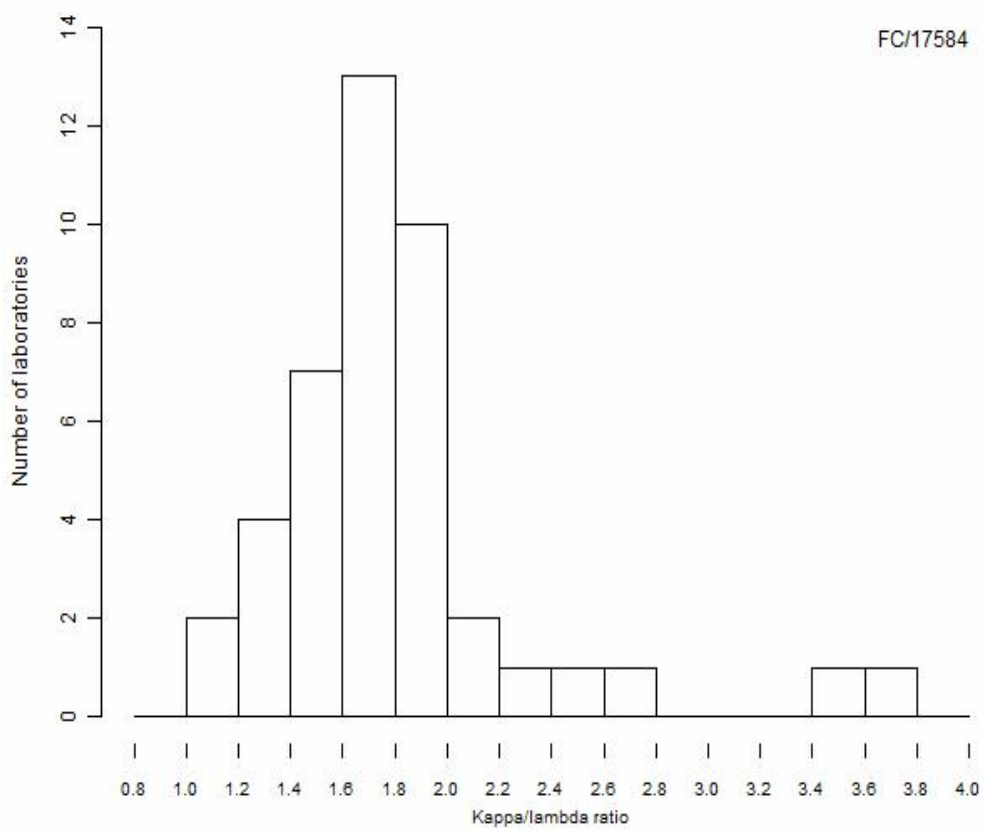


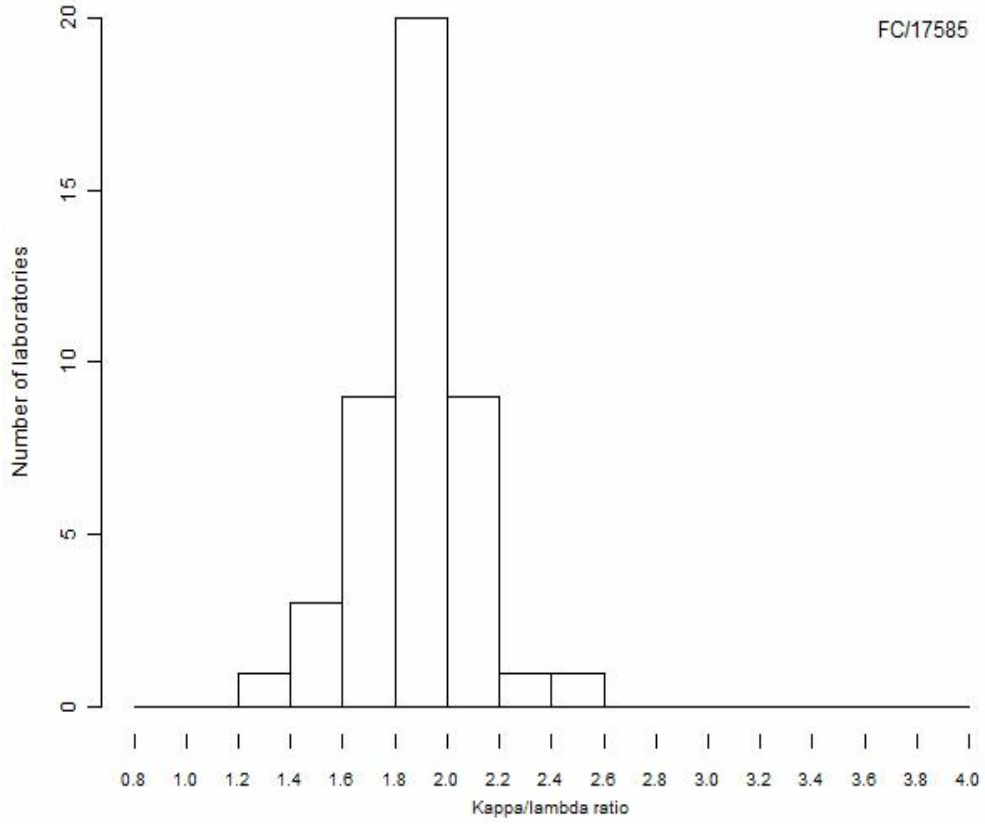
Lambda % B lymphocytes



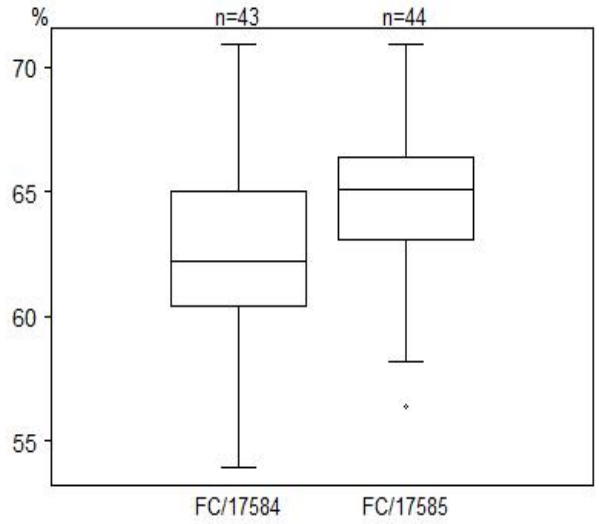


Kappa/lambda





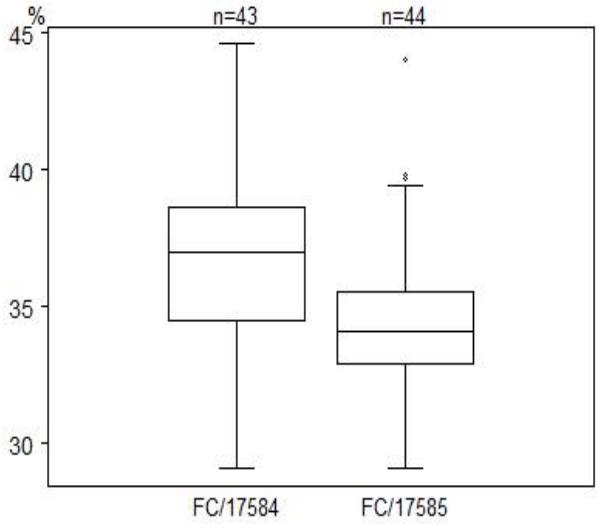
Kappa % B lymphocytes



Results not represented on the graph

FC/17584 = 50 %
FC/17584 = 51.7 %
FC/17584 = 53 %
FC/17584 = 75.1 %
FC/17584 = 76.9 %
FC/17584 = 79 %
FC/17585 = 50 %
FC/17585 = 72 %

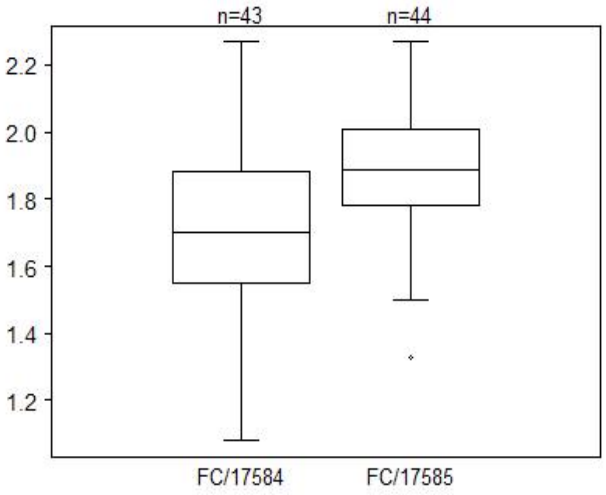
Lambda % B lymphocytes



Results not represented on the graph

FC/17584 = 21 %
FC/17584 = 22.1 %
FC/17584 = 25.3 %
FC/17584 = 27.5 %
FC/17584 = 47.8 %
FC/17585 = 28 %
FC/17585 = 28 %

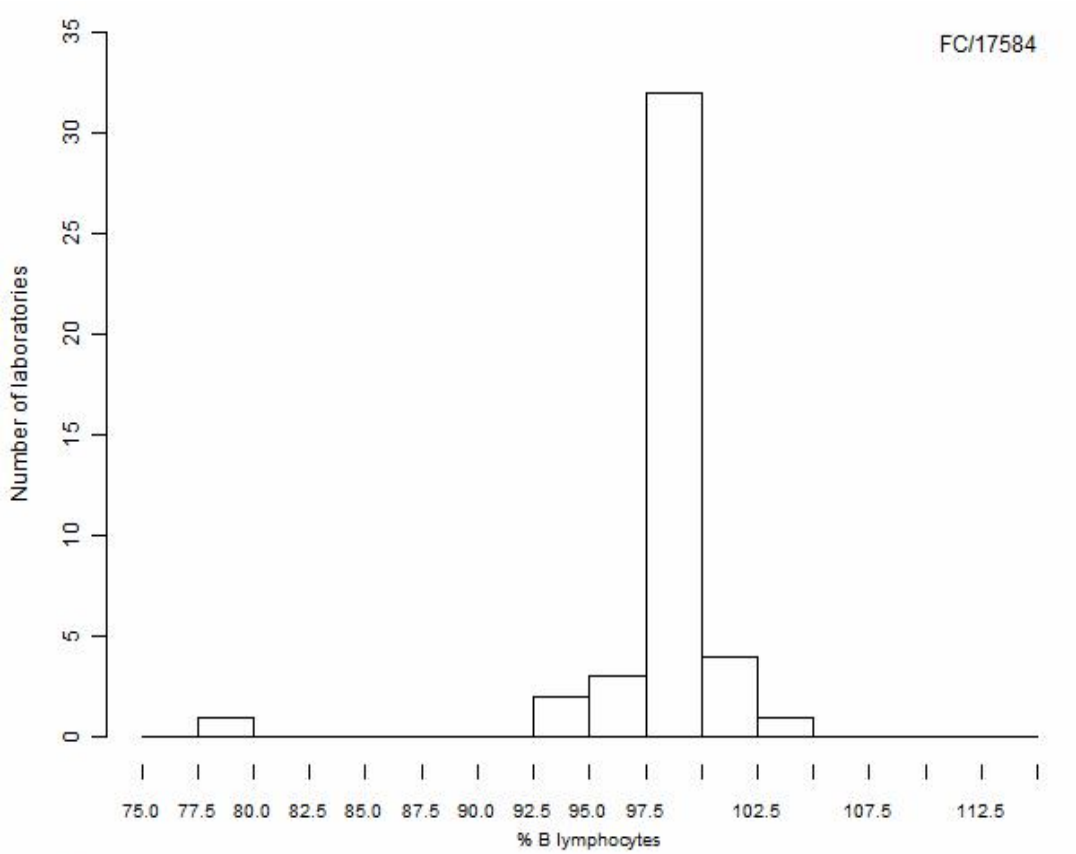
Kappa/lambda

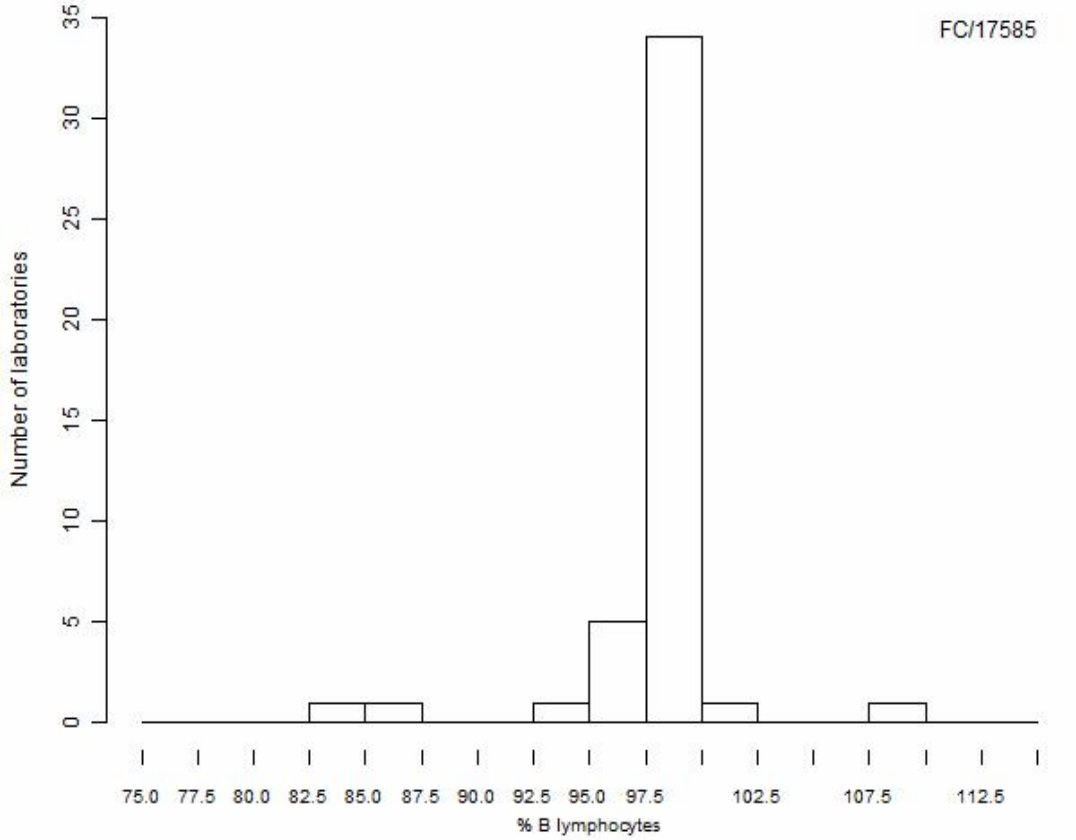


Results not represented on the graph

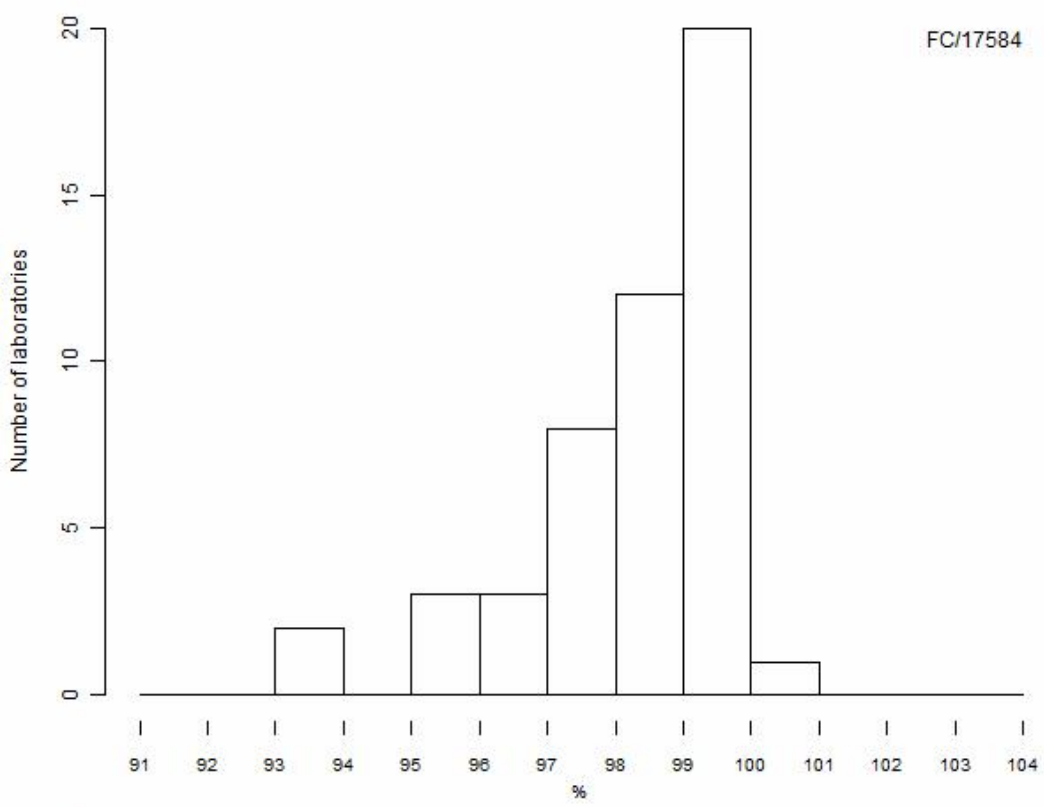
FC/17584 = 2.44
FC/17584 = 2.73
FC/17584 = 3.48
FC/17584 = 3.76
FC/17585 = 2.57

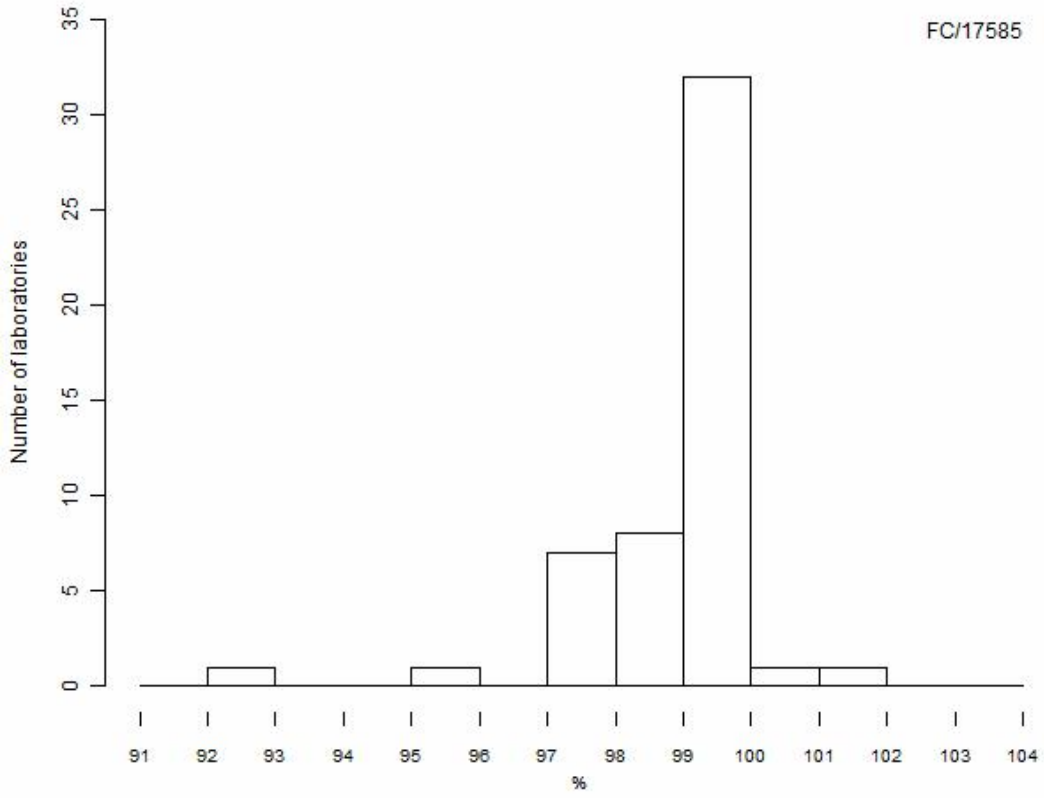
Sum K+L % B lymphocytes



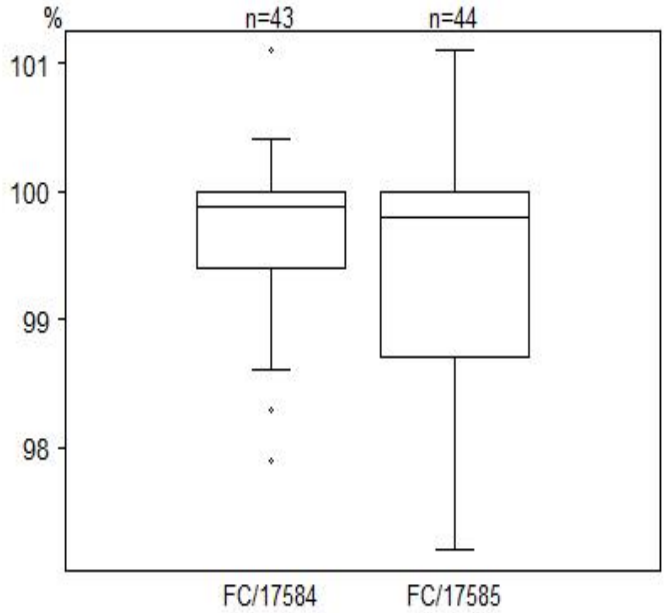


Lymphosum %


FC/17584
Number of laboratories
0
5
10
15
20
91
92
93
94
95
96
97
98
99
100
101
102
103
104
%


FC/17585
Number of laboratories
0
5
10
15
20
25
30
35
91
92
93
94
95
96
97
98
99
100
101
102
103
104
%

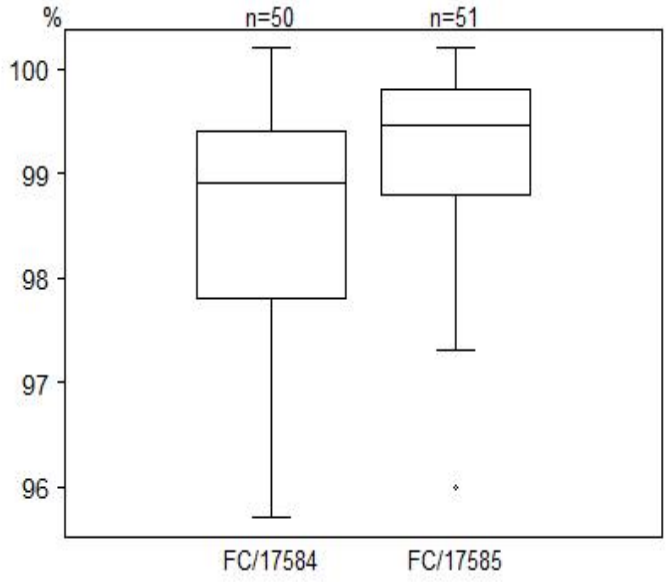
Sum K+L % B lymphocytes



Results not represented on the graph

FC/17584 = 79.2 %
FC/17584 = 93 %
FC/17584 = 94.4 %
FC/17584 = 95.6 %
FC/17584 = 95.7 %
FC/17584 = 95.9 %
FC/17584 = 102.6 %
FC/17585 = 84.4 %
FC/17585 = 87.5 %
FC/17585 = 94 %
FC/17585 = 95.4 %
FC/17585 = 95.6 %
FC/17585 = 96 %
FC/17585 = 110 %

Lymphosum %



Results not represented on the graph

FC/17584 = 83.8 %
FC/17584 = 93.3 %
FC/17584 = 93.5 %
FC/17585 = 92.5 %
FC/17585 = 102 %

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%.

Comparison between % lymphocytes count on haematology analyser and flow cytometer

Introduction

It was suggested that the percentage lymphocytes counted on the haematology analyser and the flow cytometer are alike. It was further suggested that their percentage difference would be less than 5%.

The data reported for survey 2020/3 of flow cytometry was taken to investigate the differences between the haematology analyser and flow cytometer.

Difference between haematology analyser and flow cytometer

The absolute value of percentage deviation of the haematology analyser with respect to the flow cytometer was calculated for every laboratory separately and is shown for both samples in Figure 1. Only a minority of the deviations is below 5%. In fact, 36.2 % of the deviations is below 5% for sample FC/17584 and 33.3% is below 5% form sample FC/17585.

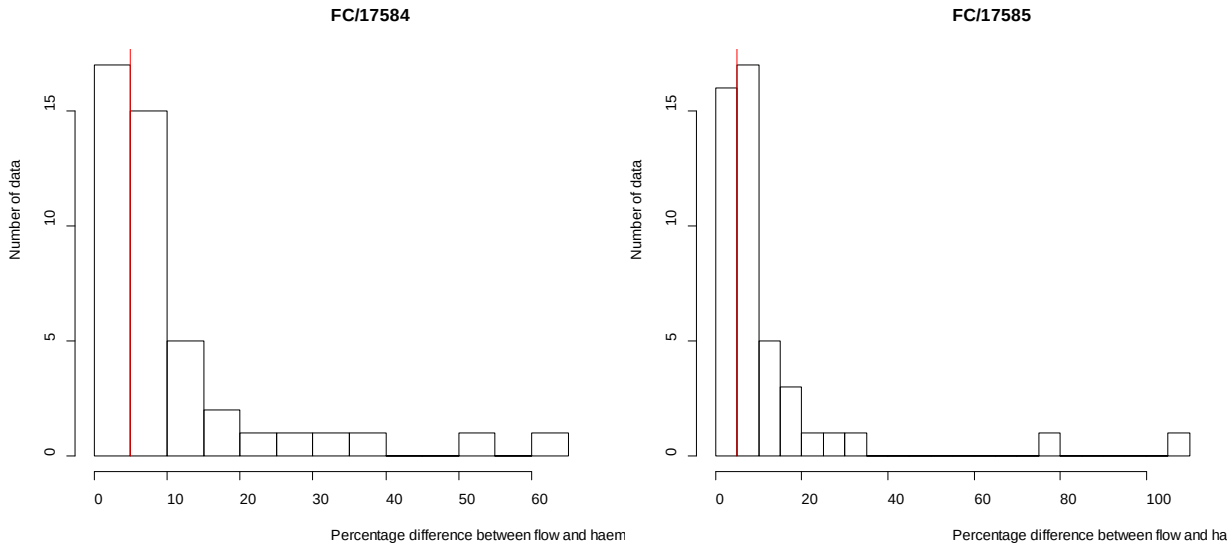


Figure 1: histogram of percentage deviations between the flow cytometer and haematology analyser for % lymphocyte count for the two samples of survey 2020/3. Red line is drawn at 5% deviation.

In order to investigate the higher than expected deviations, a scatter plot was made in which the results of both analysers are plotted against each other. Outliers were identified using a sequential Grubbs test and the horizontal and vertical axes were set at the same scale. The result is shown in Figure 2.

The scatter of points is much larger in the vertical direction, indicating that the flow cytometer has a larger variability than the haematology analyser.

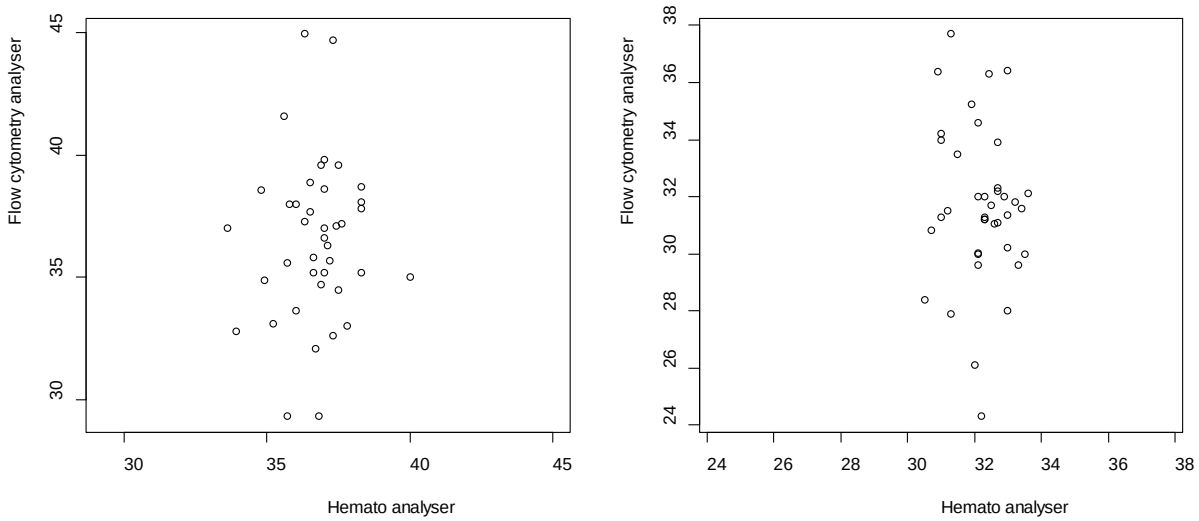


Figure 2: scatter plot of the results reported by individual laboratories for % lymphocytes on the haemato analyser and flow cytometer for the two samples of survey 202/3

Is it a matter of variability ?

The variability of both systems was further investigated by looking at the relation between the standard deviation and the median of the reported results since 2015. The variability between all results is considered here, without distinguishing between analytical methods. The results are shown in Figure 3. The findings from Figure 2 are confirmed: the variability of the EQA results from the flow cytometer is clearly higher than for the haematology analyser.

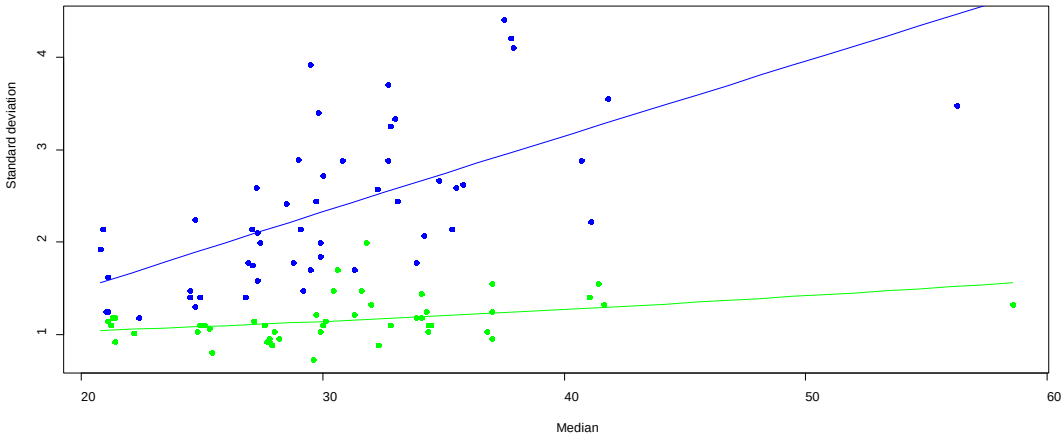


Figure 3: Standard deviation with respect to median of reported EQA results since 2015. Green line shows the results of the haematology analyser, blue line shows the results of the flow cytometer.

Is it a matter of bias ?

At last, it may be interesting to investigate the bias between both methods as well. For this purpose, the median reported value obtained for the same sample by the two methods is considered. A scatter plot is shown in Figure 4. The median of the values obtained on the flow cytometer are slightly lower. The slope of the linear regression is 0.9535, indicating a little less than 5% deviation between the mean results of the haematology analyser and the flow cytometer.

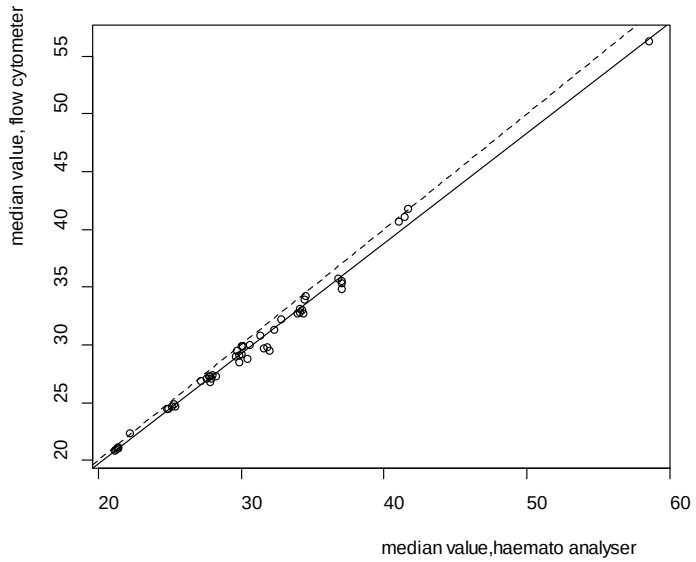


Figure 4: scatter median values of reported EQA results for both systems. Full line is the linear regression line, dashed line is the 45°-line.

Conclusion

Individual results between two systems deviate on average more than 5% from each other, and this is largely due to the high variability that is observed for the flow cytometer. The bias between both methods is a little less than 5%.

Dr Wim Coucke

NEXT SURVEY

The next survey is scheduled for **February 22, 2021**.

END