

**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 2020/2

Sciensano/Flow cytometry/74-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:
 29/09/2020

Authorization to release the report:	By Lobna Bouacida, scheme coordinator, on 30/09/2020.
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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

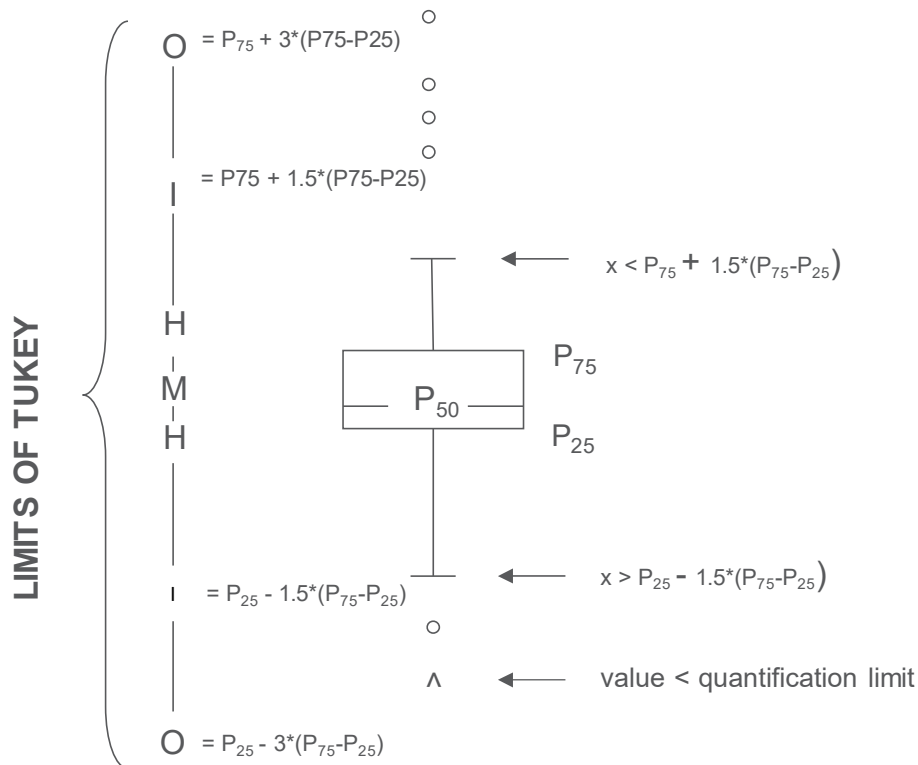
https://www.wiv-isp.be/QML/activities/external_quality/brochures/_fr/brochures.htm

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

Three blood samples (FC/17261, FC/17262 and FC/17263) collected on K2EDTA were sent to the laboratories. The three samples were identical.

These three samples were collected from one healthy and voluntary blood donor at the 'Dienst voor het Bloed' 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):

FC17261

	%	10 ⁹ /L
Leukocytes		5.4
Lymphocytes	28.6	
CD3⁺ cells	58.9	0.94
CD4⁺CD3⁺ cells	29.5	0.47
CD8⁺CD3⁺ cells	26.9	0.43
CD19⁺ cells	8.4	0.13
NK cells	29.9	0.48
κ % B lymphocytes	55.2	
λ % B lymphocytes	44.8	
κ/λ ratio	1.23	

FC17262

	%	10 ⁹ /L
Leukocytes		5.3
Lymphocytes	28.0	
CD3⁺ cells	60.5	0.95
CD4⁺CD3⁺ cells	29.5	0.46
CD8⁺CD3⁺ cells	27.0	0.42
CD19⁺ cells	8.9	0.14
NK cells	27.2	0.43
κ % B lymphocytes	58.5	
λ % B lymphocytes	41.4	
κ/λ ratio	1.41	

FC17263

	%	10⁹/L
Leukocytes		5.2
Lymphocytes	28.4	
CD3⁺ cells	61.5	0.96
CD4⁺CD3⁺ cells	27.7	0.43
CD8⁺CD3⁺ cells	27.1	0.42
CD19⁺ cells	8.0	0.12
NK cells	27.7	0.43
κ % B lymphocytes	56.6	
λ % B lymphocytes	43.1	
κ/λ ratio	1.31	

PARTICIPATION

Fifty-four laboratories (1 Latvian, 1 Canadian and 52 Belgian clinical laboratories) participated in the survey 2020/2 (send-out of blood samples on July 8, 2020 (day 0)).

RESULTS

98% of the Belgian laboratories received the samples on day 1 or 2. Forty-one laboratories (79%) received the samples on day 1 and ten (19%) received them on day 2.

61% of the Belgian laboratories (n=32) performed the analyses on day 1, 33% on day 2 (n=17) and 6% beyond day 2 (n=3).

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

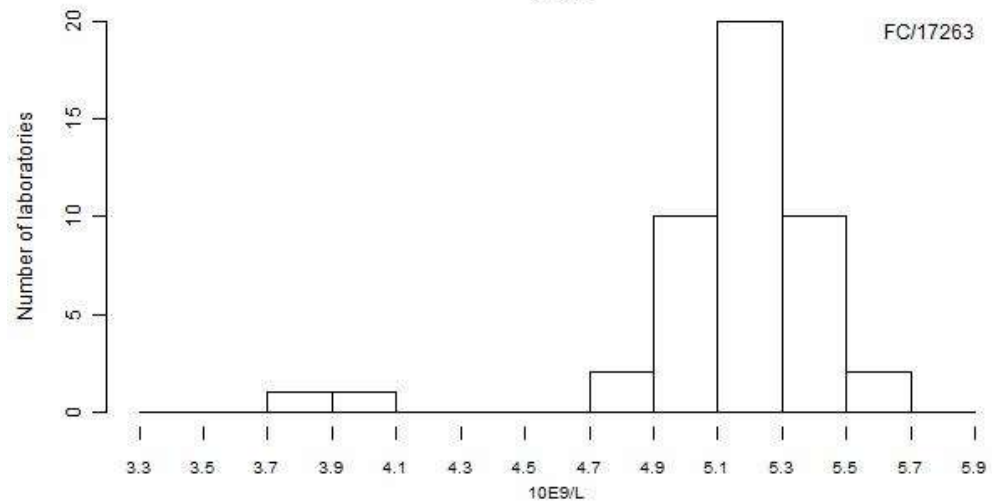
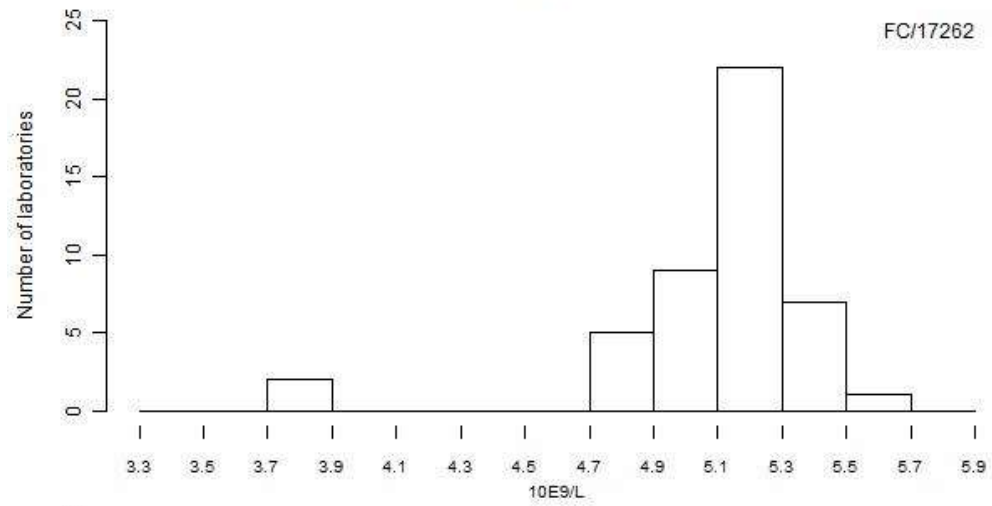
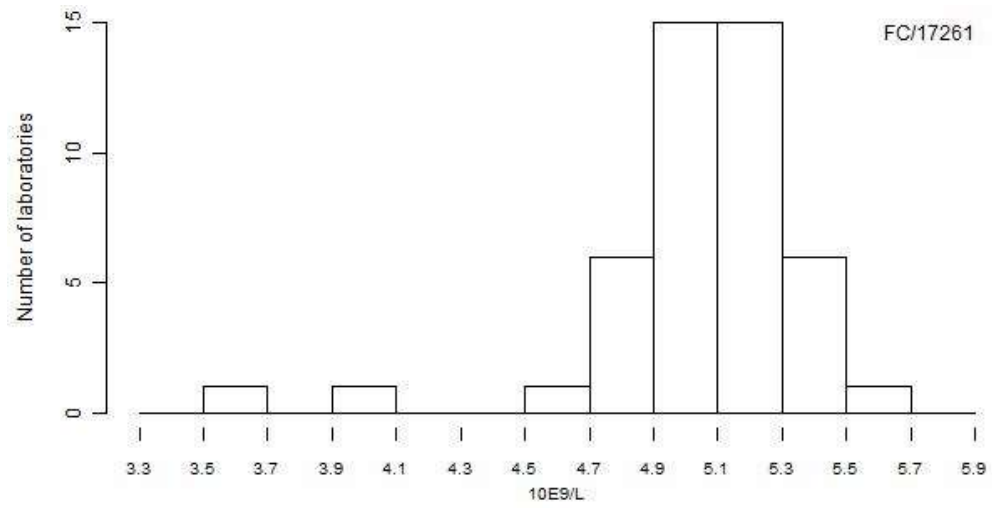
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=52). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=49).

The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/17261, FC/17262 and FC/17263:

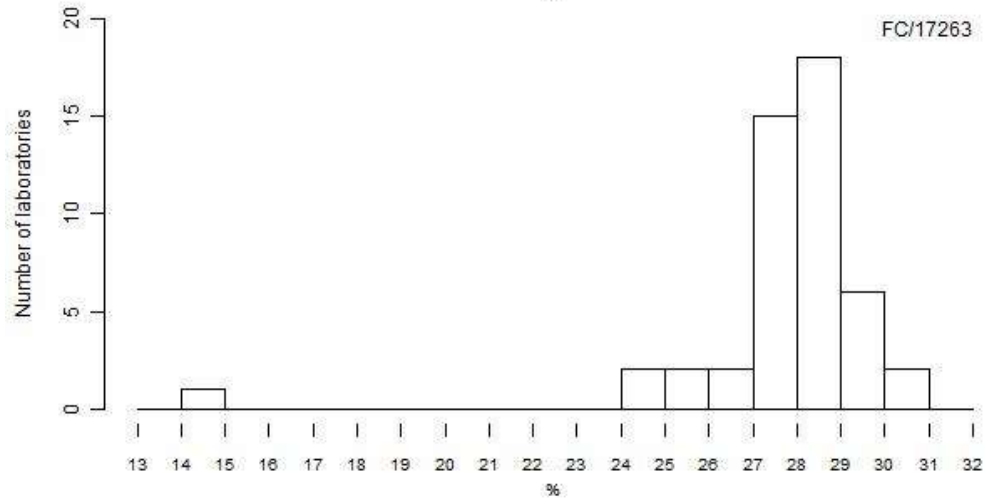
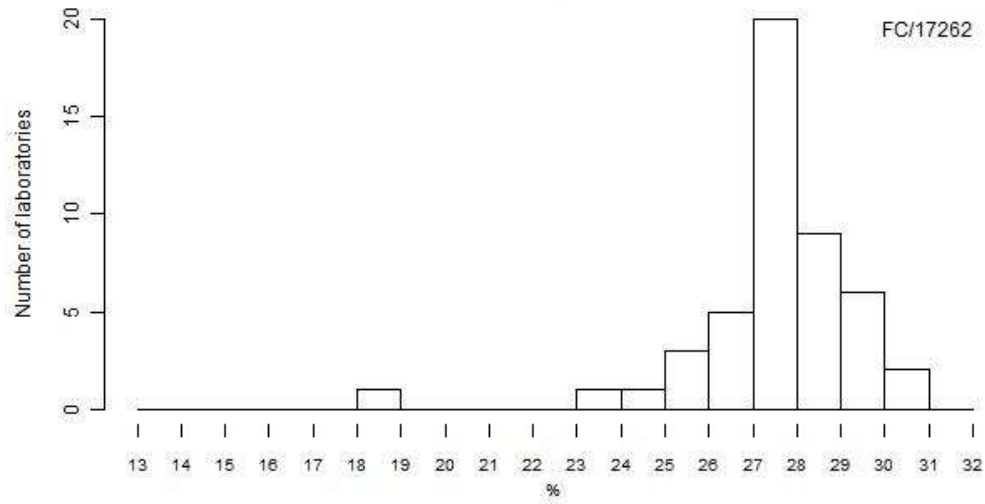
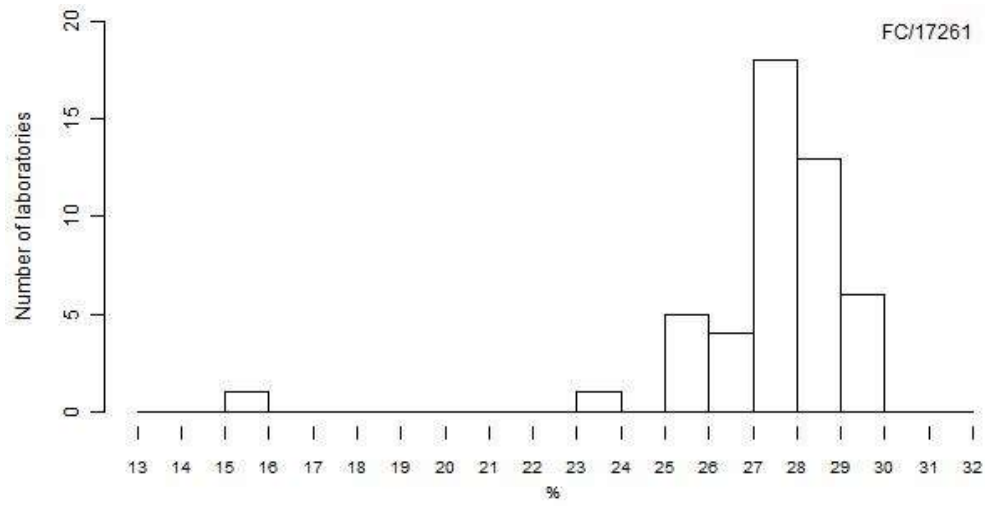
FC17261 / FC17262 / FC17263

	Median			SD			CV,%			N		
	17261	17262	17263	17261	17262	17263	17261	17262	17263	17261	17262	17263
Leukocytes 10⁹/L	5.11	5.16	5.20	0.24	0.19	0.20	4.8	3.6	3.8	46	46	46
Lymphocytes % Haematology analyser	28.0	27.9	28.2	1.0	0.9	1.0	3.7	3.2	3.4	45	45	45
Lymphocytes % Flow cytometer	27.4	27.1	27.3	2.0	2.1	2.6	7.3	7.9	9.5	44	44	44
CD3 %	61.4	61.3	61.3	1.5	1.6	1.5	2.4	2.5	2.4	51	51	51
CD3 10⁹/L	0.890	0.885	0.890	0.100	0.078	0.090	11.2	8.9	10.1	46	46	46
CD4 %	29.0	29.0	28.7	1.0	1.4	1.3	3.6	4.8	4.4	51	51	51
CD4 10⁹/L	0.422	0.419	0.420	0.037	0.042	0.056	8.8	10.1	13.2	46	46	46
CD8 %	26.4	26.7	26.7	1.3	1.5	2.1	5.0	5.5	8.0	51	51	51
CD8 10⁹/L	0.380	0.380	0.380	0.048	0.036	0.071	12.7	9.5	18.7	46	46	46
CD19 %	8.3	8.4	8.2	0.7	0.9	0.5	8.0	11.0	6.3	51	51	51
CD19 10⁹/L	0.118	0.120	0.120	0.018	0.017	0.022	15.1	14.2	18.5	46	46	46
NK cells %	29.2	29.4	29.4	2.2	1.4	1.8	7.5	4.8	6.2	51	51	51
NK cells 10⁹/L	0.419	0.420	0.433	0.073	0.059	0.045	17.3	14.1	10.4	46	46	46
κ % B lymphocytes	57.3	57.9	57.4	3.4	2.3	2.8	5.9	4.0	4.9	44	44	44
λ % B lymphocytes	42.0	40.9	41.0	2.4	2.0	2.6	5.7	4.9	6.4	44	44	44
κ/λ ratio	1.36	1.40	1.38	0.14	0.13	0.16	10.3	9.5	11.3	44	44	44
κ+λ % B lymphocytes	99.8	99.6	99.8	0.7	1.8	1.0	0.7	1.8	1.0	44	44	44
Lymphosum	99.4	99.4	99.1	1.2	1.4	1.6	1.2	1.4	1.6	51	51	51

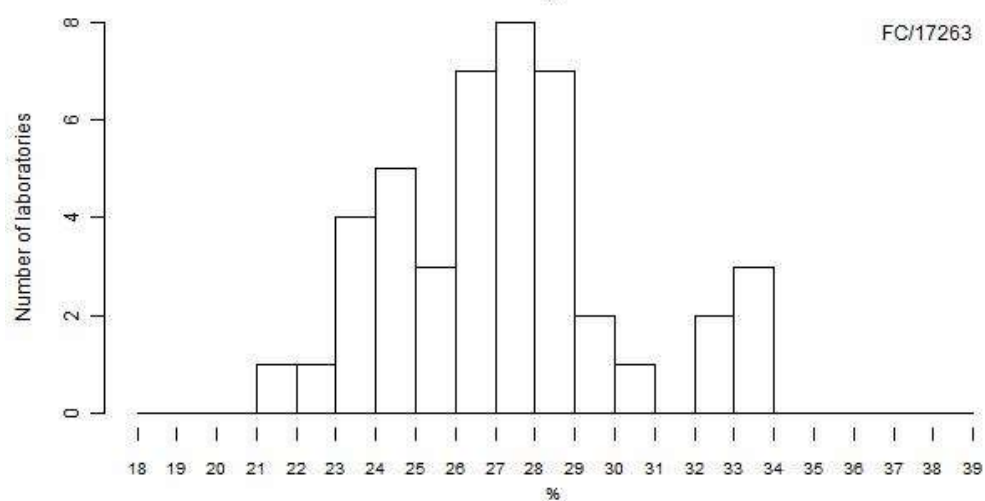
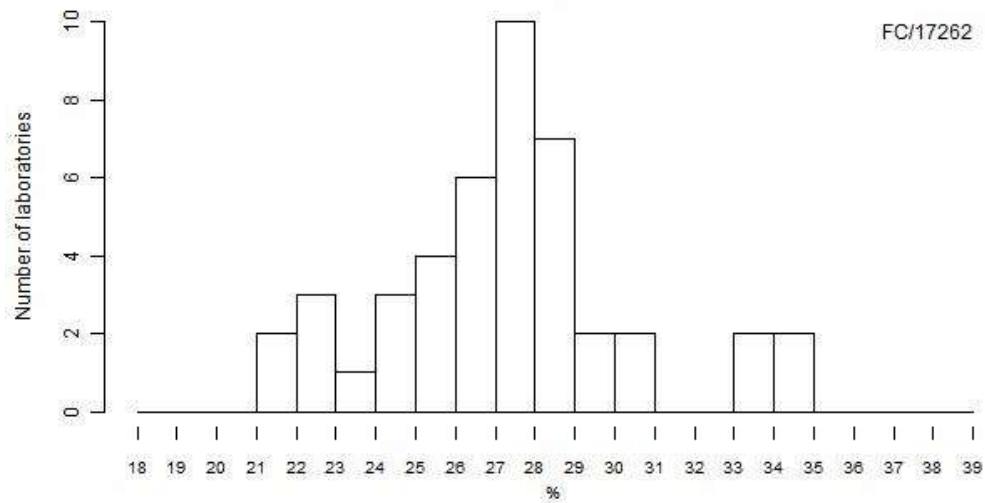
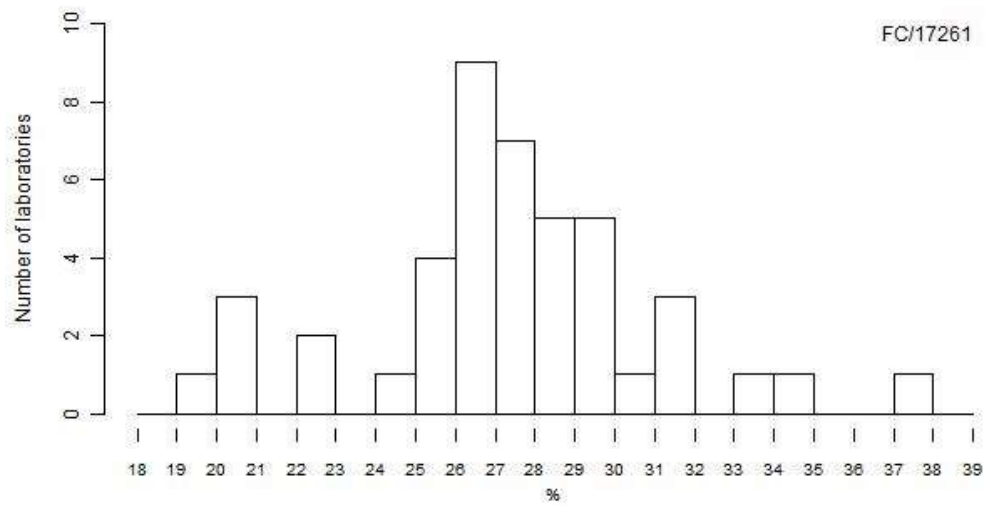
WBC 10E9/L



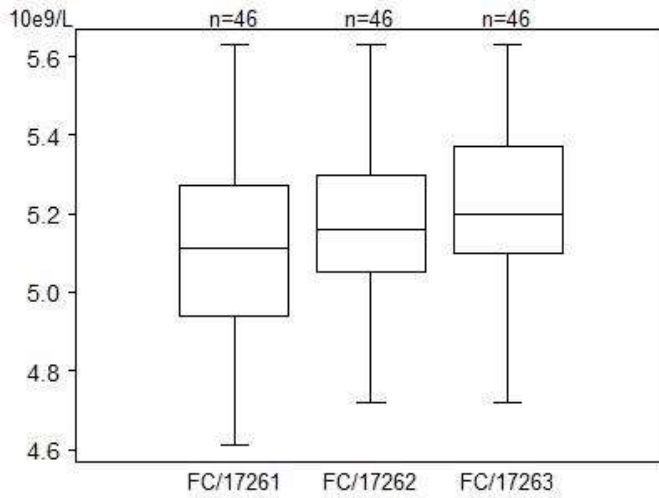
Lymphocytes % Haematology analyser



Lymphocytes % Flow cytometer



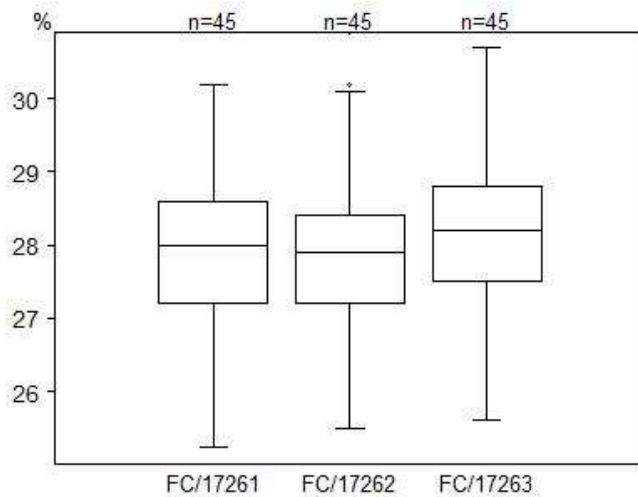
WBC 10E9/L



Results not represented on the graph

FC/17261 = 3.58 10e9/l
 FC/17261 = 3.96 10e9/l
 FC/17261 = 31.4 10e9/l
 FC/17261 = 5000 10e9/
 FC/17261 = 5320 10e9/
 FC/17262 = 3.82 10e9/l
 FC/17262 = 3.9 10e9/L
 FC/17262 = 31.9 10e9/l
 FC/17262 = 4800 10e9/
 FC/17262 = 5130 10e9/
 FC/17263 = 3.86 10e9/l
 FC/17263 = 3.91 10e9/l
 FC/17263 = 31.7 10e9/l
 FC/17263 = 5100 10e9/
 FC/17263 = 5220 10e9/

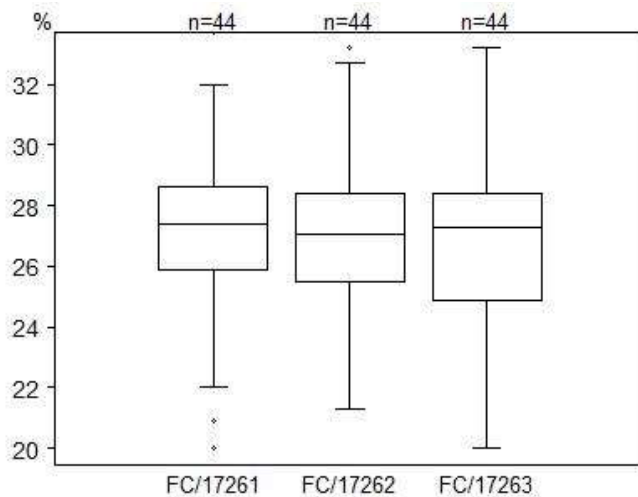
Lymphocytes % haematology analyser



Results not represented on the graph

FC/17261 = 15.9 %
 FC/17261 = 23.3 %
 FC/17262 = 18.2 %
 FC/17262 = 23.4 %
 FC/17262 = 24.7 %
 FC/17262 = 30.9 %
 FC/17263 = 14.6 %
 FC/17263 = 24.2 %
 FC/17263 = 24.2 %

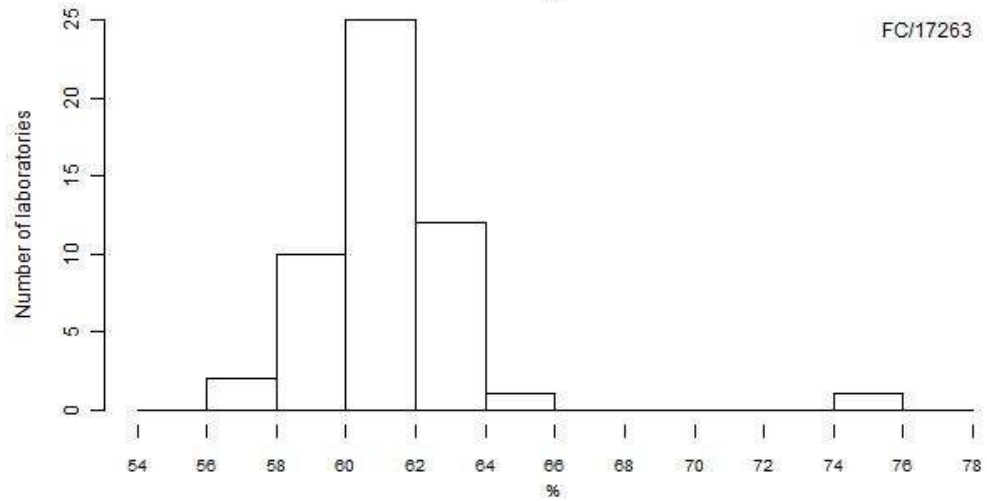
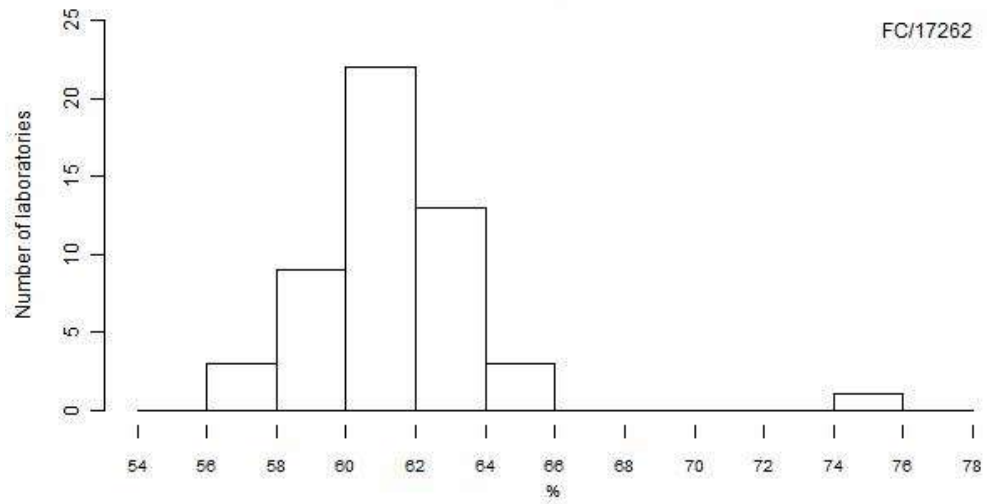
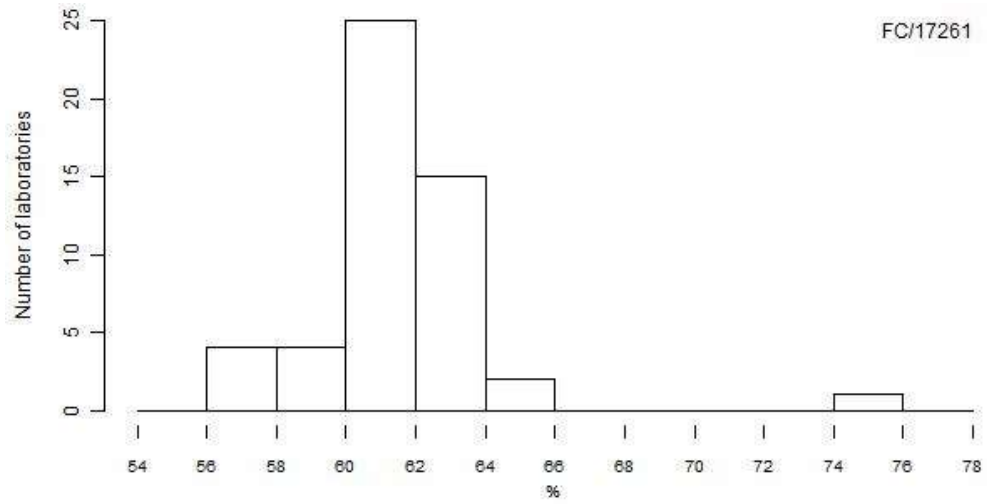
Lymphocytes % flow cytometer



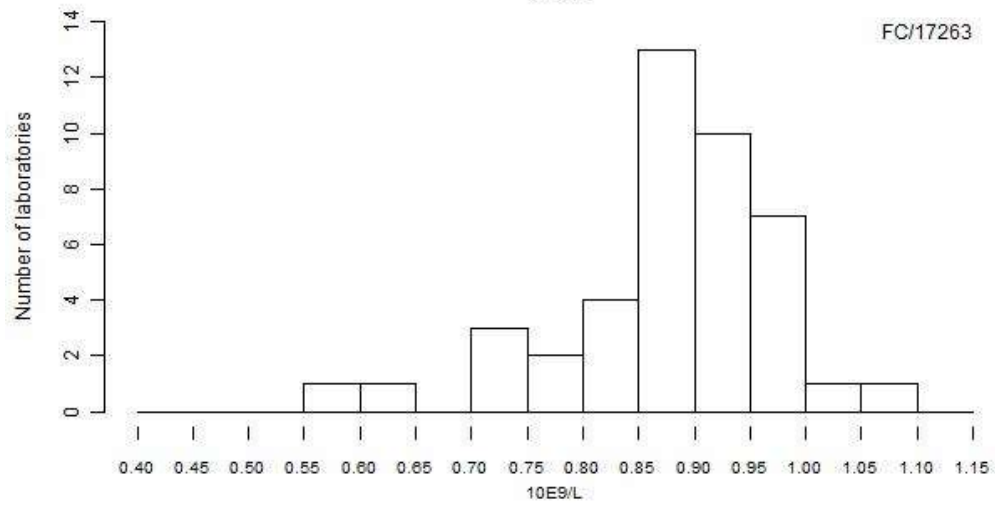
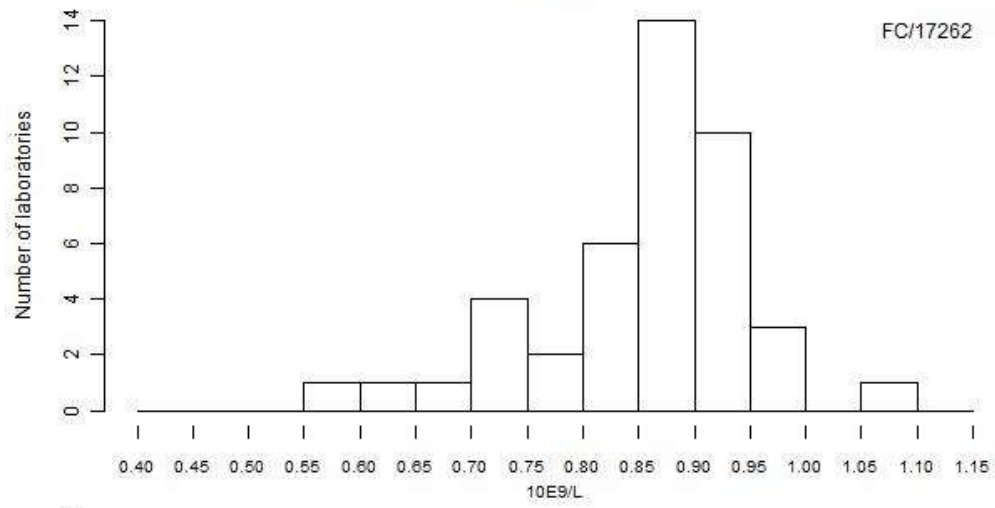
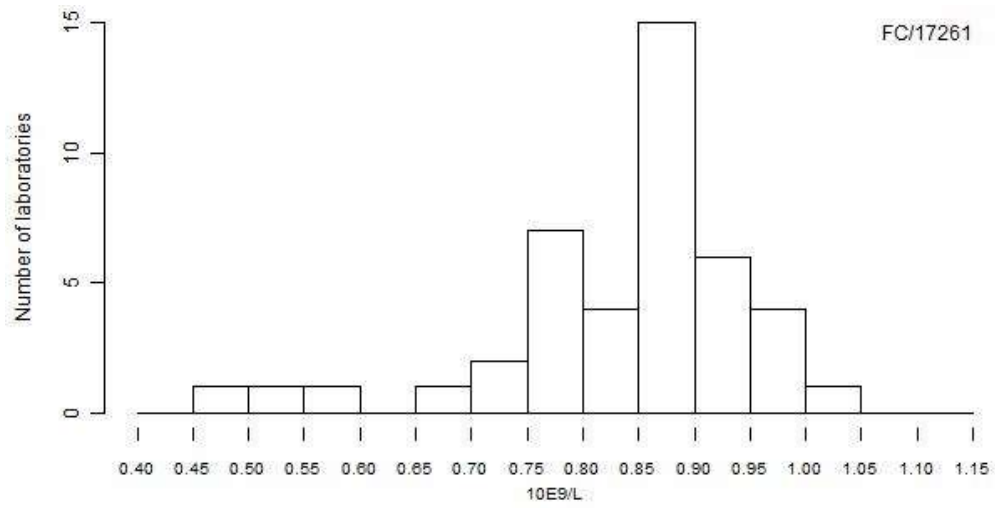
Results not represented on the graph

FC/17261 = 33.7 %
 FC/17261 = 34.3 %
 FC/17261 = 37.6 %
 FC/17262 = 34.5 %
 FC/17262 = 34.6 %
 FC/17263 = 33.9 %

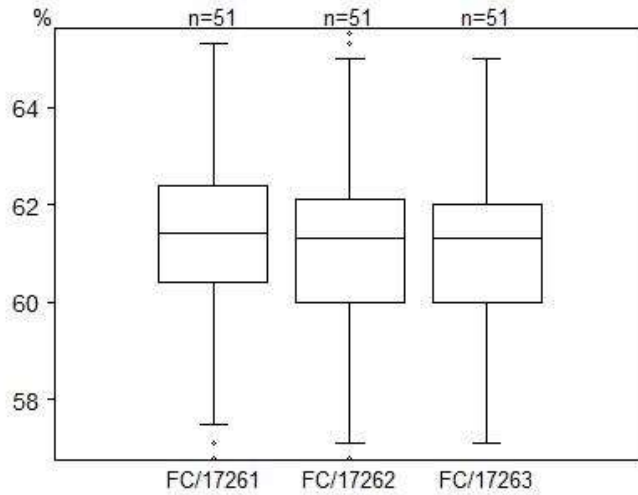
CD3 %



CD3 10E9/L



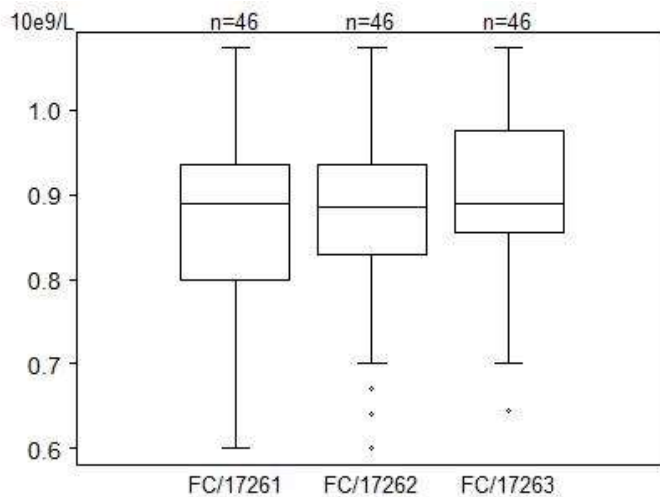
CD3 %



Results not represented on the graph

FC/17261 = 56.5 %
 FC/17261 = 74.1 %
 FC/17262 = 74.1 %
 FC/17263 = 74.8 %

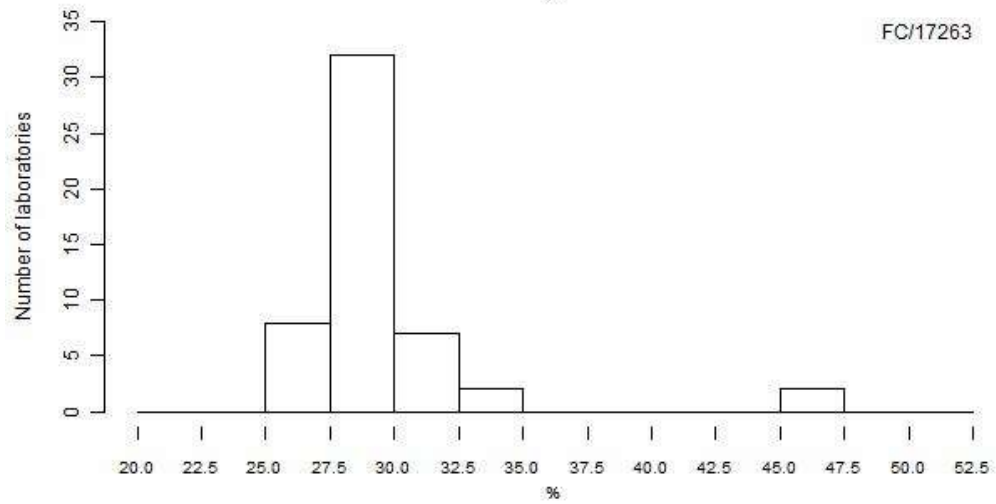
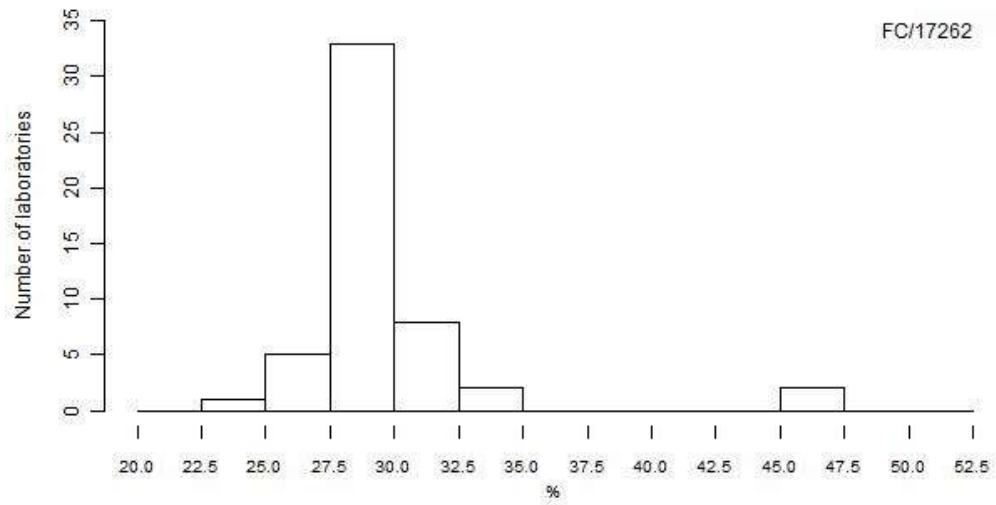
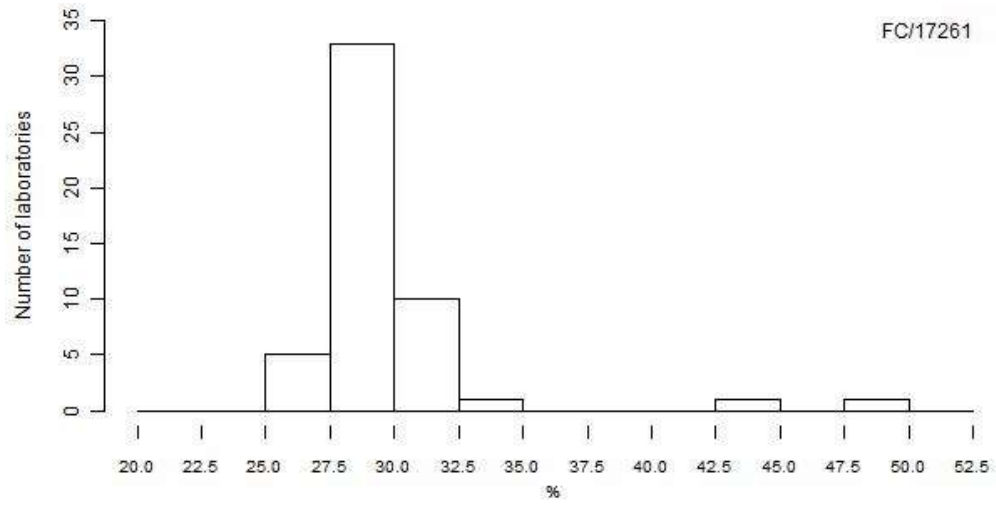
CD3 10E9/L



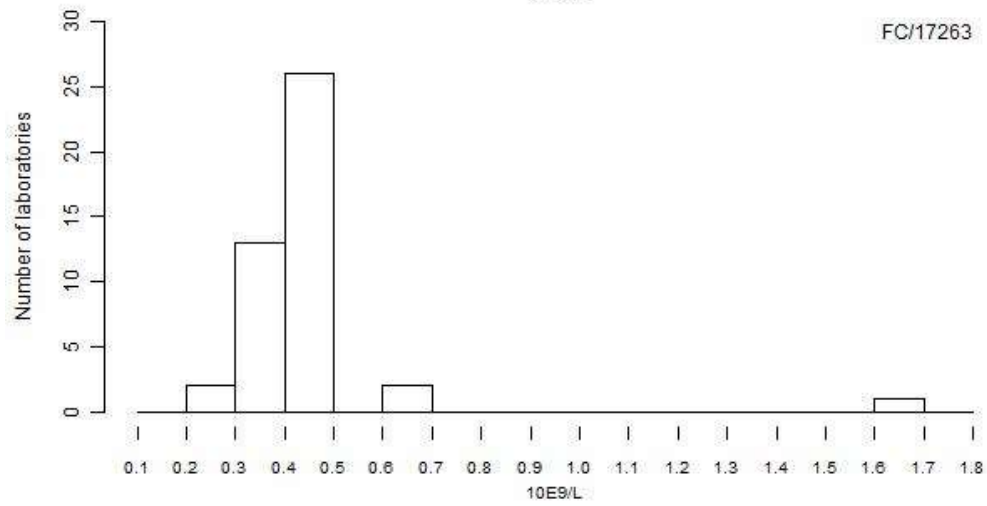
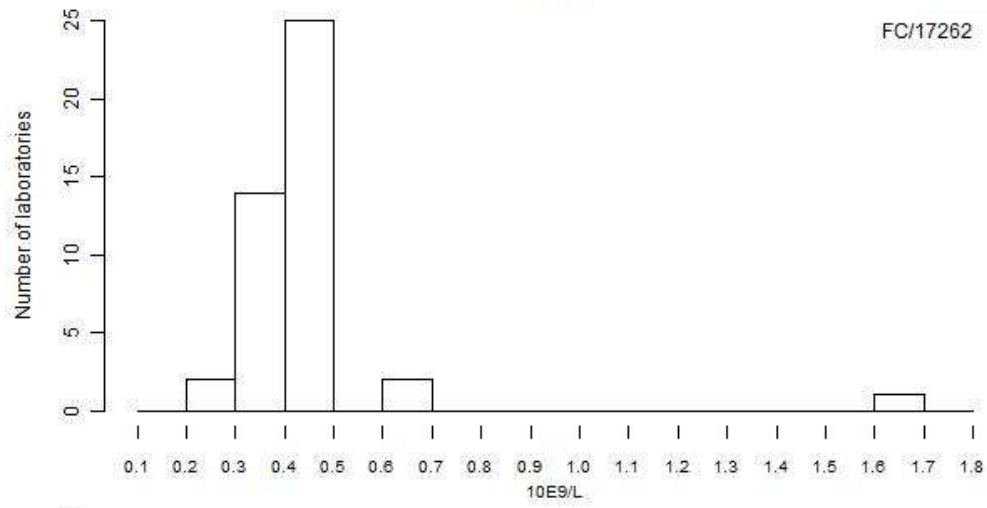
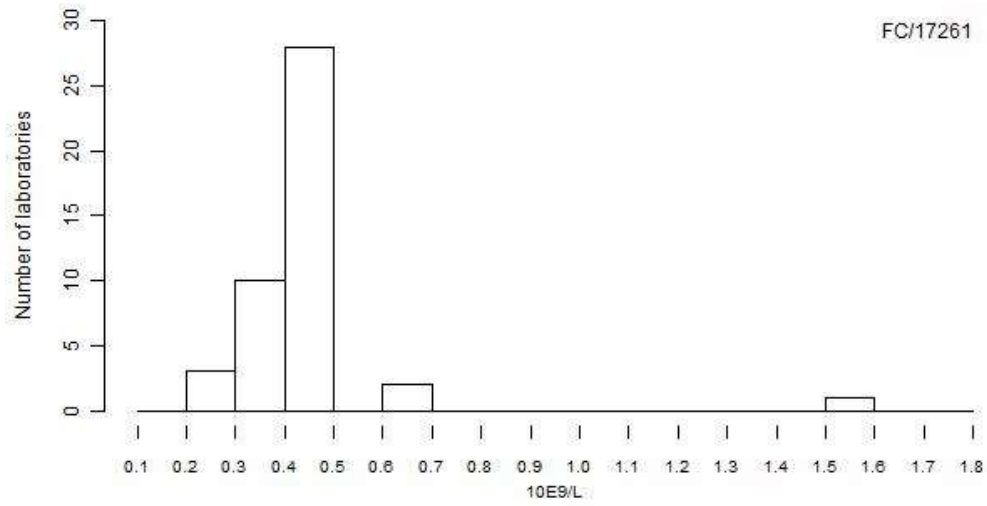
Results not represented on the graph

FC/17261 = 0.493 10e9
 FC/17261 = 0.53 10e9/l
 FC/17261 = 3.076 10e9
 FC/17261 = 695 10e9/L
 FC/17261 = 821 10e9/L
 FC/17261 = 841 10e9/L
 FC/17261 = 903 10e9/L
 FC/17261 = 7540 10e9/
 FC/17262 = 3.2 10e9/L
 FC/17262 = 719 10e9/L
 FC/17262 = 861 10e9/L
 FC/17262 = 882 10e9/L
 FC/17262 = 884 10e9/L
 FC/17262 = 8337 10e9/
 FC/17263 = 0.57 10e9/l
 FC/17263 = 3.25 10e9/L
 FC/17263 = 713 10e9/L

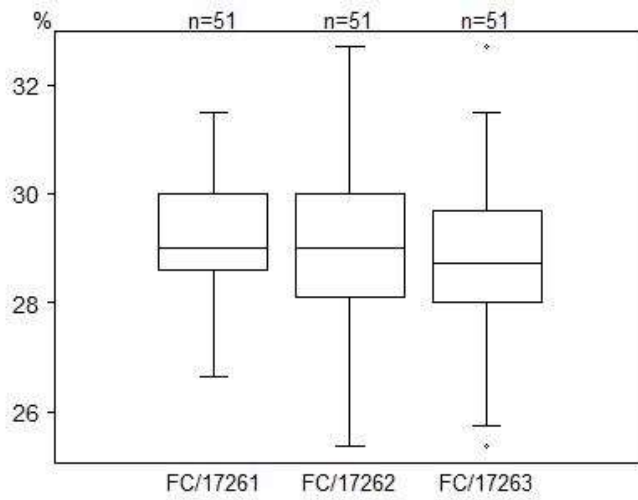
CD4 %



CD4 10E9/L



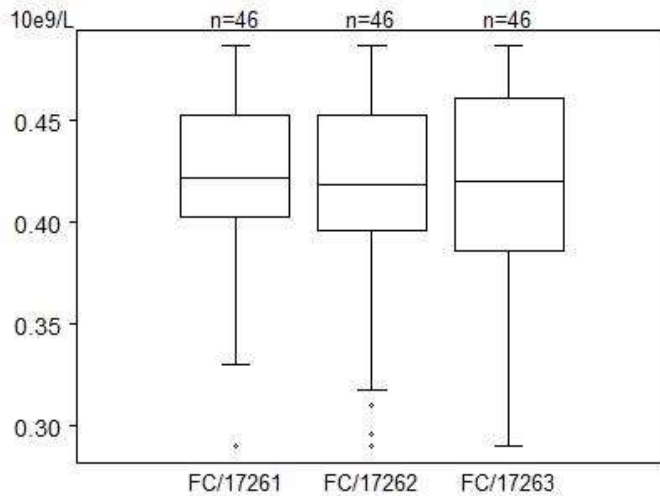
CD4 %



Results not represented on the graph

FC/17261 = 33 %
 FC/17261 = 44.2 %
 FC/17261 = 48.5 %
 FC/17262 = 24.8 %
 FC/17262 = 34.5 %
 FC/17262 = 45.4 %
 FC/17262 = 46.5 %
 FC/17263 = 33.2 %
 FC/17263 = 45.7 %
 FC/17263 = 45.9 %

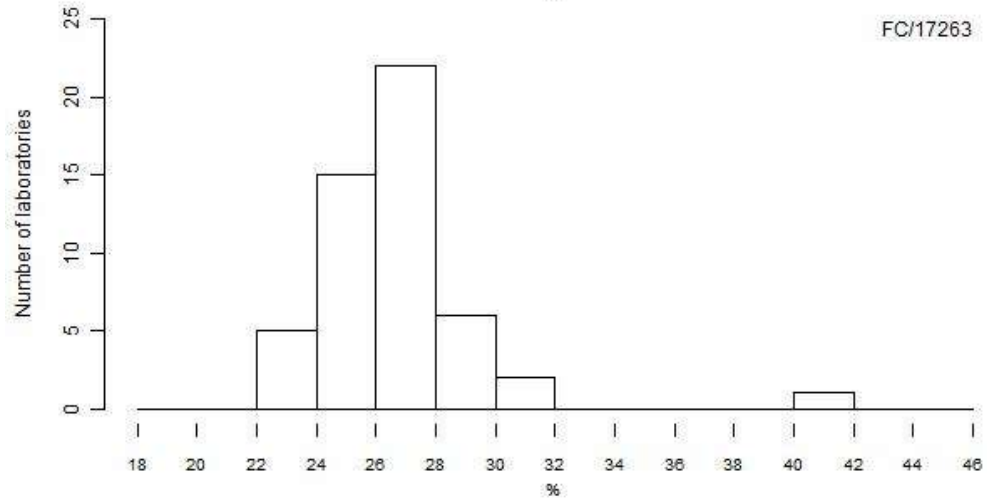
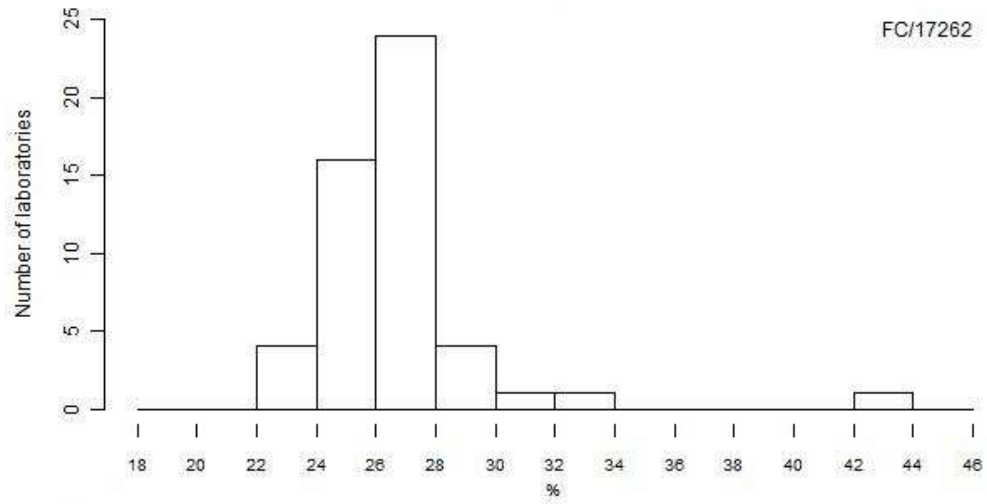
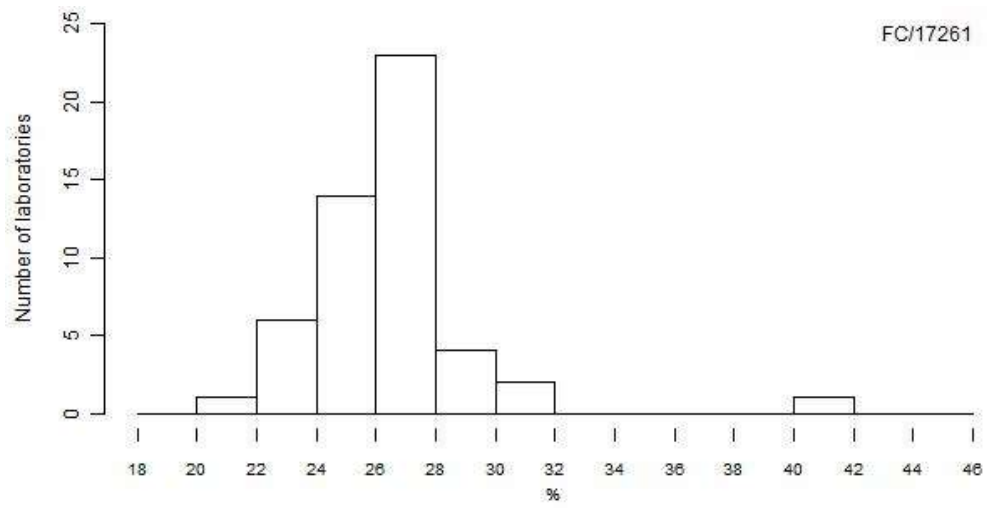
CD4 10E9/L



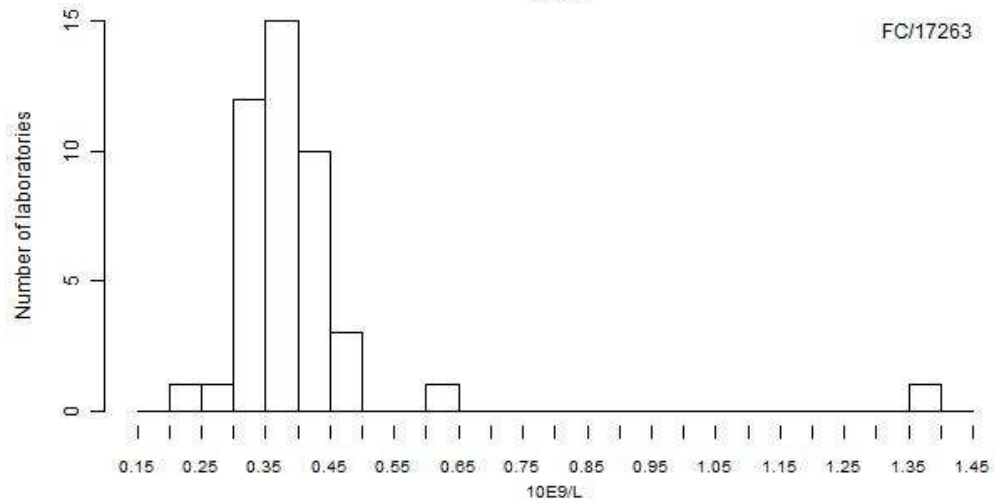
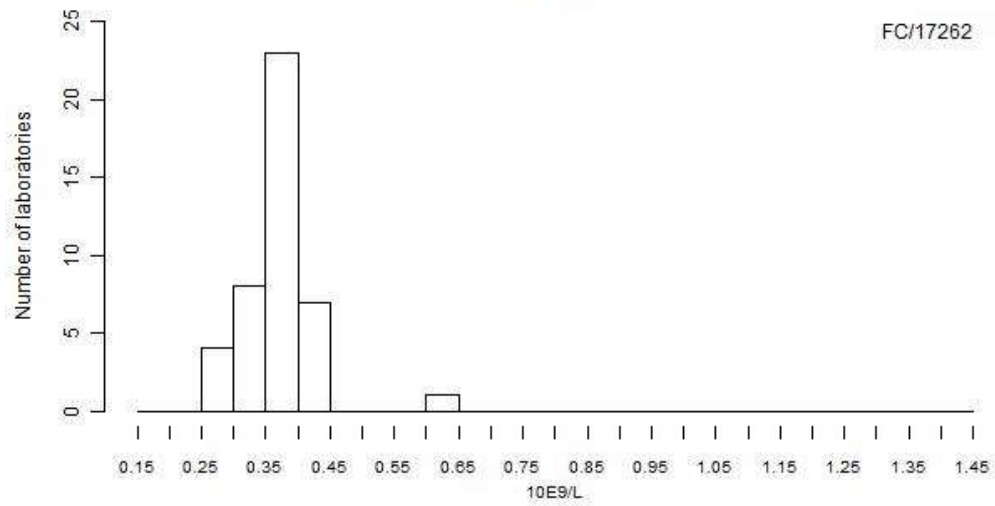
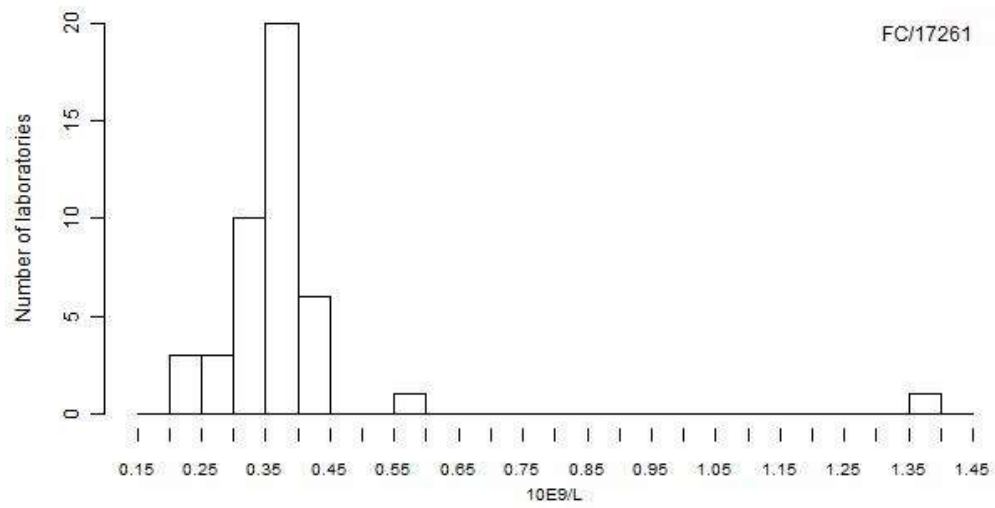
Results not represented on the graph

FC/17261 = 0.237 10e9
 FC/17261 = 0.25 10e9/l
 FC/17261 = 0.624 10e9
 FC/17261 = 0.67 10e9/l
 FC/17261 = 1.552 10e9
 FC/17261 = 318 10e9/L
 FC/17261 = 378 10e9/L
 FC/17261 = 397 10e9/L
 FC/17261 = 430 10e9/L
 FC/17261 = 3998 10e9/
 FC/17262 = 0.658 10e9
 FC/17262 = 0.68 10e9/l
 FC/17262 = 1.674 10e9
 FC/17262 = 327 10e9/L
 FC/17262 = 382 10e9/L
 FC/17262 = 399 10e9/L
 FC/17262 = 417 10e9/L

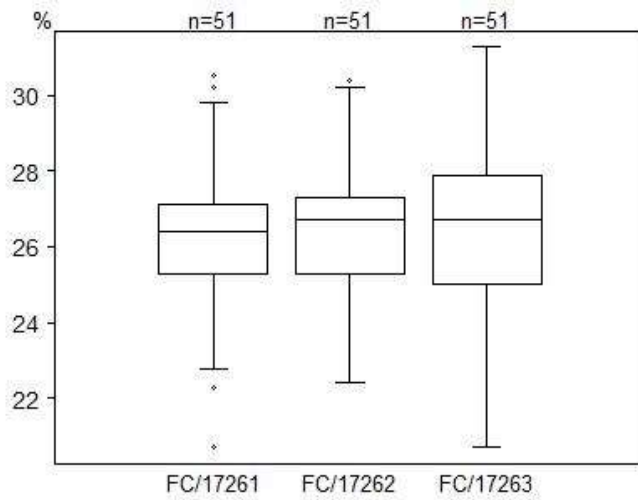
CD8 %



CD8 10E9/L



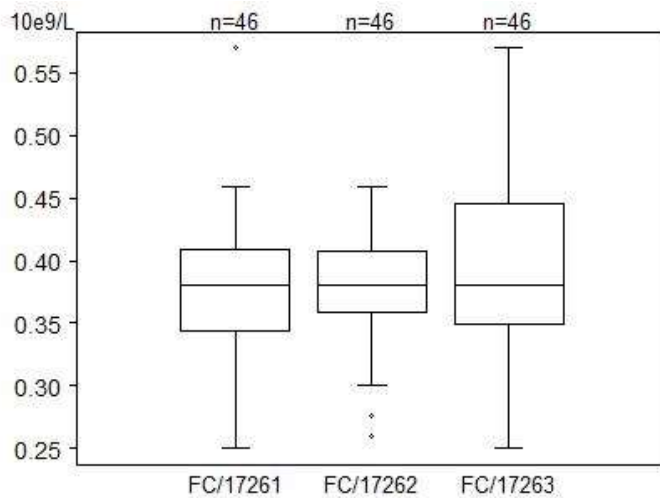
CD8 %



Results not represented
on the graph

FC/17261 = 41.3 %
FC/17262 = 32.9 %
FC/17262 = 42.7 %
FC/17263 = 40.9 %

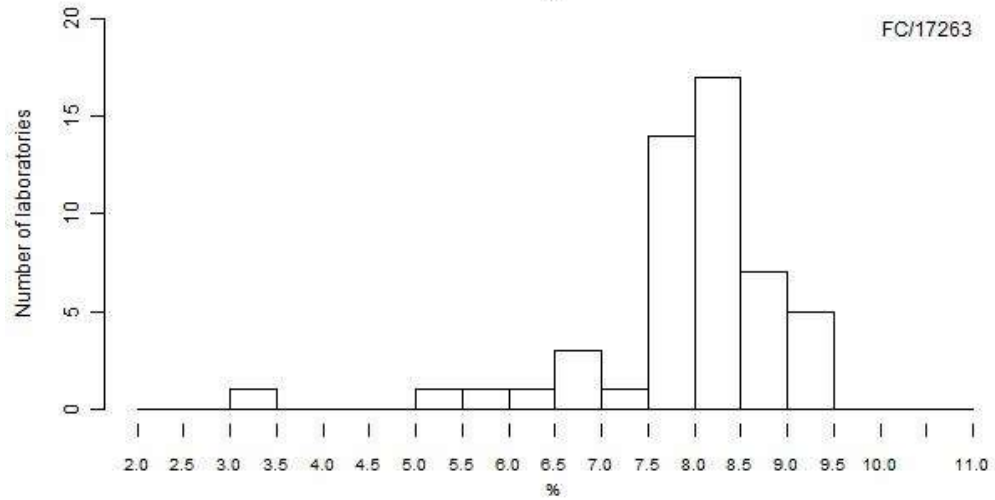
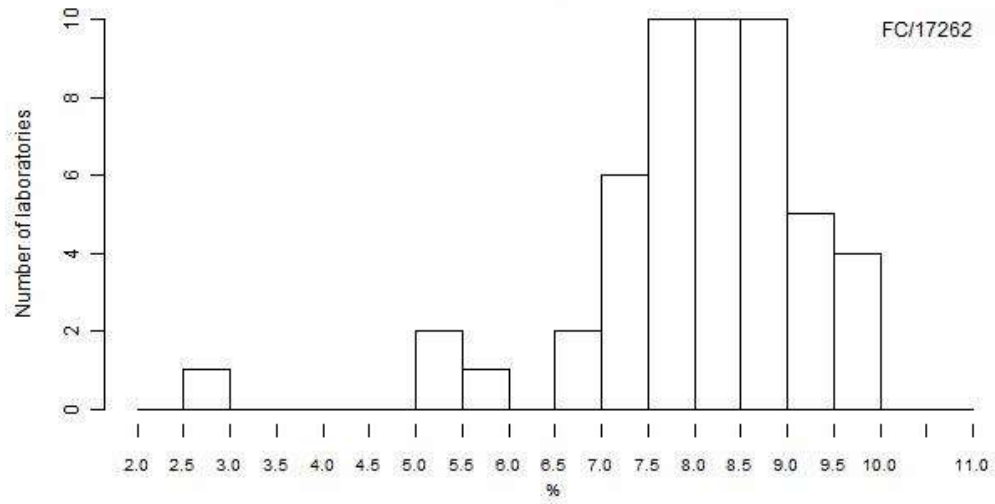
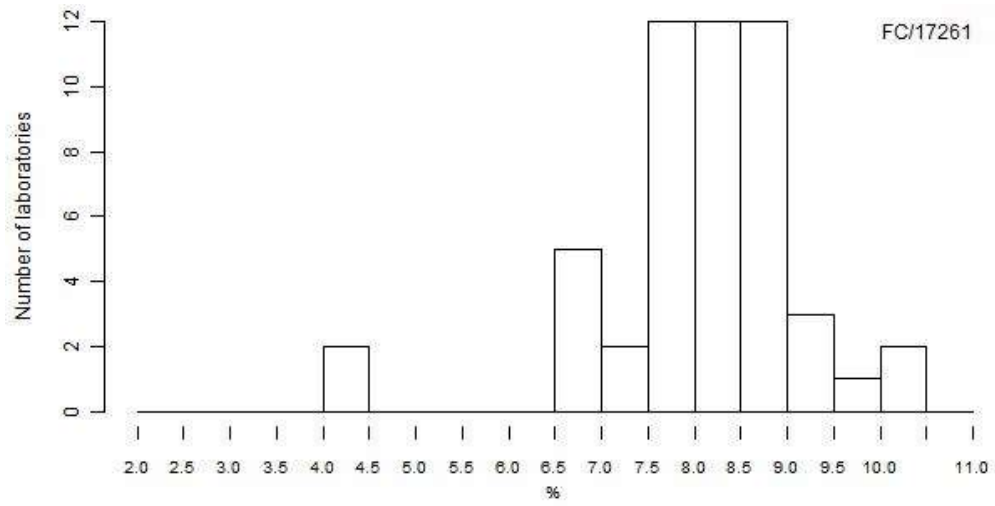
CD8 10E9/L



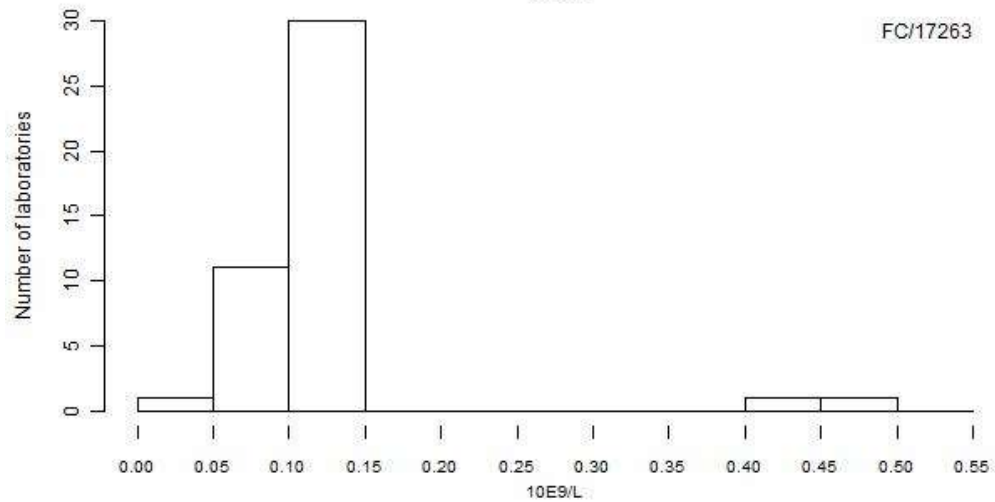
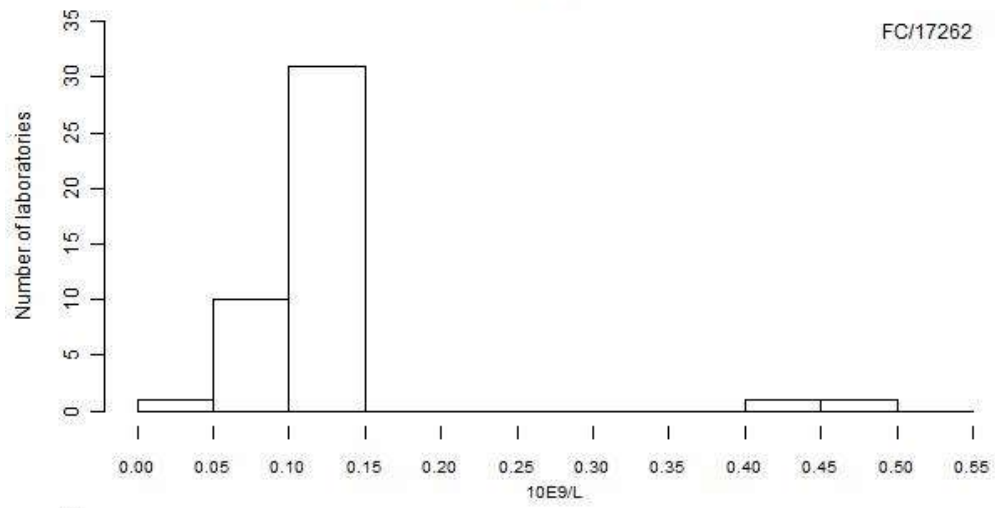
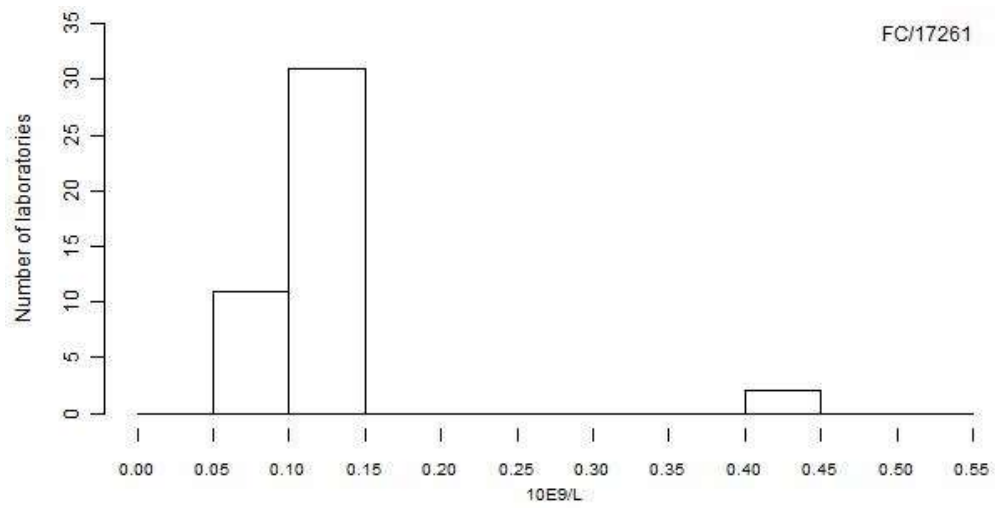
Results not represented
on the graph

FC/17261 = 0.202 10e9
FC/17261 = 1.356 10e9
FC/17261 = 303 10e9/L
FC/17261 = 364 10e9/L
FC/17261 = 364 10e9/L
FC/17261 = 376 10e9/L
FC/17261 = 3347 10e9/
FC/17262 = 0.63 10e9/l
FC/17262 = 1.685 10e9
FC/17262 = 311 10e9/L
FC/17262 = 386 10e9/L
FC/17262 = 392 10e9/L
FC/17262 = 392 10e9/L
FC/17262 = 3743 10e9/
FC/17263 = 0.61 10e9/l
FC/17263 = 1.39 10e9/l
FC/17263 = 312 10e9/L

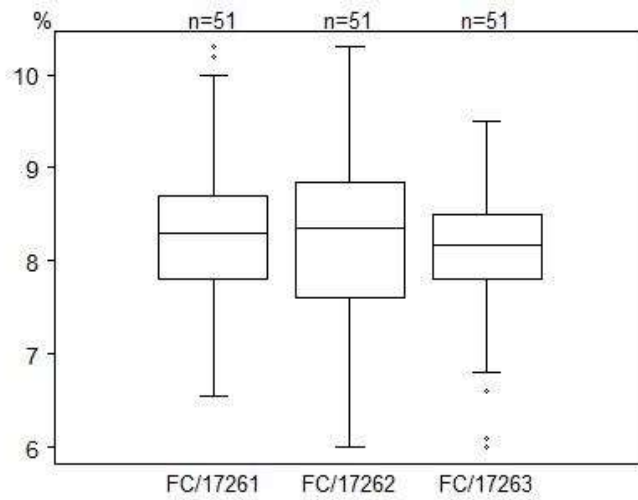
CD19 %



CD19 10E9/L



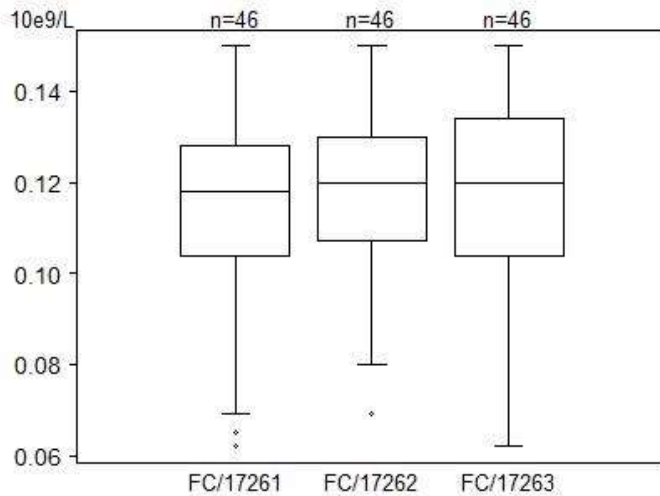
CD19 %



Results not represented on the graph

FC/17261 = 4.4 %
 FC/17261 = 4.4 %
 FC/17262 = 2.9 %
 FC/17262 = 5.1 %
 FC/17263 = 3.2 %
 FC/17263 = 5.3 %

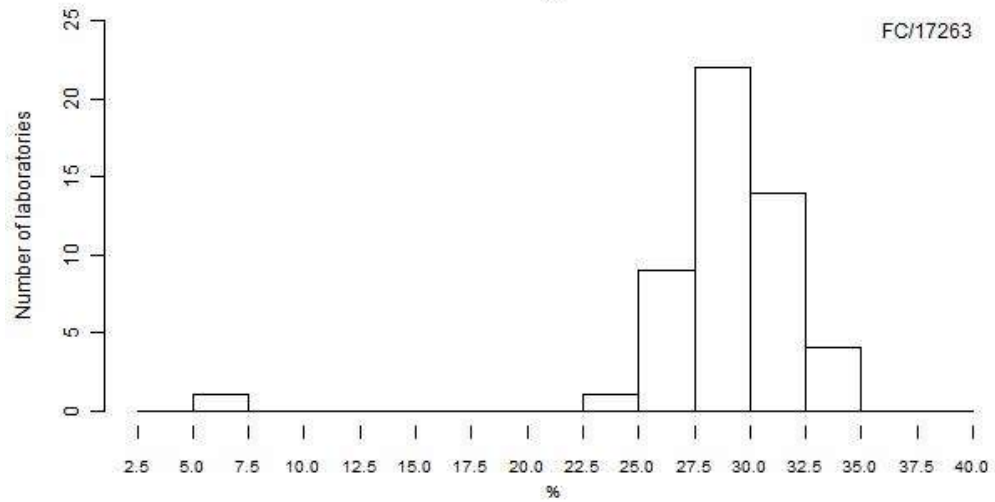
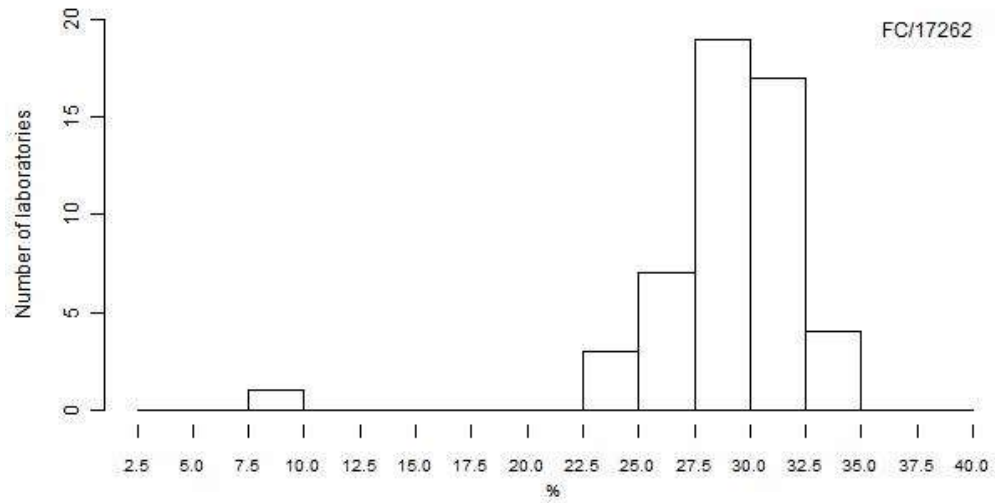
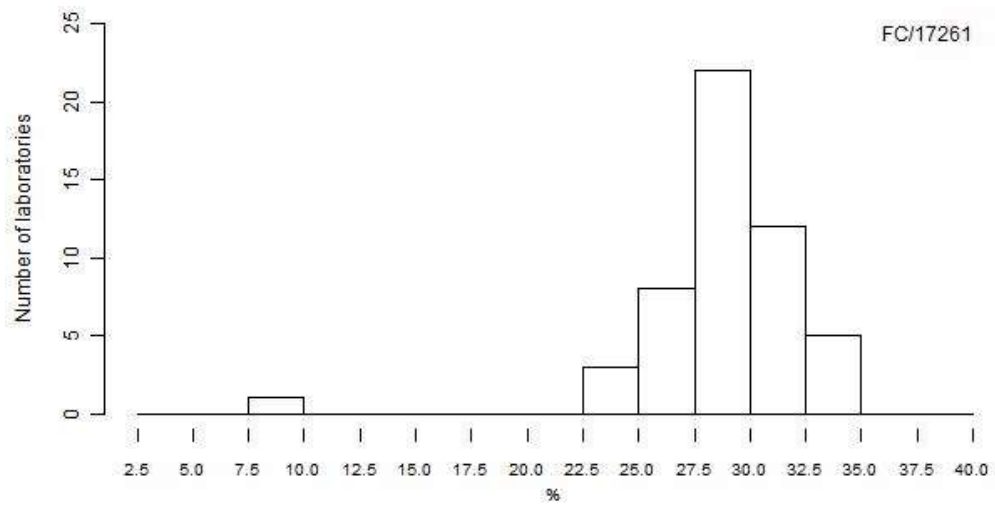
CD19 10E9/L



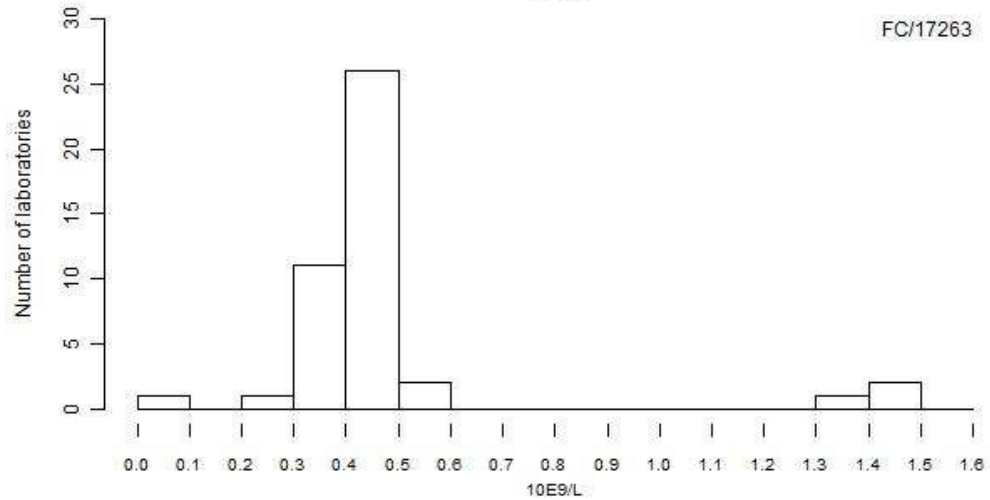
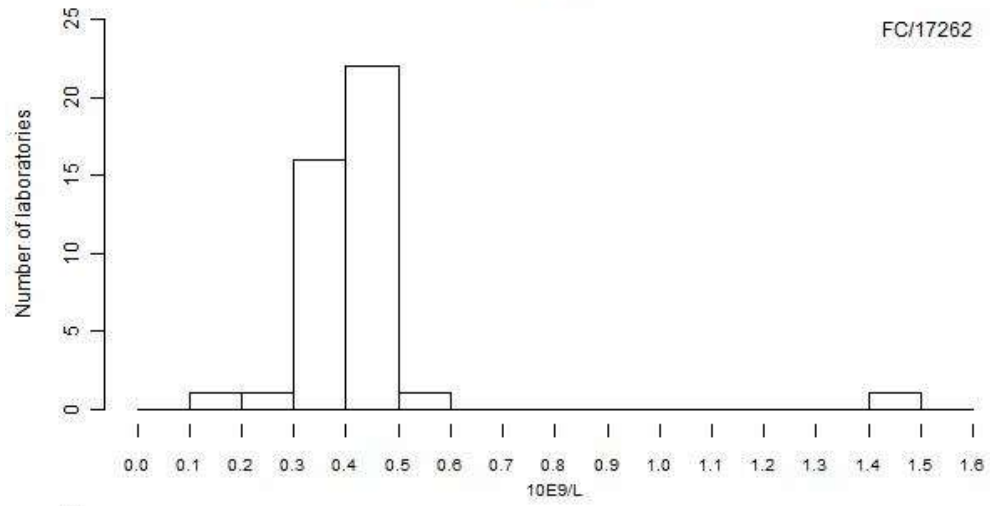
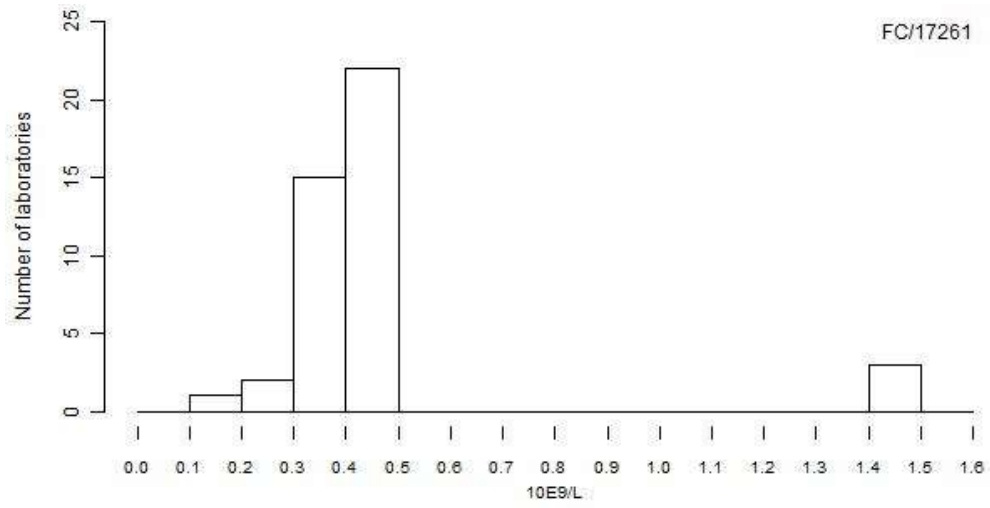
Results not represented on the graph

FC/17261 = 0.434 10e9
 FC/17261 = 0.44 10e9/l
 FC/17261 = 93 10e9/L
 FC/17261 = 108 10e9/L
 FC/17261 = 123 10e9/L
 FC/17261 = 137 10e9/L
 FC/17261 = 899 10e9/L
 FC/17262 = 0.042 10e9
 FC/17262 = 0.43 10e9/l
 FC/17262 = 0.454 10e9
 FC/17262 = 95 10e9/L
 FC/17262 = 109 10e9/L
 FC/17262 = 126 10e9/L
 FC/17262 = 130 10e9/L
 FC/17262 = 964 10e9/L
 FC/17263 = 0.045 10e9
 FC/17263 = 0.45 10e9/l

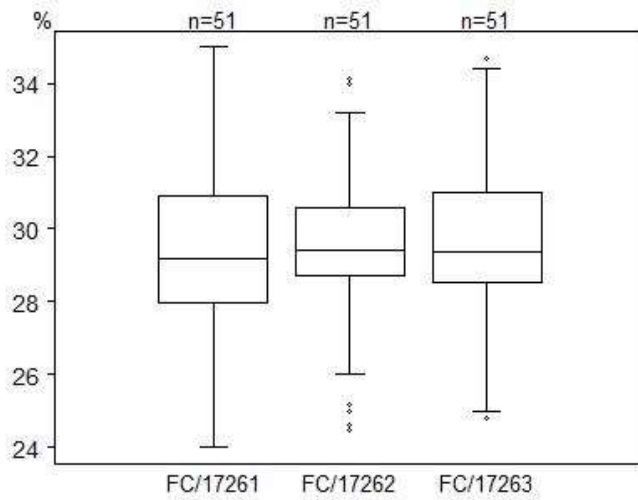
NKcells %



NKcells 10E9/L



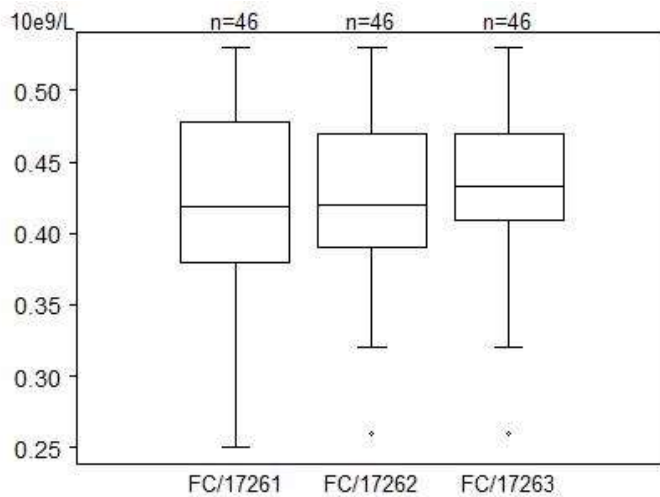
NKcells %



Results not represented on the graph

FC/17261 = 7.7 %
 FC/17262 = 7.8 %
 FC/17263 = 7 %

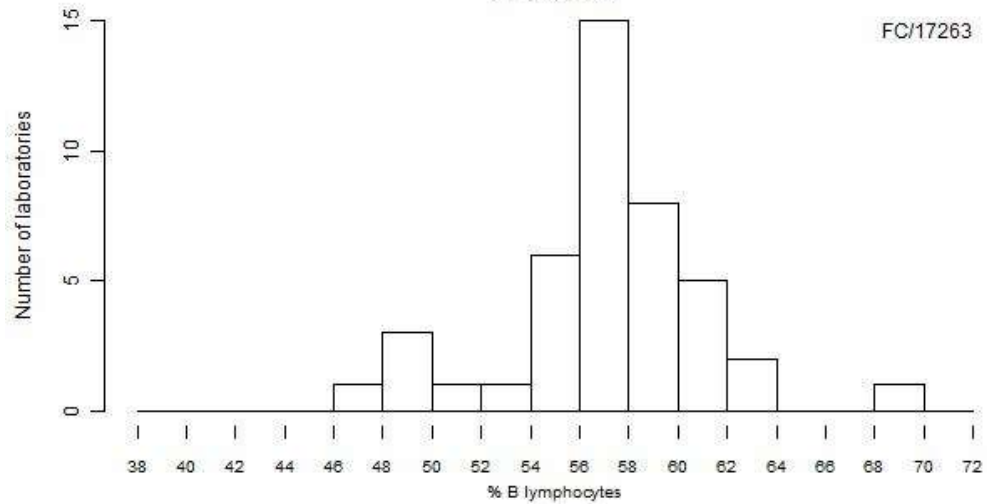
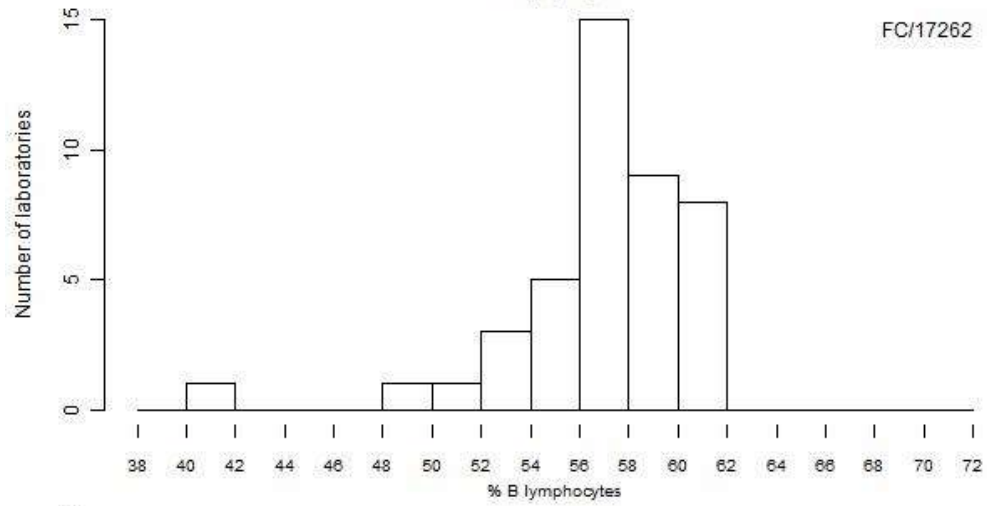
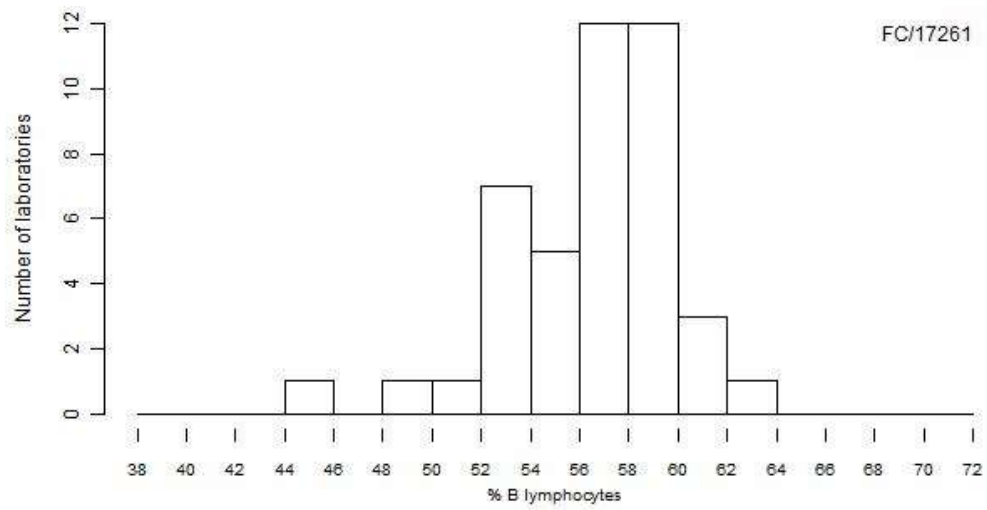
NKcells 10E9/L



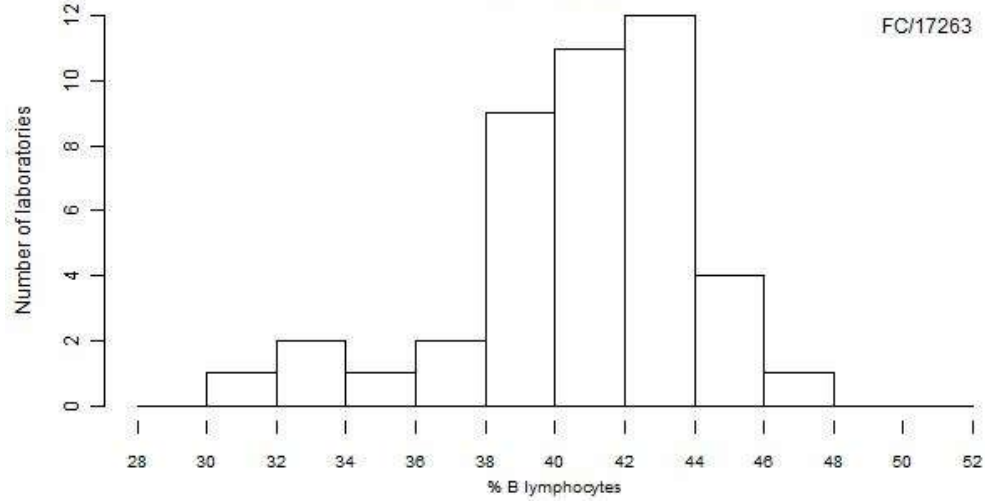
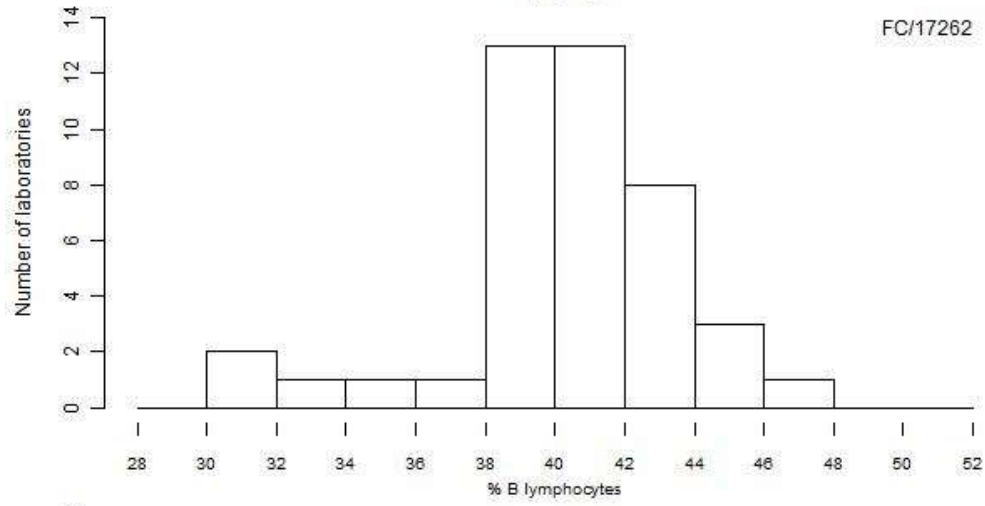
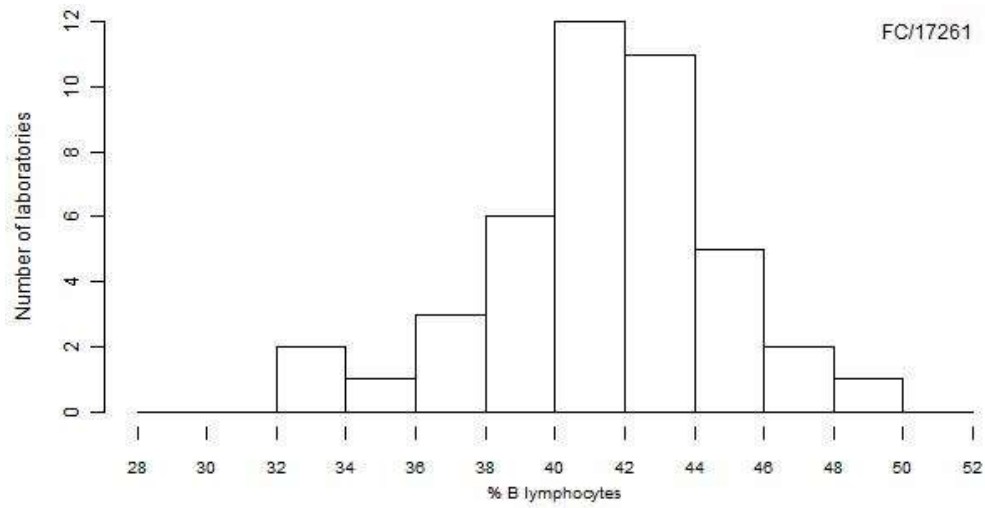
Results not represented on the graph

FC/17261 = 0.109 10e9
 FC/17261 = 1.405 10e9
 FC/17261 = 1.409 10e9
 FC/17261 = 1.409 10e9
 FC/17261 = 323 10e9/L
 FC/17261 = 338 10e9/L
 FC/17261 = 405 10e9/L
 FC/17261 = 458 10e9/L
 FC/17261 = 488 10e9/L
 FC/17261 = 4558 10e9/
 FC/17262 = 0.113 10e9
 FC/17262 = 1.408 10e9
 FC/17262 = 1.623 10e9
 FC/17262 = 1.623 10e9
 FC/17262 = 328.3 10e9
 FC/17262 = 349 10e9/L
 FC/17262 = 414 10e9/L

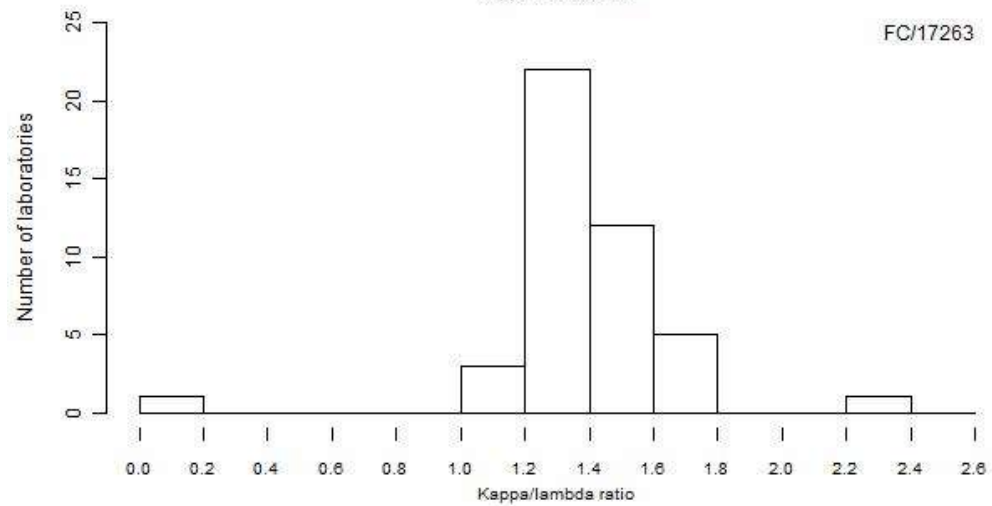
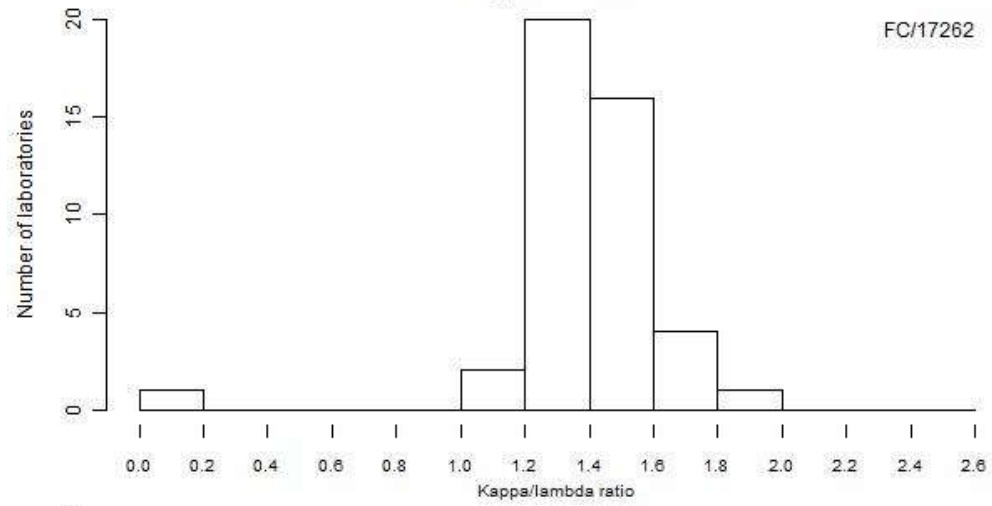
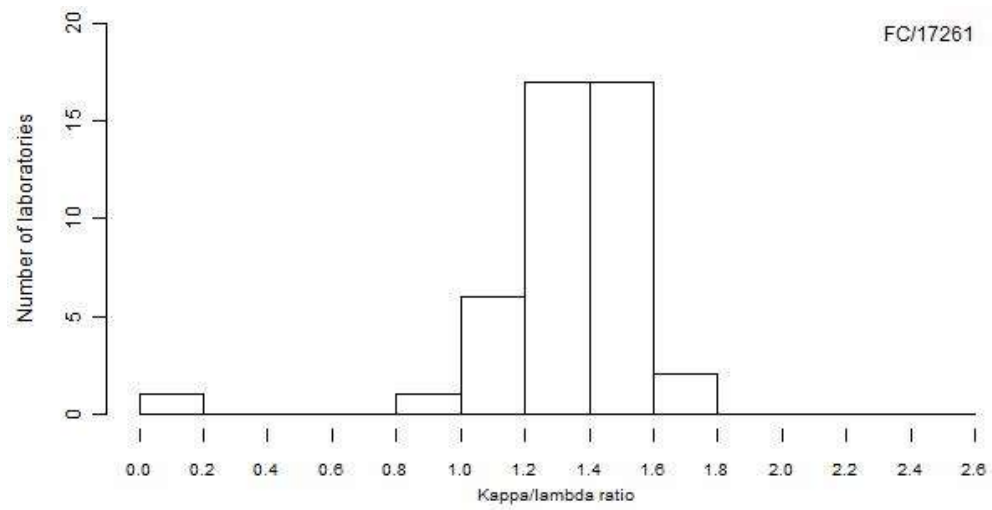
Kappa % B lymphocytes



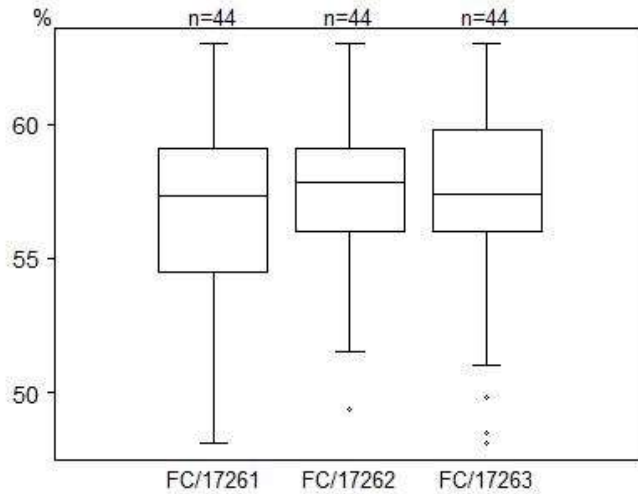
Lambda % B lymphocytes



Kappa/lambda



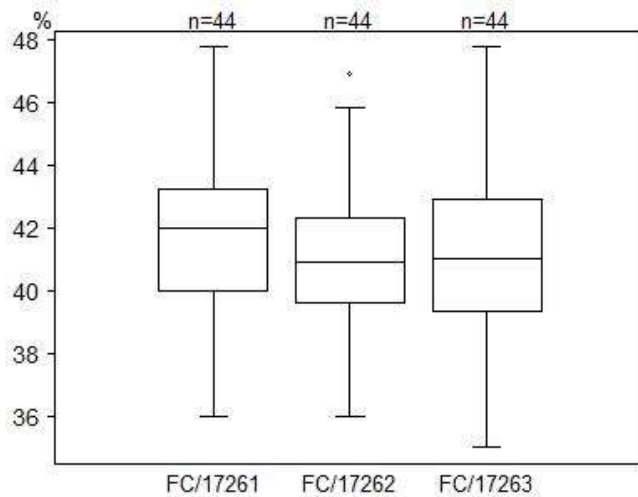
Kappa % B lymphocytes



Results not represented on the graph

FC/17261 = 2.5 %
 FC/17261 = 45 %
 FC/17262 = 1.4 %
 FC/17262 = 42 %
 FC/17263 = 3.3 %
 FC/17263 = 47 %
 FC/17263 = 69 %

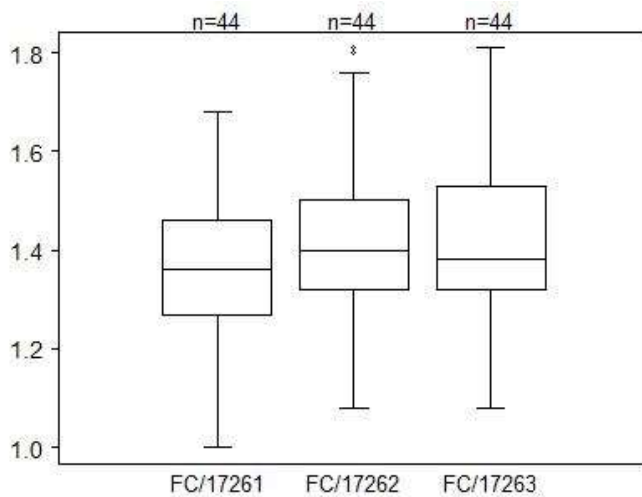
Lambda % B lymphocytes



Results not represented on the graph

FC/17261 = 32.6 %
 FC/17261 = 34 %
 FC/17261 = 48.5 %
 FC/17261 = 97.5 %
 FC/17262 = 31 %
 FC/17262 = 32 %
 FC/17262 = 32.5 %
 FC/17262 = 98.2 %
 FC/17263 = 31 %
 FC/17263 = 33 %
 FC/17263 = 34 %
 FC/17263 = 96.8 %

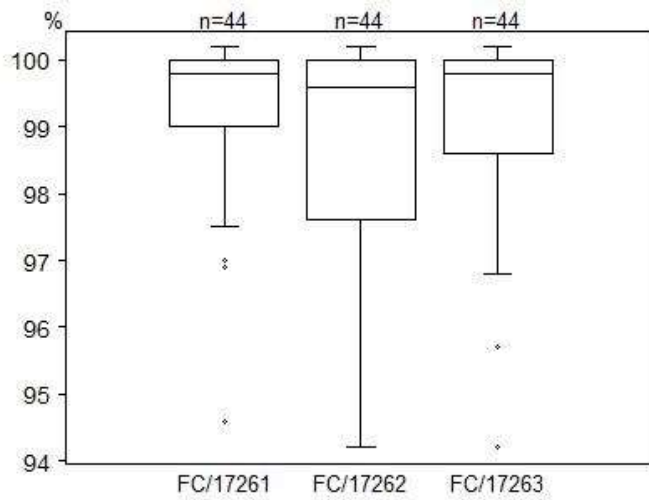
Kappa/lambda



Results not represented on the graph

FC/17261 = 0.03
 FC/17262 = 0.01
 FC/17263 = 0.03
 FC/17263 = 2.23

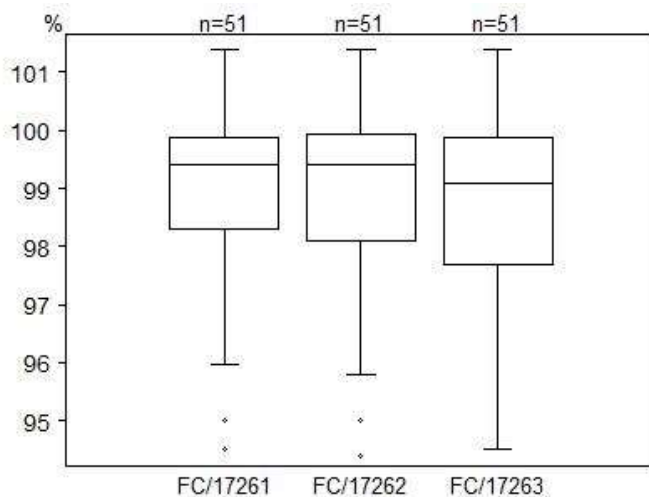
Sum K+L % B lymphocytes



Results not represented on the graph

FC/17261 = 79 %
 FC/17261 = 85.3 %
 FC/17261 = 91 %
 FC/17261 = 91 %
 FC/17261 = 93.3 %
 FC/17261 = 93.7 %
 FC/17262 = 73 %
 FC/17262 = 89.8 %
 FC/17262 = 90 %
 FC/17262 = 93 %
 FC/17262 = 93 %
 FC/17262 = 93.1 %
 FC/17262 = 93.2 %
 FC/17263 = 82 %
 FC/17263 = 87 %
 FC/17263 = 89 %
 FC/17263 = 91 %

Lymphosum %

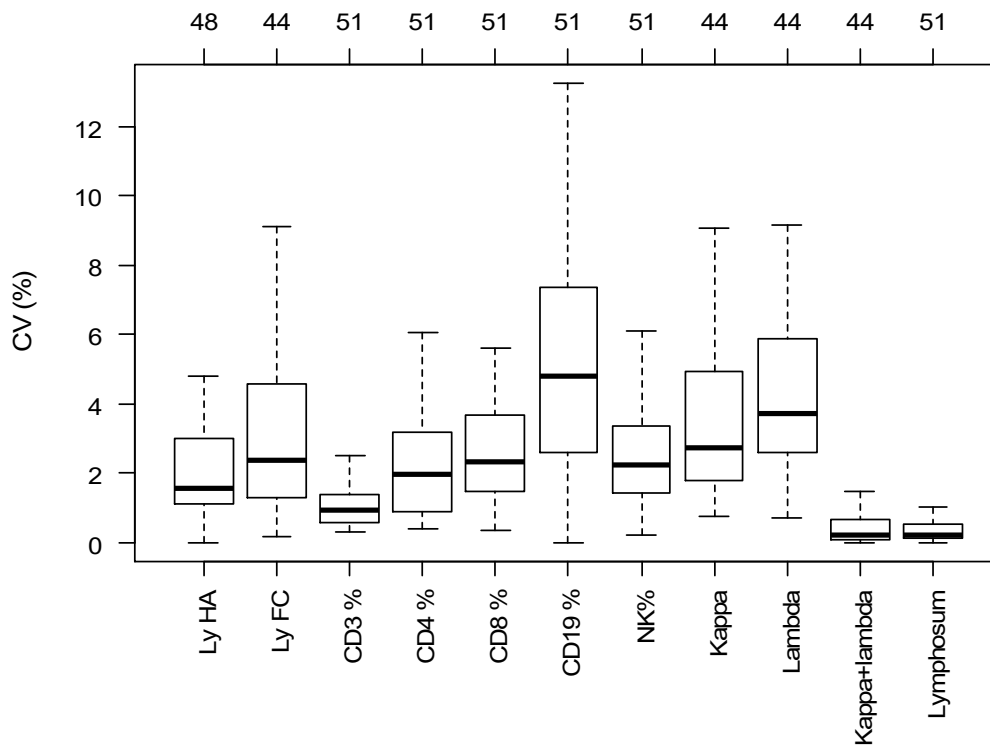
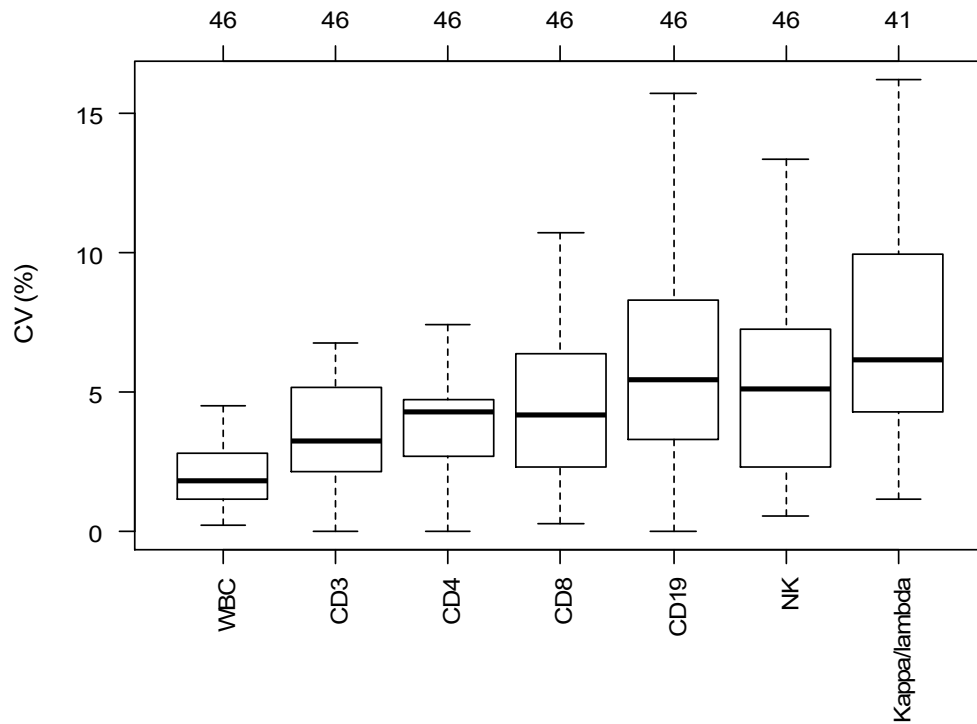


Results not represented on the graph

FC/17261 = 86.2 %
 FC/17261 = 94 %
 FC/17262 = 84.8 %
 FC/17263 = 85 %

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%.

Following figures represent the box-and-whisker plots of the intra-laboratory variability based on the results of the three identical samples (FC/17261, FC/17262 and FC/17263). The intra-laboratory variability is estimated by the coefficient of variation of the three results calculated for each laboratory and each parameter.



NEXT SURVEY

The next survey is scheduled for **November 23, 2020**.

END

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