

**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 2020/1**

**Sciensano/Flow cytometry/73-E**

Expertise and service provision  
Quality of laboratories  
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A preliminary version of this report was submitted to the experts EQA Flow Cytometry on: 24/03/2020  
 This report could not be discussed at an expert committee meeting due to the Covid-19 crisis, the experts were invited to send their comments by email.

Authorisation to release report: by Lobna Bouacida, scheme coordinator, on 06/05/2020.

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/ nl/rapports_annee.htm)  
[https://www.wiv-isp.be/QML/activities/external\\_quality/rapports/ fr/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<sub>G</sub>):  
central value of the results obtained by all laboratories (all methods together).
- Global standard deviation (SD<sub>G</sub>):  
measure of the spread of the results obtained by all the laboratories (all methods together).
- Global median of your method (M<sub>M</sub>):  
central value of the results obtained by the laboratories using your method.
- Standard deviation of your method (SD<sub>M</sub>):  
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<sub>M/G</sub>** : median

**H<sub>M/G</sub>** : percentiles 25 en 75

**I<sub>M/G</sub>** : internal limits (M ± 2.7 SD)

**O<sub>M/G</sub>** : 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_nl.htm)

[https://www.wiv-isp.be/QML/index\\_fr.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/_nl/brochures.htm)

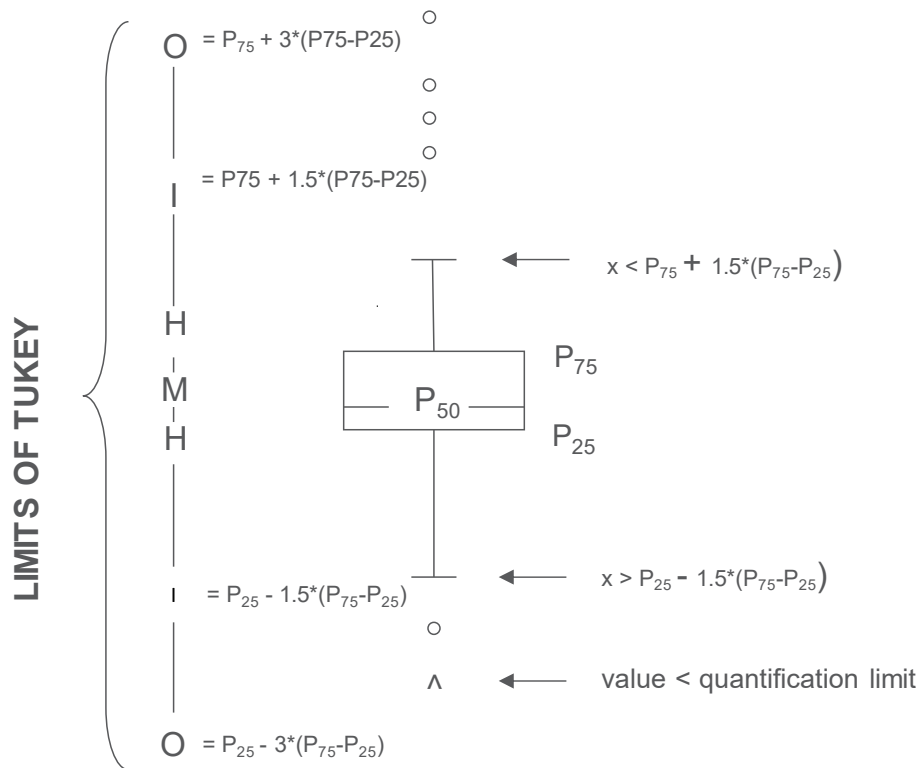
[https://www.wiv-isp.be/QML/activities/external\\_quality/brochures/\\_fr/brochures.htm](https://www.wiv-isp.be/QML/activities/external_quality/brochures/_fr/brochures.htm)

1. Informatiebrochure over de externe kwaliteitsevaluatieprogramma's voor klinische laboratoria (Algemene informatiebrochure over de externe evaluatie)/  
Brochure d'information sur les programmes d'évaluation externe de la qualité pour les laboratoires cliniques (Brochure d'information générale sur l'évaluation externe).
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/17057, FC/17058 and FC/17059) 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.

## PARTICIPATION

Fifty-four laboratories (1 Latvian, 1 Canadian and 52 Belgian clinical laboratories) participated in the survey 2020/1 (send-out of blood samples: 17<sup>th</sup> of February 2020 (day 0)).



## RESULTS

All Belgian laboratories received the samples on day 1 or 2. Forty-six laboratories (88%) received the samples on day 1 and six (12%) received them on day 2.

77% of the Belgian laboratories (n=40) performed the analyses on day 1, 21% on day 2 (n=11) and 2% on day 3 (n=1).

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

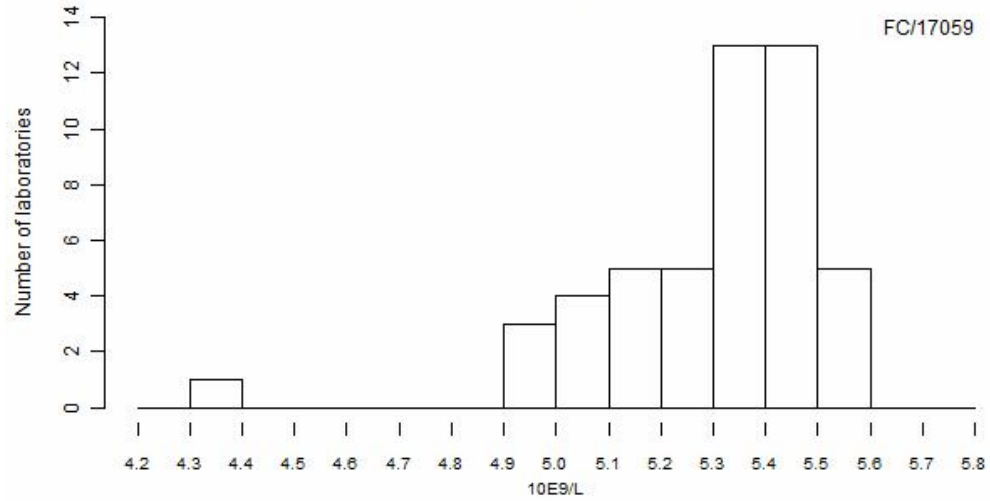
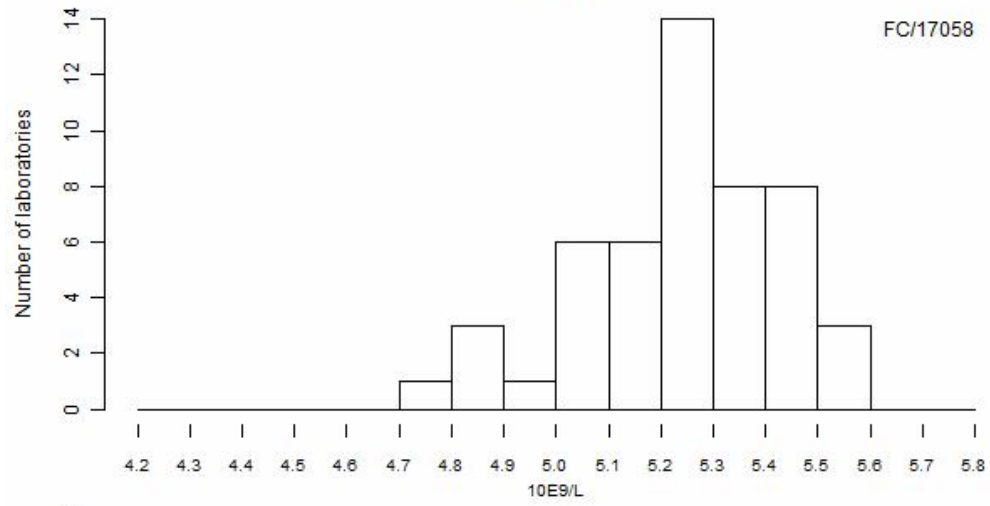
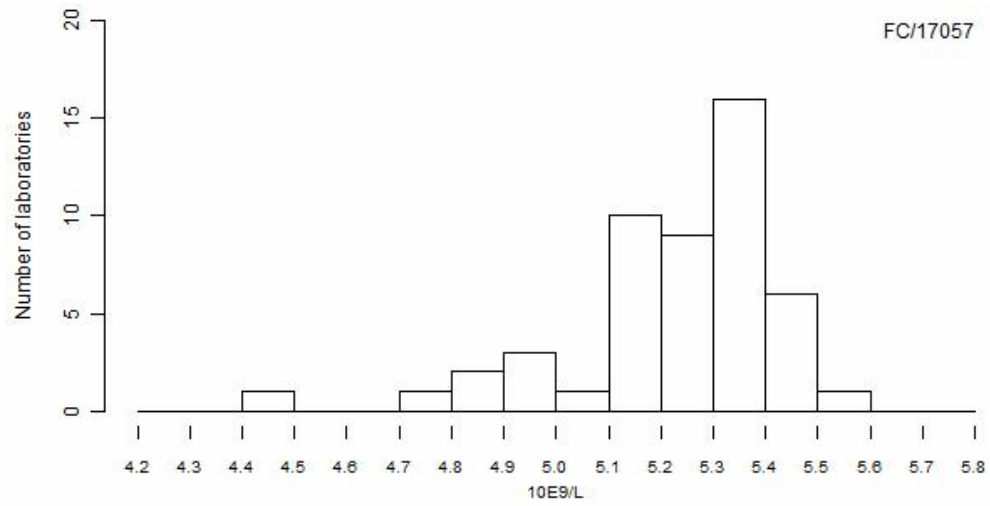
Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=52). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=51).

The following table shows the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/17057, FC/17058 and FC/17059:

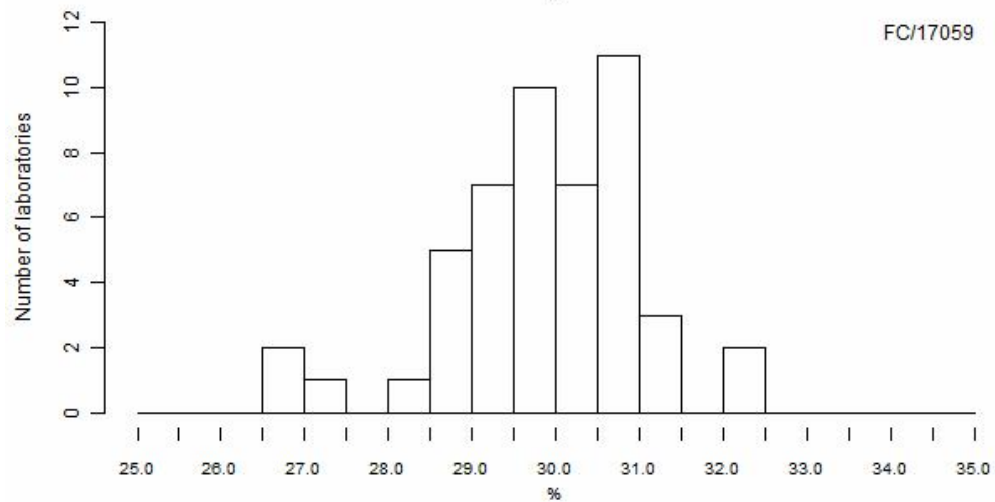
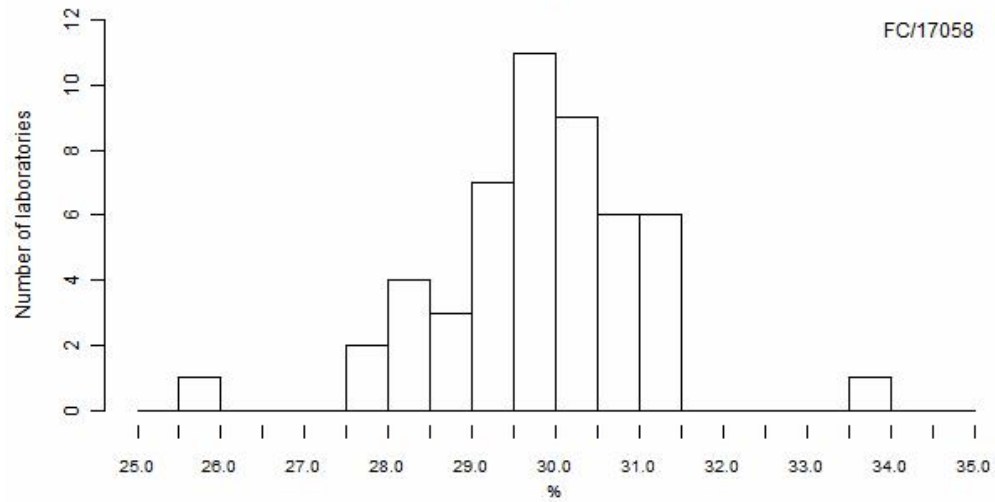
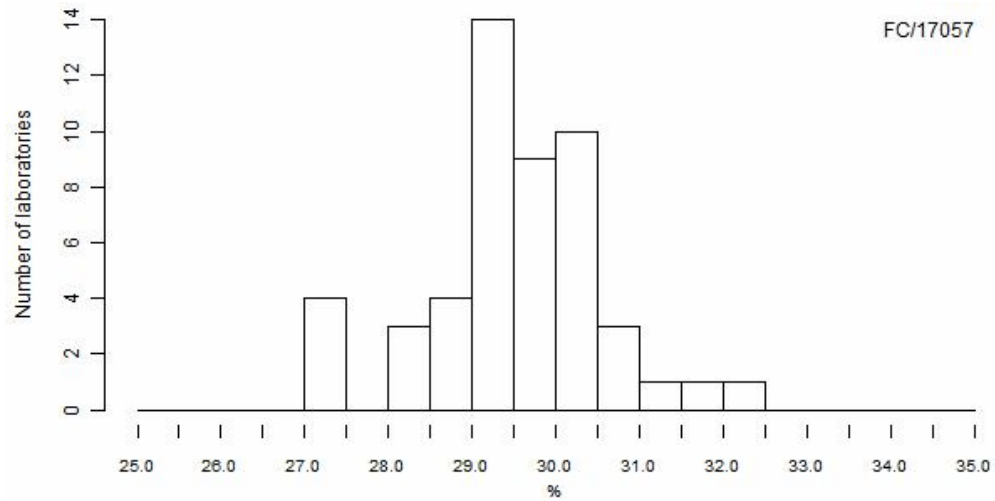
**FC17057 / FC17058 / FC17059**

	Median			SD			CV,%			N		
	17057	17058	17059	17057	17058	17059	17057	17058	17059	17057	17058	17059
<b>Leukocytes 10<sup>9</sup>/L</b>	5.30	5.29	5.38	0.13	0.20	0.19	2.5	3.8	3.6	50	50	49
<b>Lymphocytes % Haematology analyser</b>	29.6	29.9	29.9	0.7	1.0	1.0	2.5	3.5	3.5	49	49	48
<b>Lymphocytes % Flow cytometer</b>	29.0	28.5	29.1	2.9	2.4	2.1	10.0	8.5	7.4	46	46	46
<b>CD3 %</b>	89.2	89.4	89.4	1.1	0.9	1.3	1.2	1.0	1.5	52	52	52
<b>CD3 10<sup>9</sup>/L</b>	1.386	1.413	1.424	0.090	0.076	0.116	6.5	5.4	8.2	51	51	50
<b>CD4 %</b>	35.7	35.6	35.4	1.5	1.3	1.3	4.1	3.7	3.8	52	52	52
<b>CD4 10<sup>9</sup>/L</b>	0.560	0.564	0.565	0.040	0.031	0.044	7.1	5.4	7.9	51	51	50
<b>CD8 %</b>	49.6	50.5	50.0	2.3	2.0	2.4	4.6	4.0	4.9	52	52	52
<b>CD8 10<sup>9</sup>/L</b>	0.784	0.790	0.785	0.063	0.057	0.065	8.0	7.2	8.3	51	51	50
<b>CD19 %</b>	7.1	7.2	7.0	0.9	0.8	0.8	12.6	11.3	11.6	52	52	52
<b>CD19 10<sup>9</sup>/L</b>	0.110	0.111	0.110	0.018	0.021	0.016	16.1	19.0	14.1	51	51	50
<b>NK cells %</b>	3.0	2.9	3.0	0.9	0.8	1.0	29.6	28.1	32.1	52	52	52
<b>NK cells 10<sup>9</sup>/L</b>	0.047	0.044	0.047	0.016	0.013	0.015	33.9	29.4	31.8	51	51	50
<b>κ % B lymphocytes</b>	63.9	64.0	64.0	2.9	2.5	1.3	4.5	3.9	2.0	46	46	46
<b>λ % B lymphocytes</b>	35.0	34.4	35.4	2.5	2.1	1.7	7.2	6.0	4.8	46	46	46
<b>κ/λ ratio</b>	1.85	1.86	1.81	0.19	0.18	0.16	10.0	9.6	9.0	46	46	46
<b>κ+λ % B lymphocytes</b>	99.8	99.7	99.7	0.7	0.7	0.6	0.7	0.7	0.6	46	46	46
<b>Lymphosum</b>	99.5	99.6	99.5	0.6	0.7	1.0	0.6	0.7	1.0	52	52	52

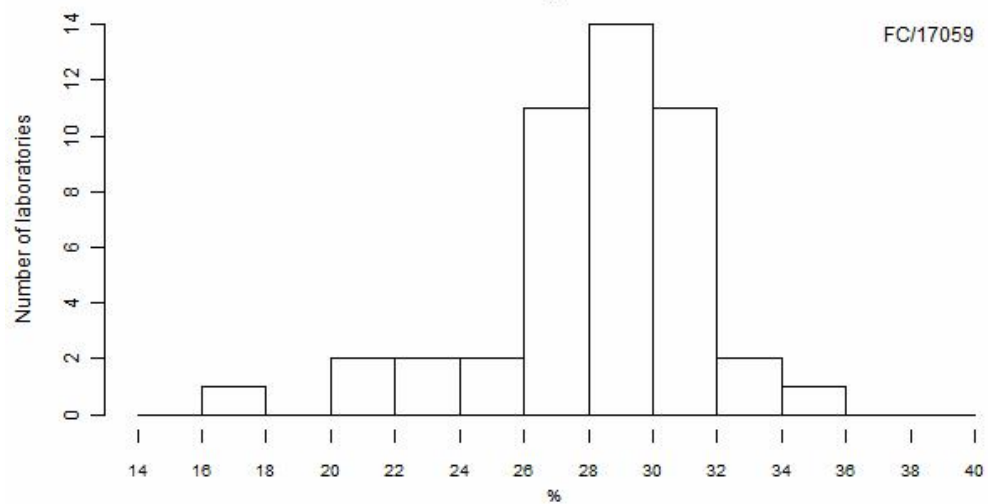
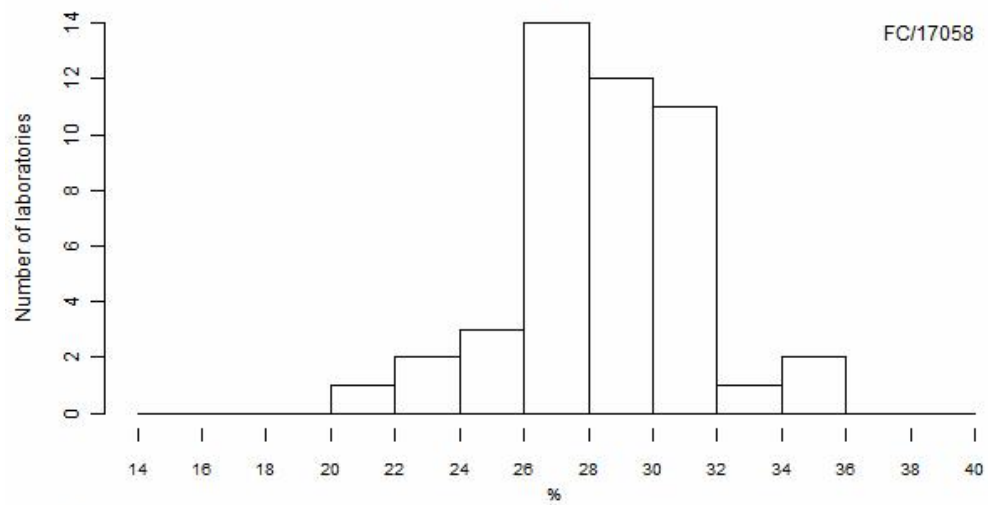
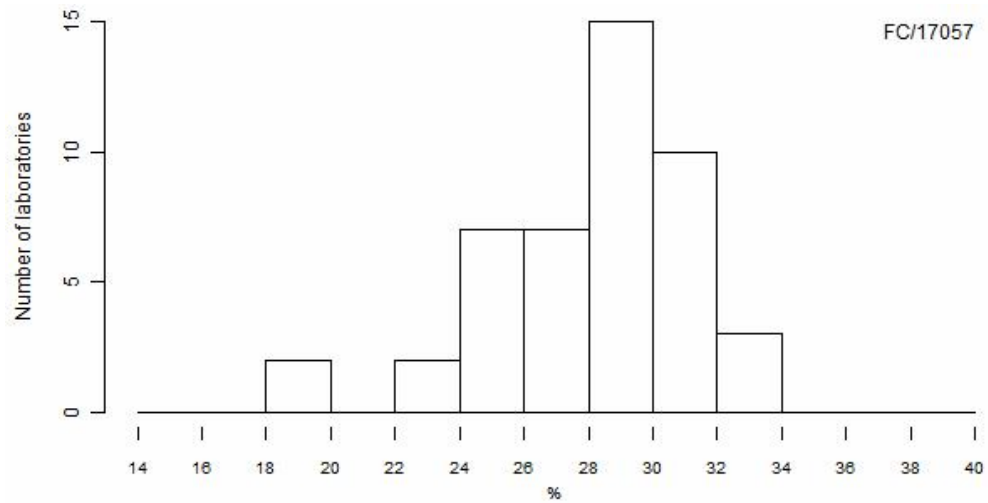
# WBC 10E9/L



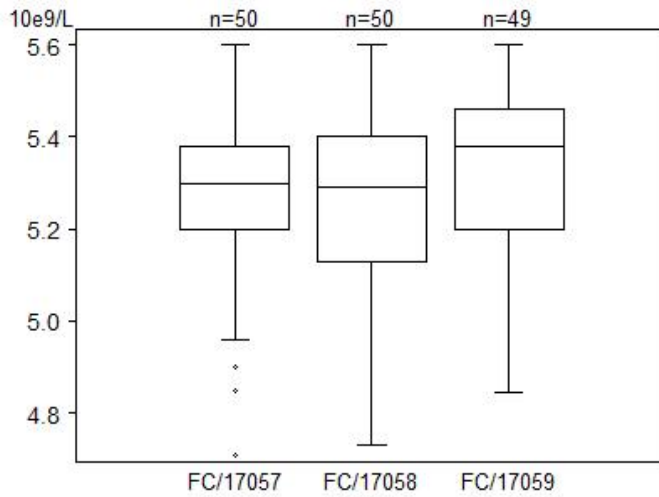
## Lympho% haematology analyser



## Lympho% flow cytometer



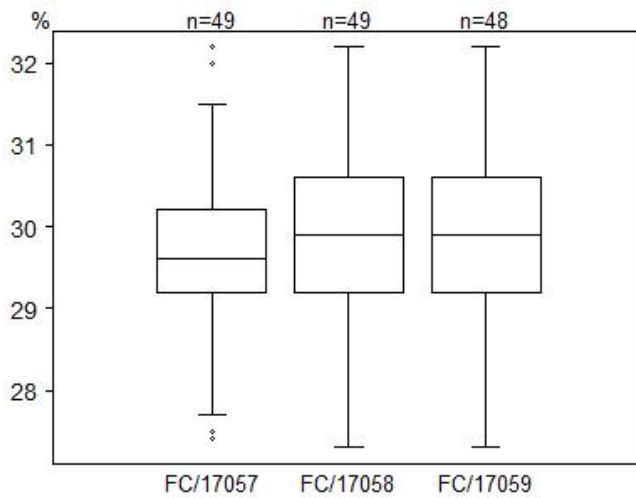
## WBC 10E9/L



Results not represented on the graph

FC/17057 = 4.5 10e9/L  
 FC/17057 = 5200 10e9/  
 FC/17058 = 5200 10e9/  
 FC/17059 = 4.34 10e9/l  
 FC/17059 = 5200 10e9/

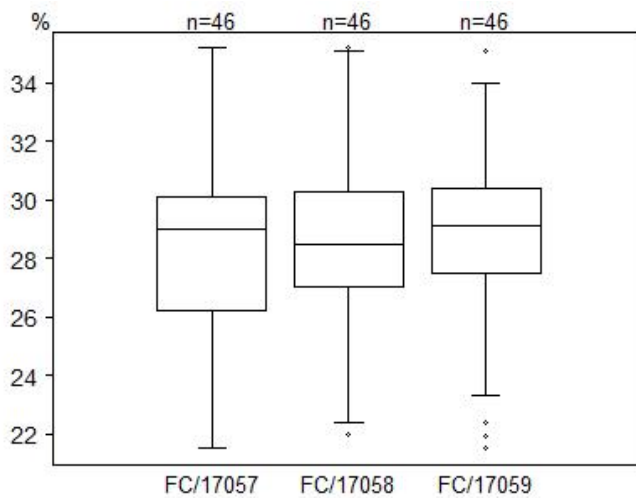
## Lympho% haematology analyser



Results not represented on the graph

FC/17058 = 25.9 %  
 FC/17058 = 34 %  
 FC/17059 = 26.8 %  
 FC/17059 = 27 %

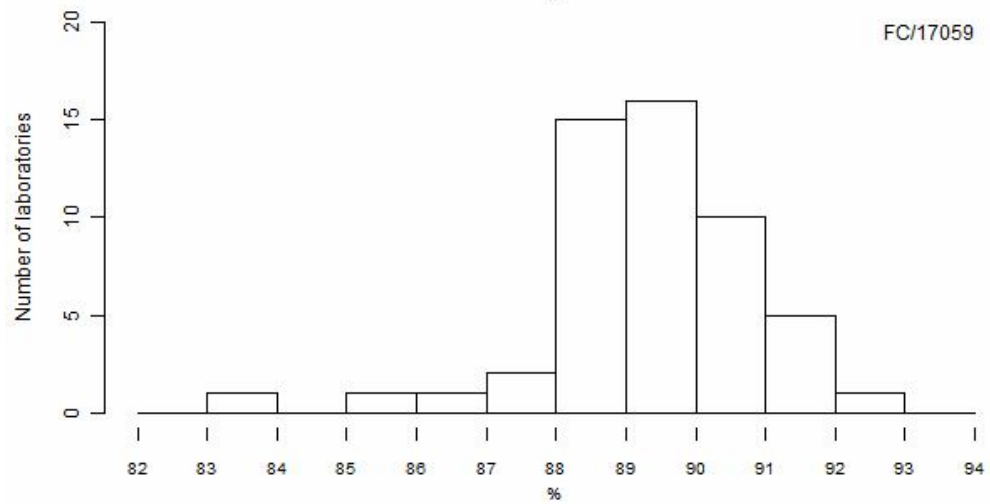
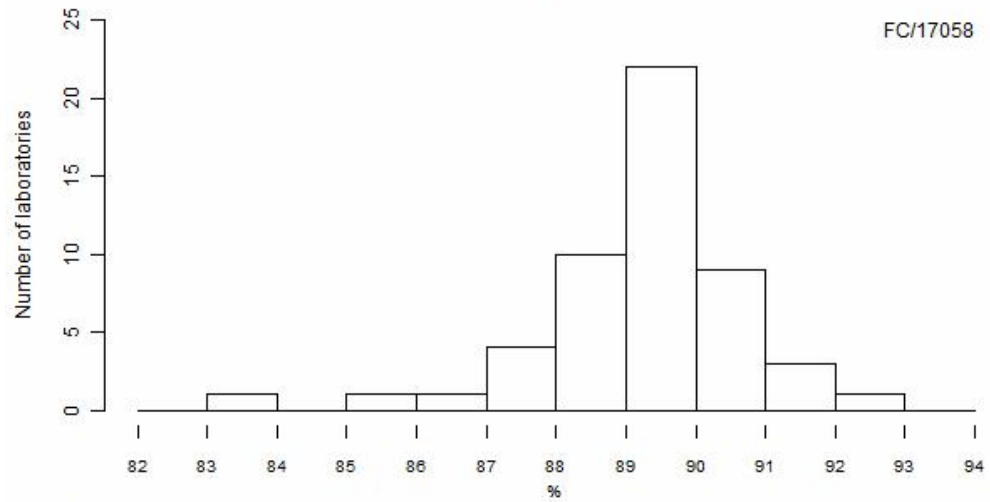
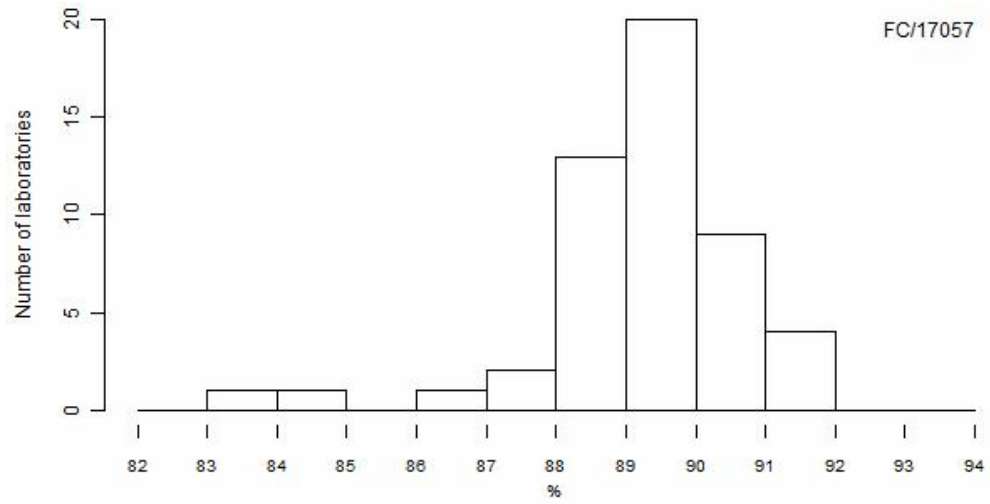
## Lympho% flow cytometer



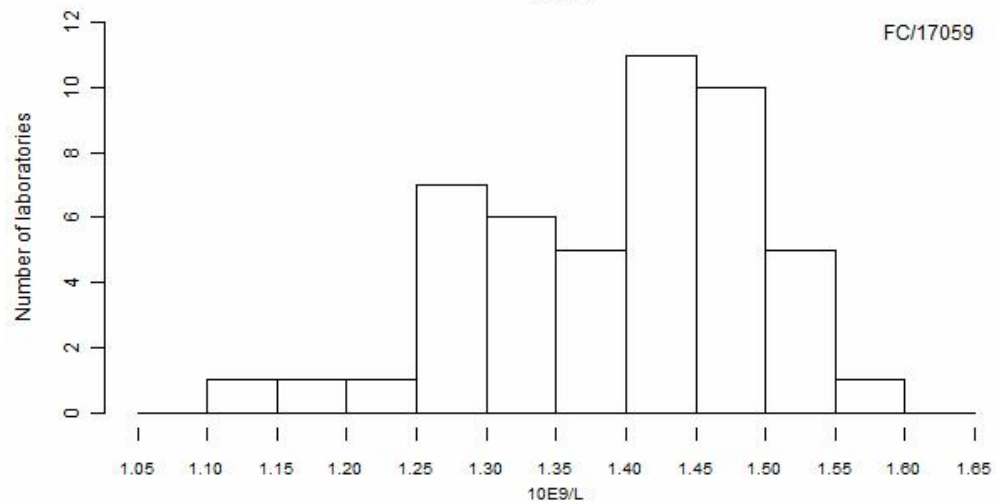
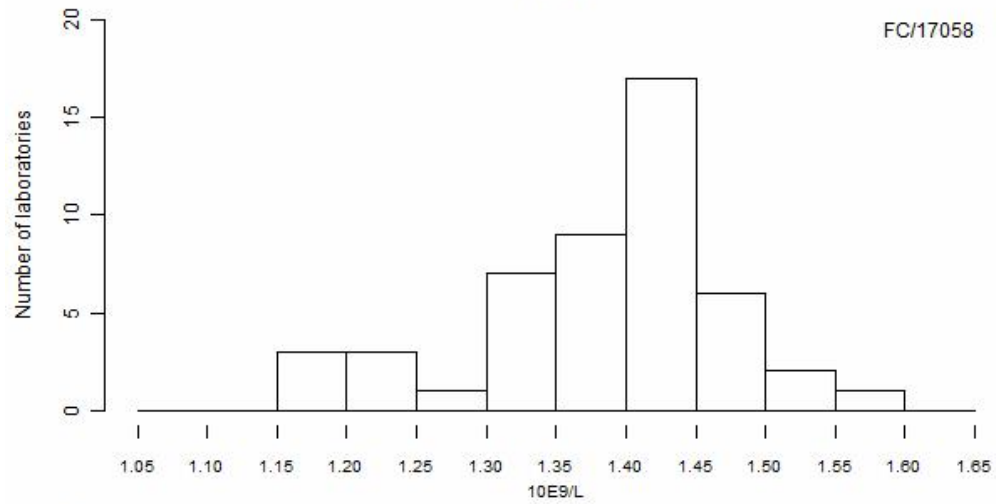
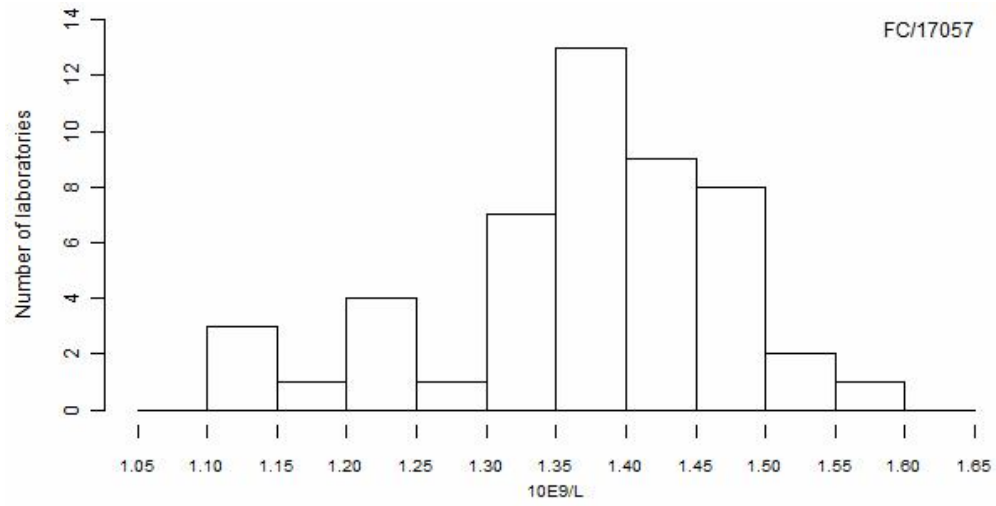
Results not represented on the graph

FC/17057 = 19.5 %  
 FC/17057 = 19.8 %  
 FC/17058 = 36 %  
 FC/17059 = 16.2 %

# CD3 %

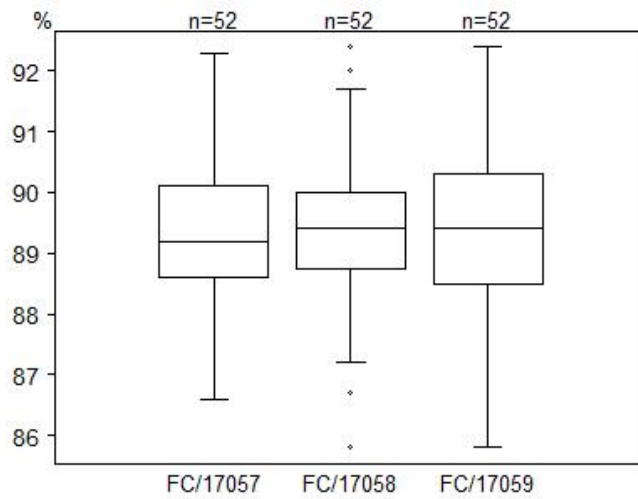


# CD3 10E9/L





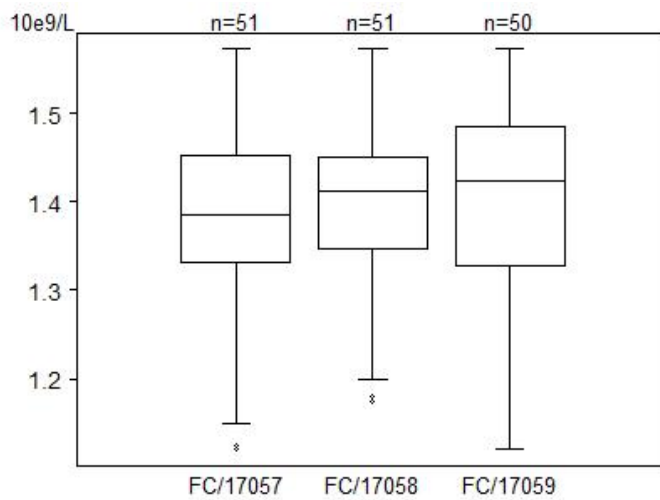
## CD3 %



Results not represented  
on the graph

FC/17057 = 74 %  
 FC/17057 = 83.9 %  
 FC/17057 = 84.6 %  
 FC/17058 = 83.2 %  
 FC/17059 = 83.9 %

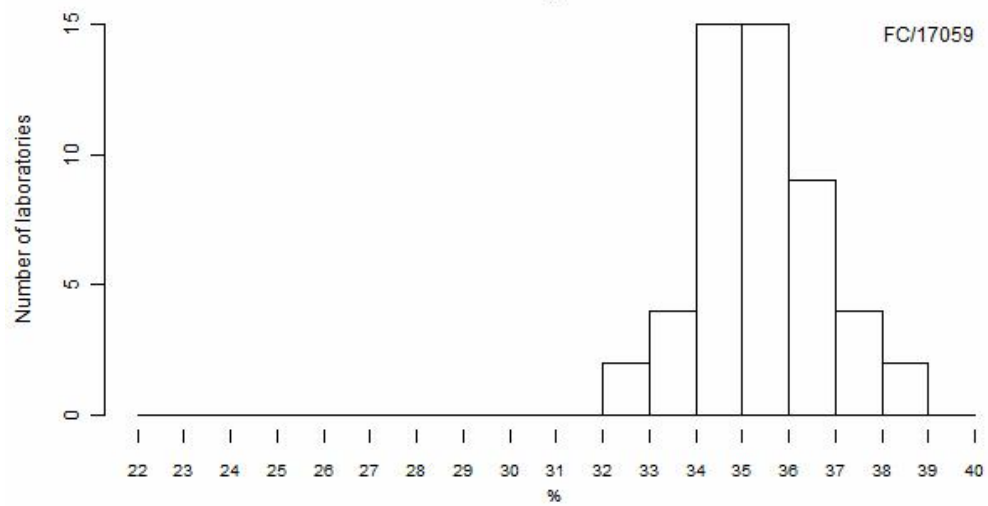
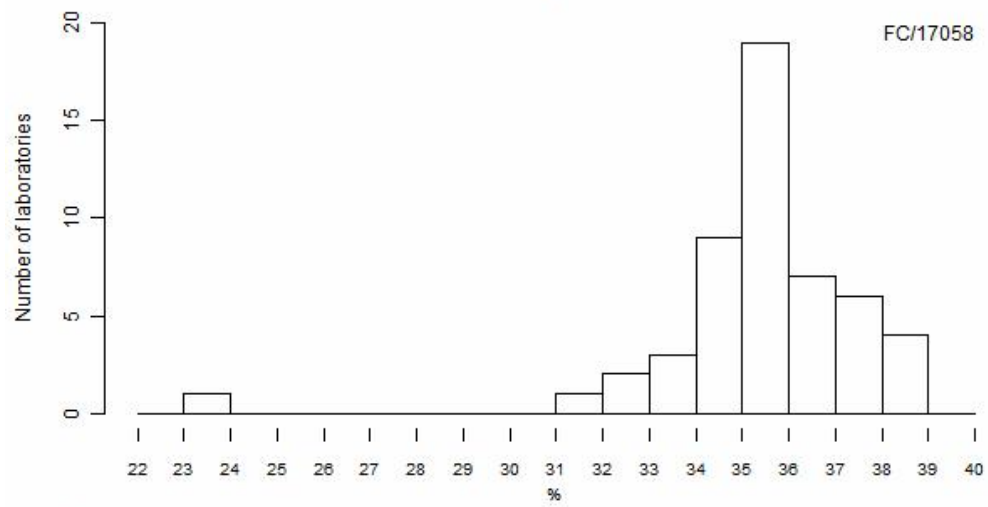
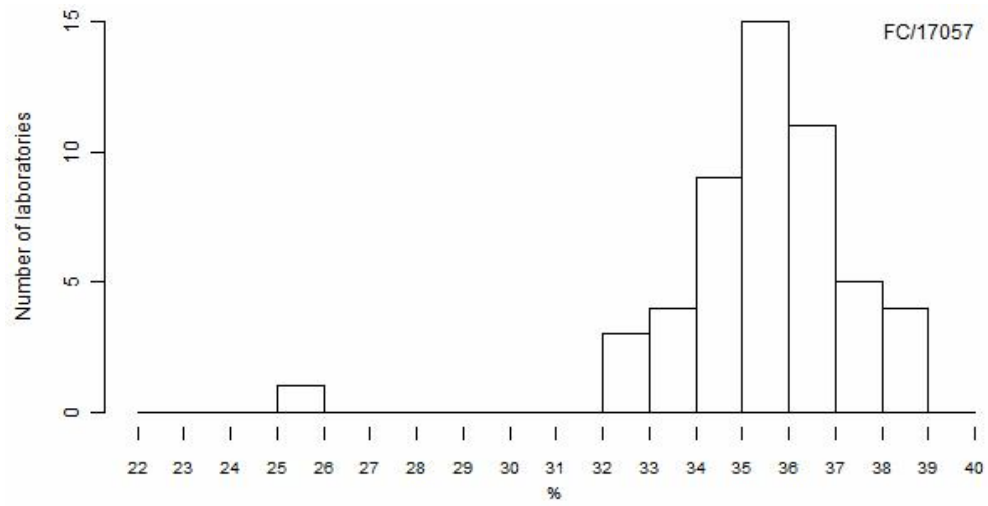
## CD3 10E9/L



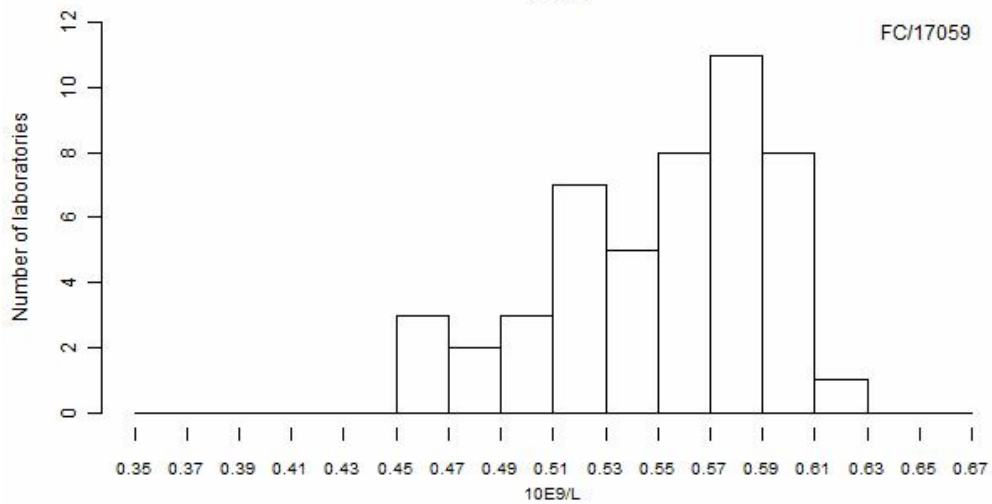
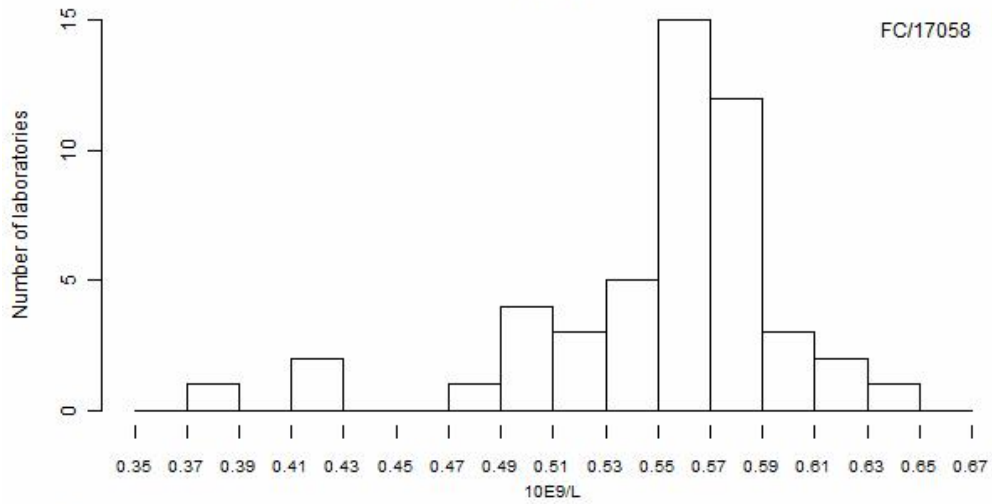
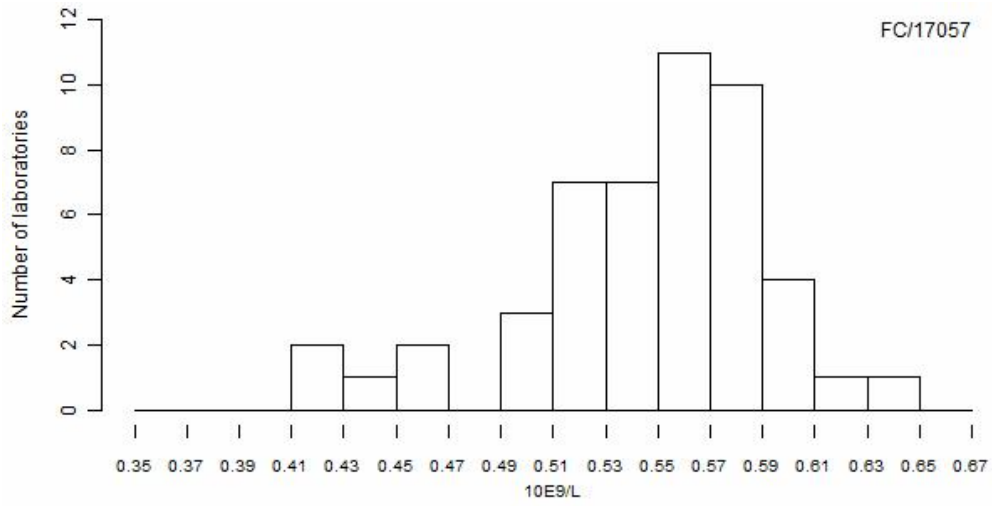
Results not represented  
on the graph

FC/17057 = 4.69 10e9/l  
 FC/17057 = 1419.5 10e  
 FC/17057 = 1471.77 10  
 FC/17058 = 4.84 10e9/l  
 FC/17058 = 1449.19 10  
 FC/17058 = 1554.5 10e  
 FC/17059 = 4.87 10e9/l  
 FC/17059 = 1517.38 10  
 FC/17059 = 1523 10e9/

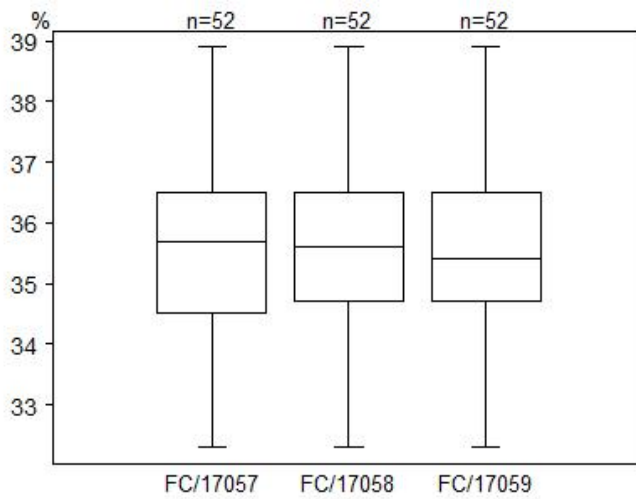
# CD4 %



# CD4 10E9/L



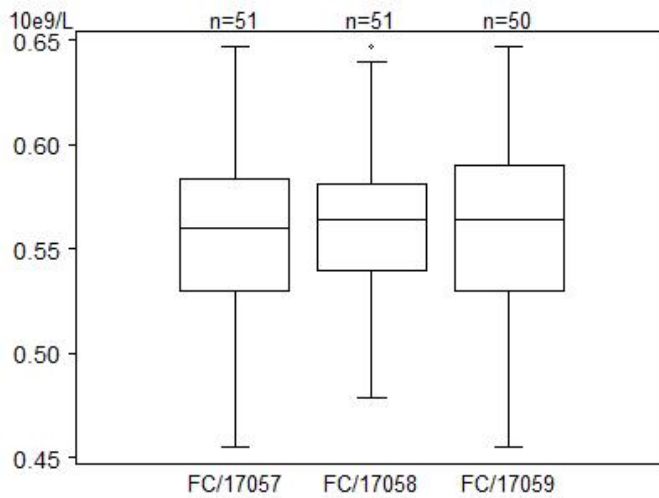
## CD4 %



Results not represented on the graph

FC/17057 = 25.9 %  
FC/17058 = 23.2 %  
FC/17058 = 31.3 %  
FC/17059 = 69 %

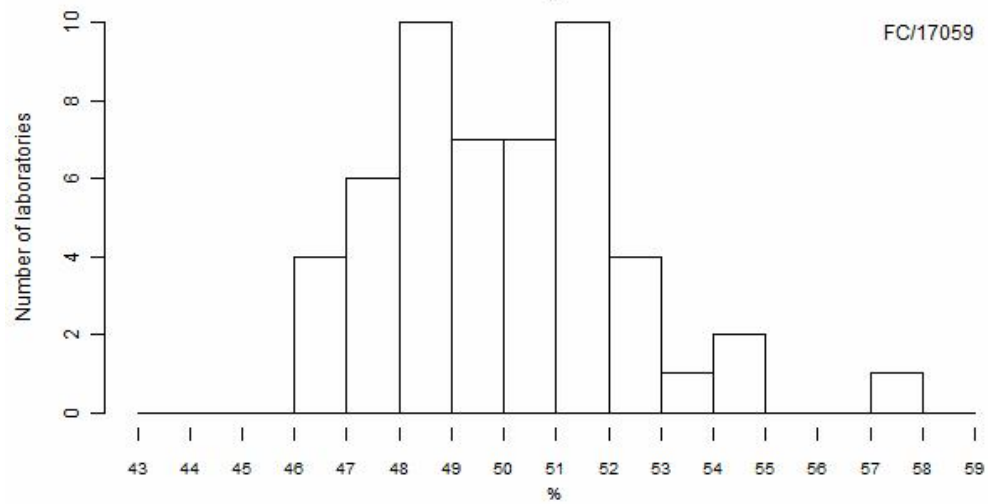
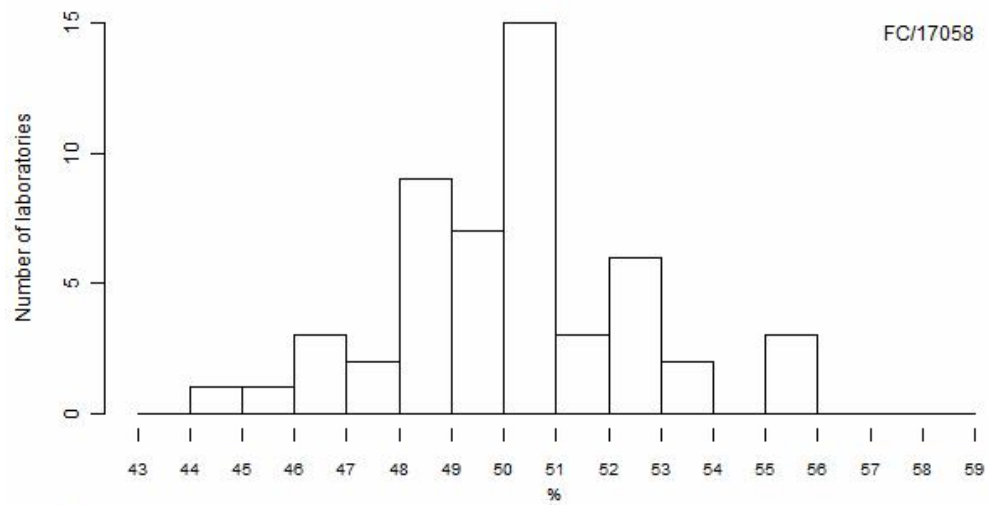
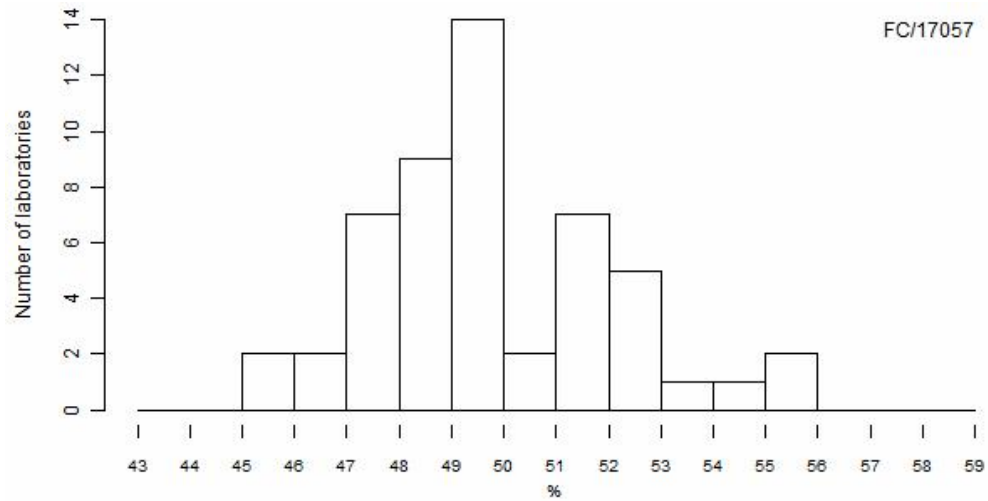
## CD4 10E9/L



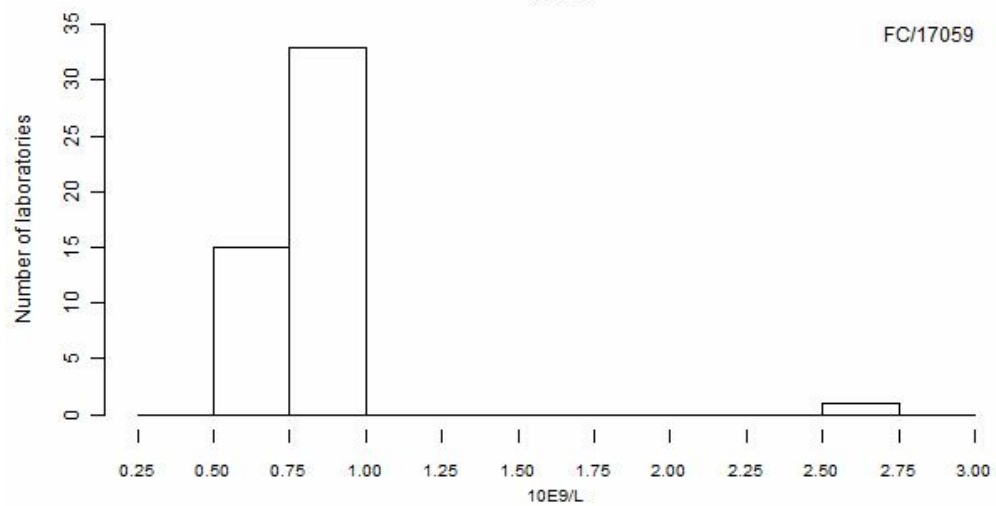
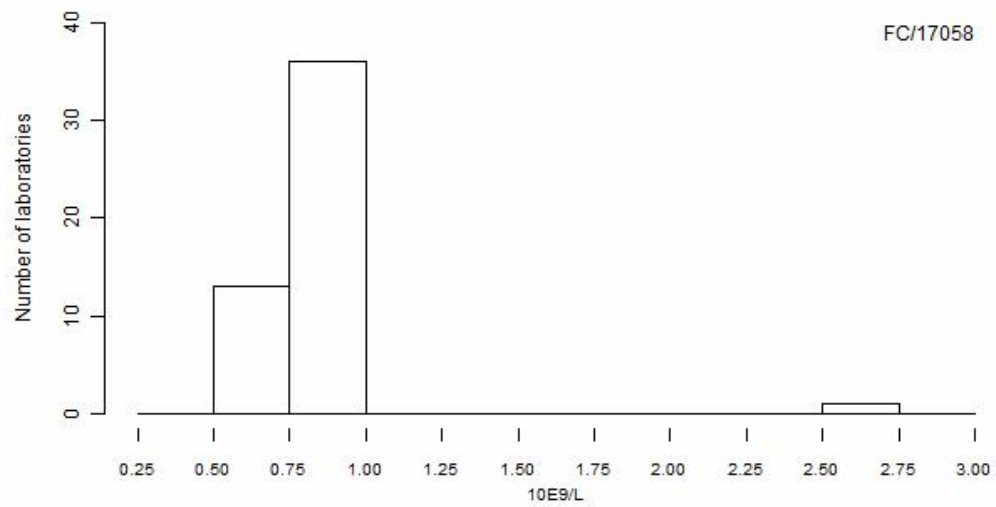
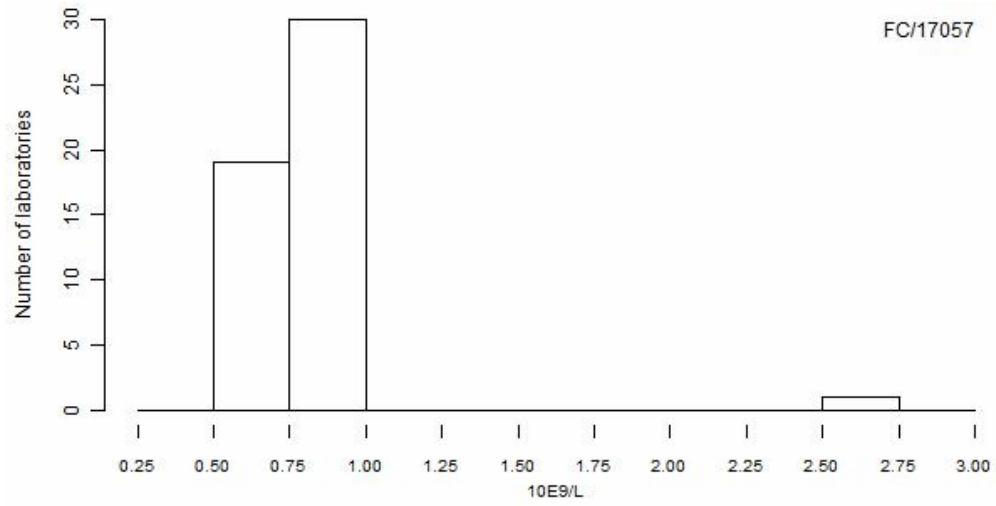
Results not represented on the graph

FC/17057 = 0.412 10e9  
FC/17057 = 0.413 10e9  
FC/17057 = 0.44 10e9/l  
FC/17057 = 1.72 10e9/l  
FC/17057 = 561.7 10e9  
FC/17057 = 605.71 10e  
FC/17058 = 0.383 10e9  
FC/17058 = 0.426 10e9  
FC/17058 = 0.43 10e9/l  
FC/17058 = 1.85 10e9/l  
FC/17058 = 592.02 10e  
FC/17058 = 599.99 10e  
FC/17059 = 1.88 10e9/l  
FC/17059 = 602.04 10e  
FC/17059 = 634.85 10e

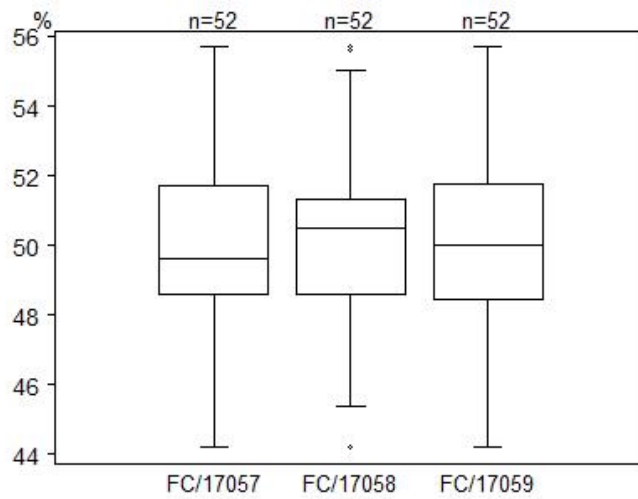
# CD8 %



# CD8 10E9/L

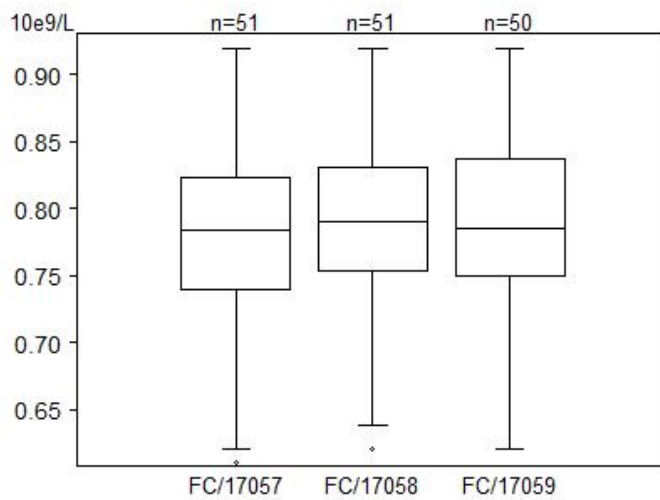


## CD8 %



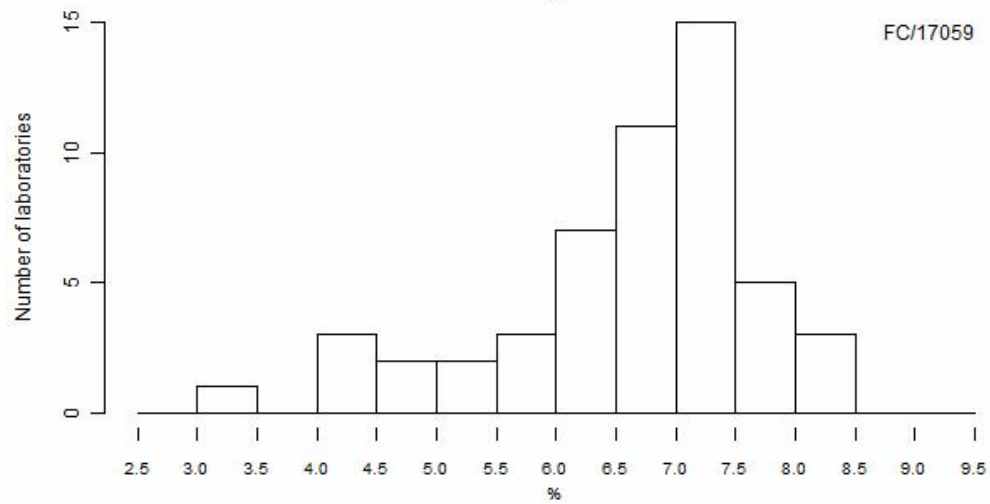
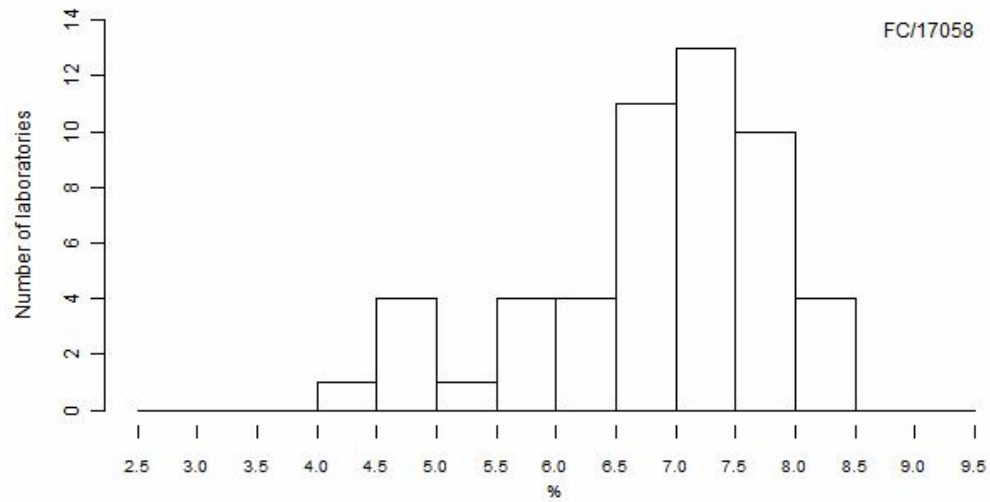
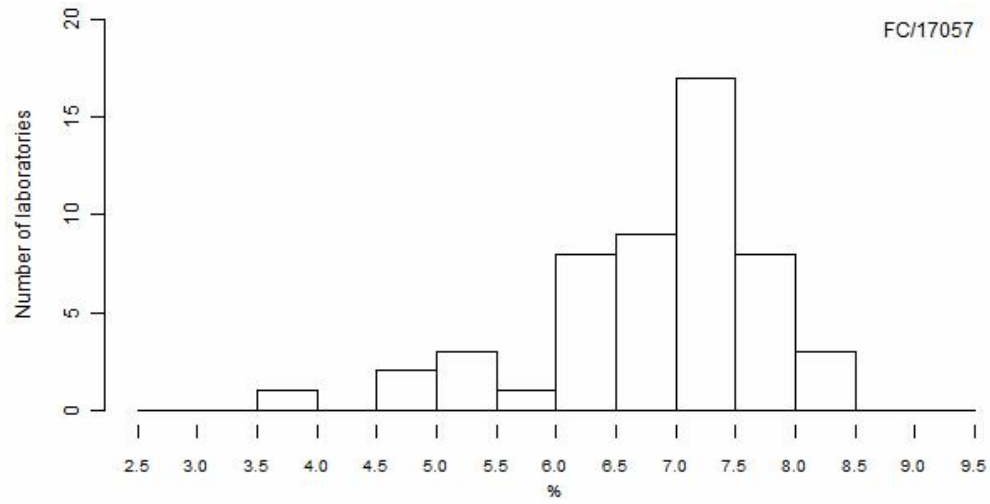
Results not represented  
on the graph  
FC/17059 = 57.5 %

## CD8 10E9/L



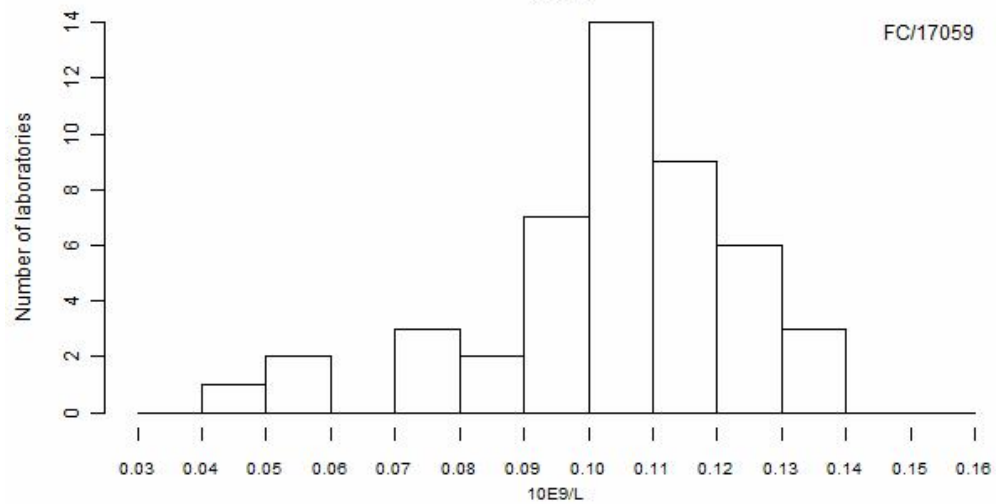
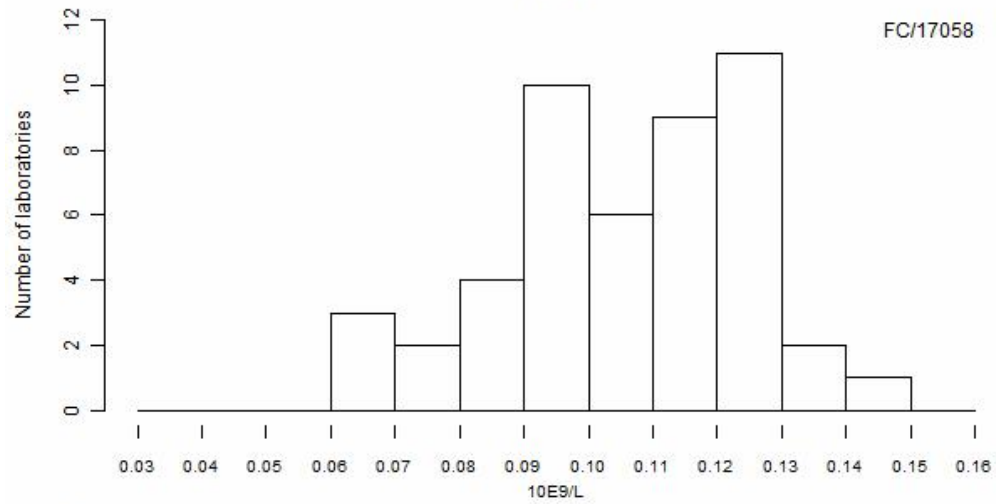
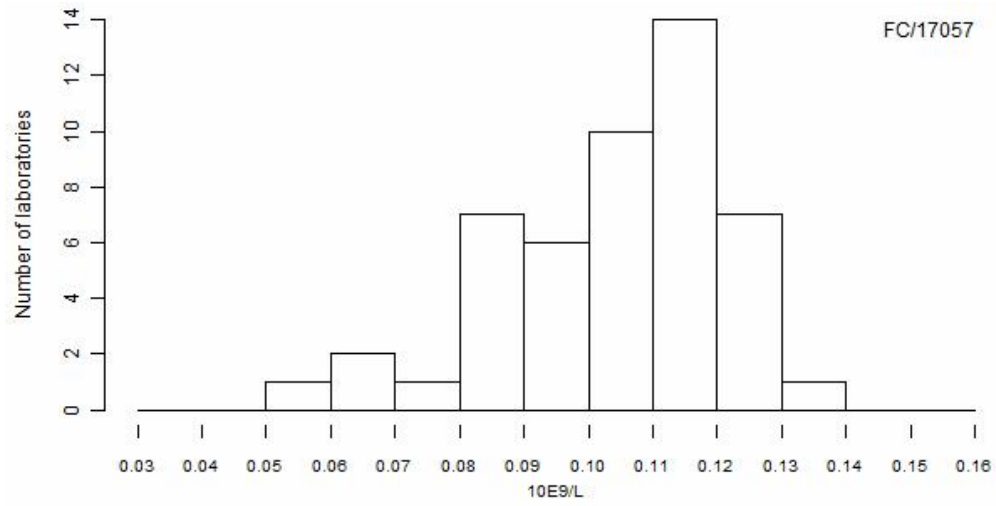
Results not represented  
on the graph  
FC/17057 = 0.591 10e9  
FC/17057 = 2.7 10e9/L  
FC/17057 = 781.5 10e9  
FC/17057 = 824.48 10e  
FC/17058 = 2.72 10e9/L  
FC/17058 = 814.93 10e  
FC/17058 = 871.6 10e9  
FC/17059 = 2.7 10e9/L  
FC/17059 = 832.14 10e  
FC/17059 = 840.17 10e

# CD19 %

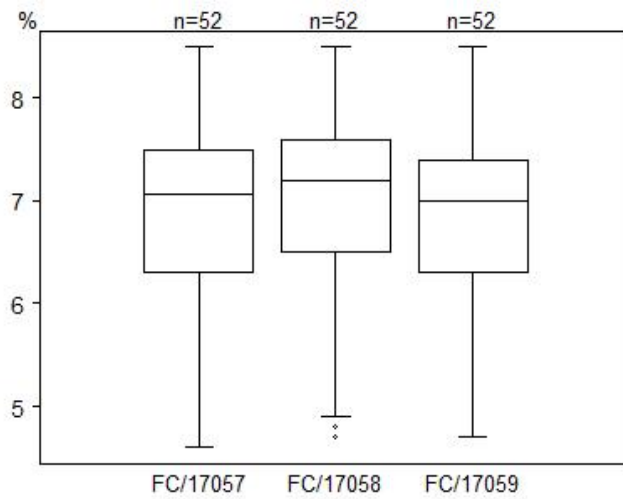




# CD19 10E9/L



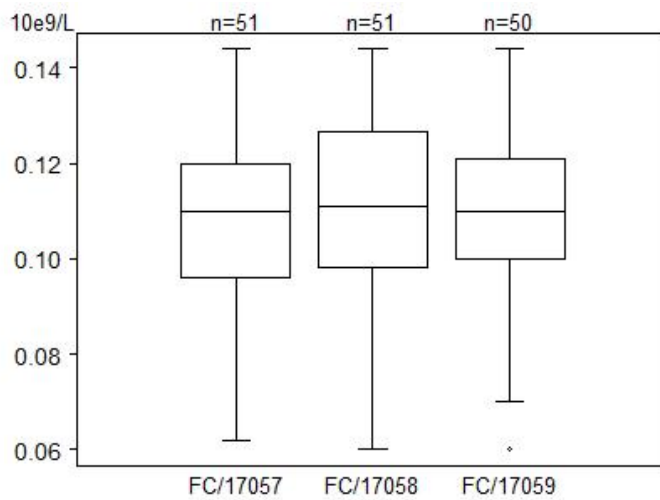
## CD19 %



Results not represented  
on the graph

FC/17057 = 3.7 %  
 FC/17058 = 4.1 %  
 FC/17059 = 3.2 %  
 FC/17059 = 4.3 %  
 FC/17059 = 4.3 %  
 FC/17059 = 4.4 %

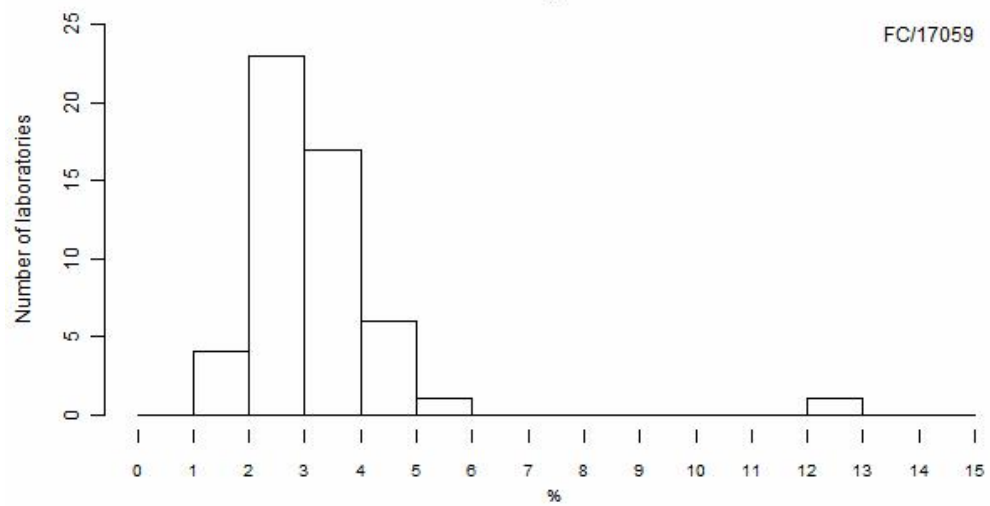
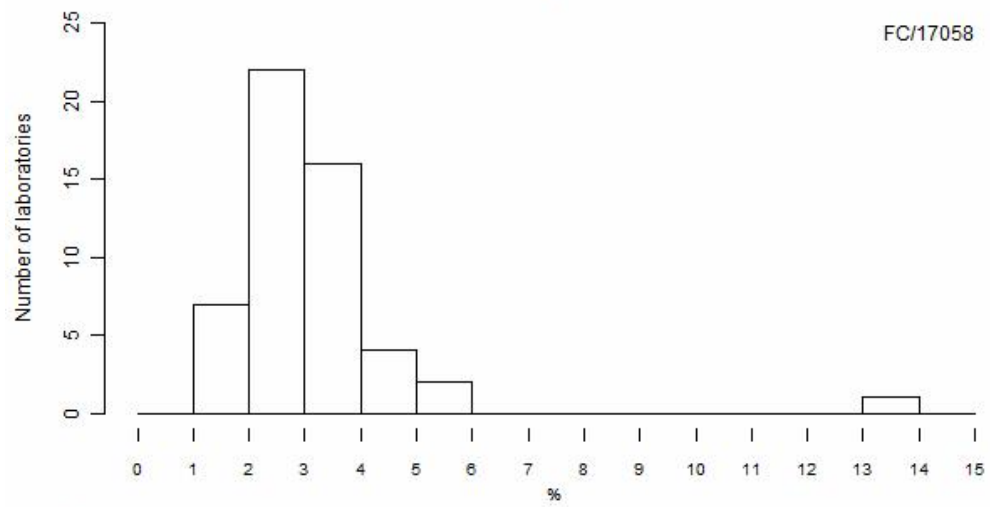
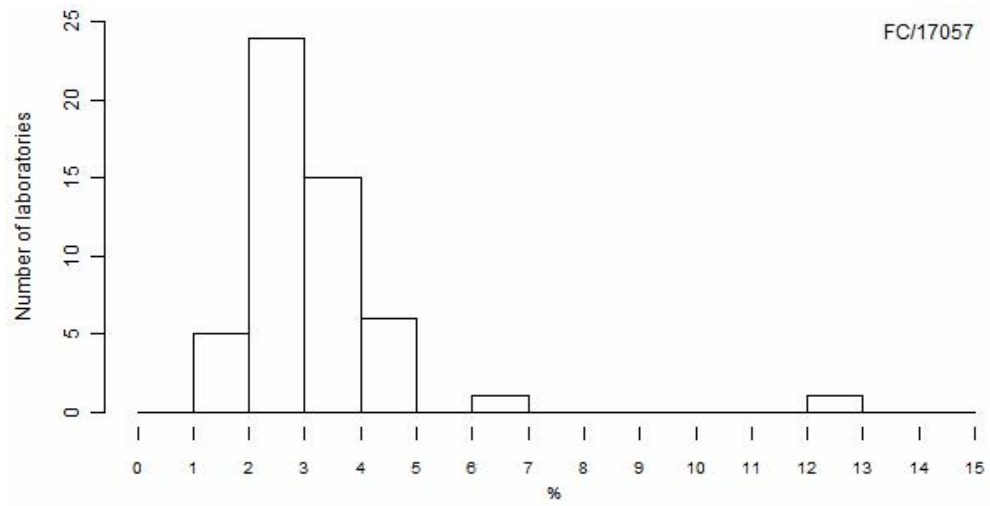
## CD19 10E9/L



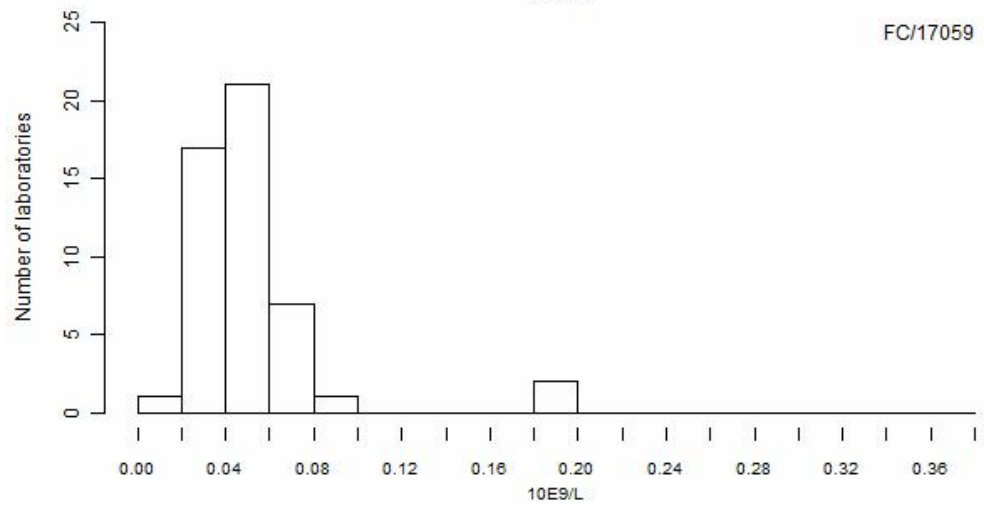
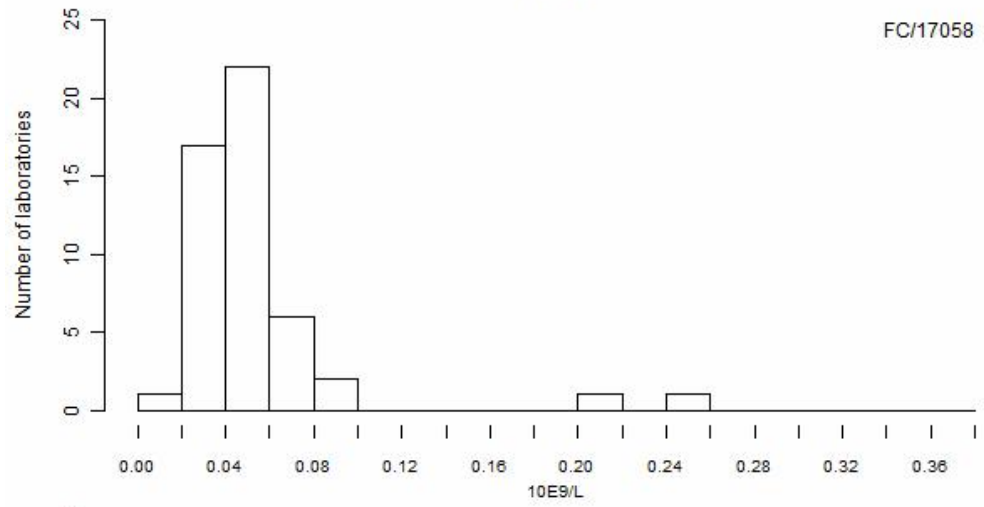
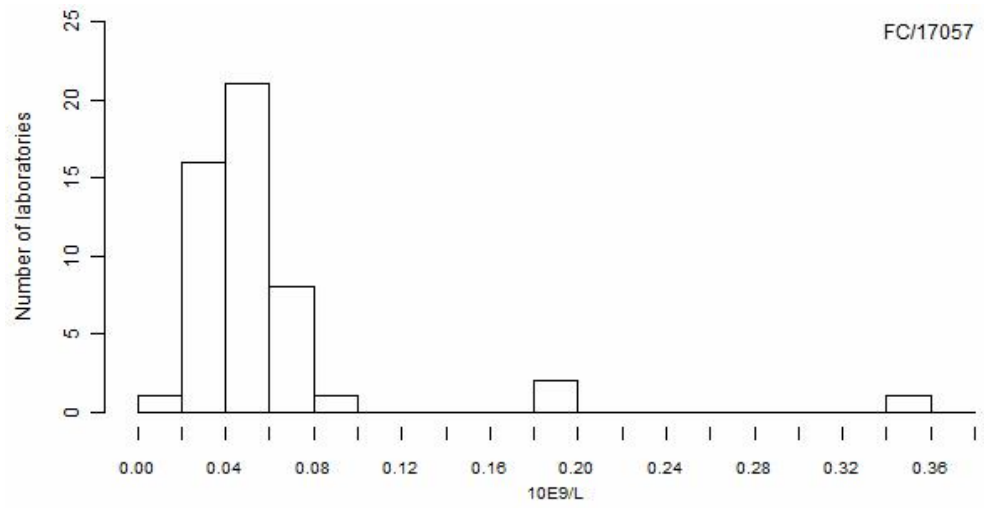
Results not represented  
on the graph

FC/17057 = 0.055 10e9  
 FC/17057 = 0.33 10e9/L  
 FC/17057 = 113 10e9/L  
 FC/17057 = 113.39 10e  
 FC/17058 = 0.36 10e9/L  
 FC/17058 = 0.76 10e9/L  
 FC/17058 = 121.44 10e  
 FC/17058 = 124.8 10e9  
 FC/17059 = 0.049 10e9  
 FC/17059 = 0.053 10e9  
 FC/17059 = 0.37 10e9/L  
 FC/17059 = 0.73 10e9/L  
 FC/17059 = 120.74 10e  
 FC/17059 = 121.2 10e9

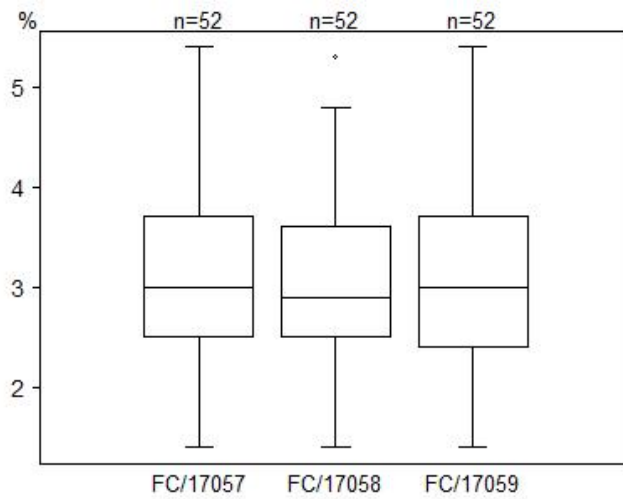
# NKcells %



# NKcells 10E9/L



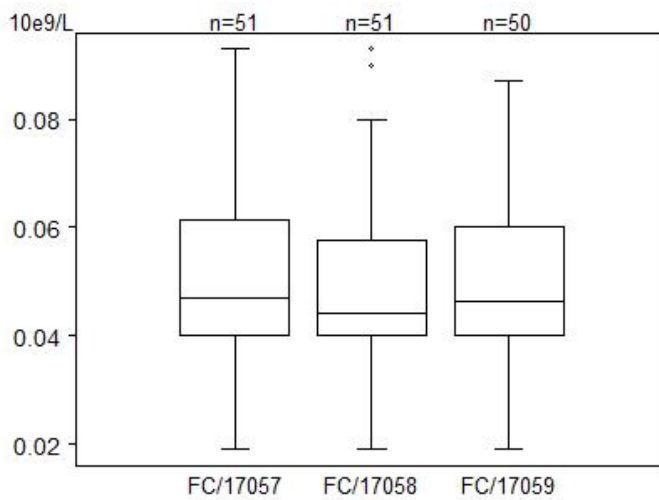
## NKcells %



Results not represented on the graph

FC/17057 = 6.1 %  
 FC/17057 = 12.7 %  
 FC/17058 = 5.9 %  
 FC/17058 = 13.6 %  
 FC/17059 = 12.8 %

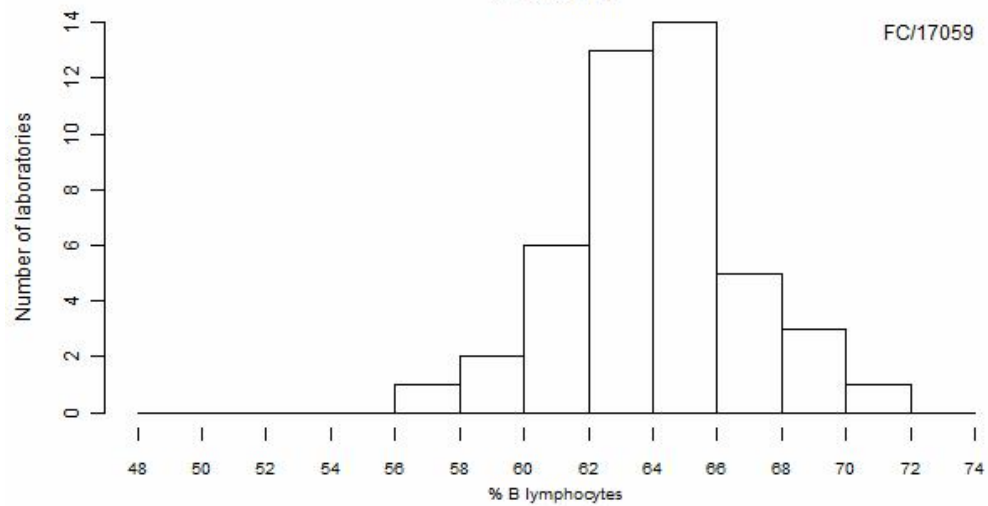
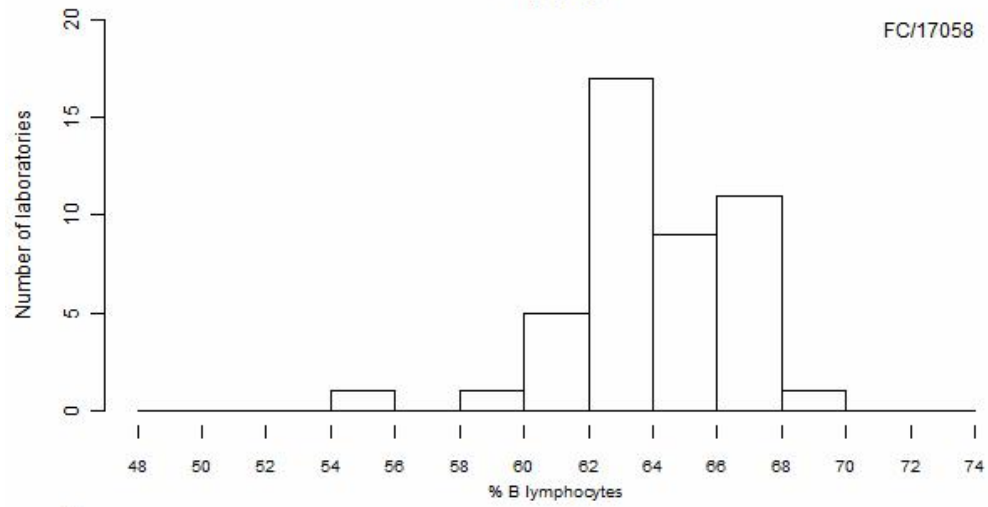
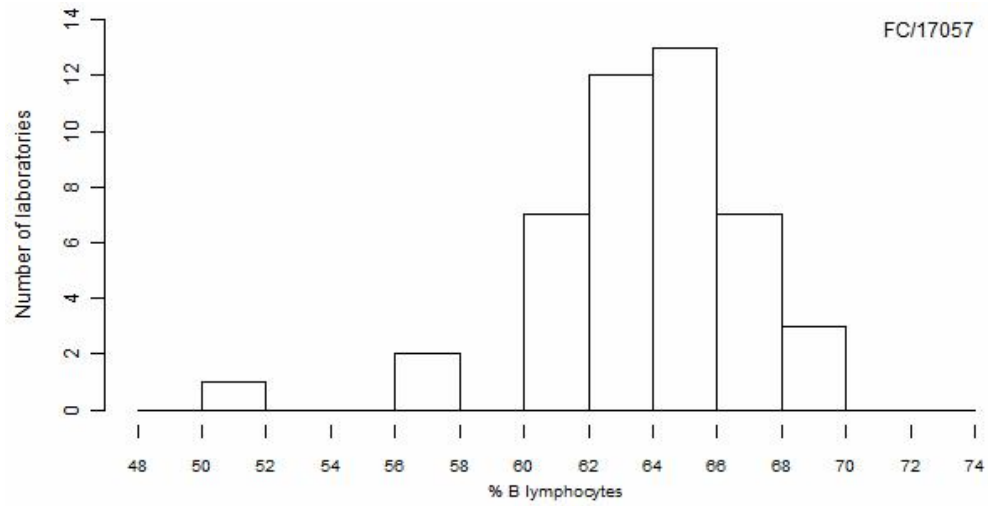
## NKcells 10E9/L



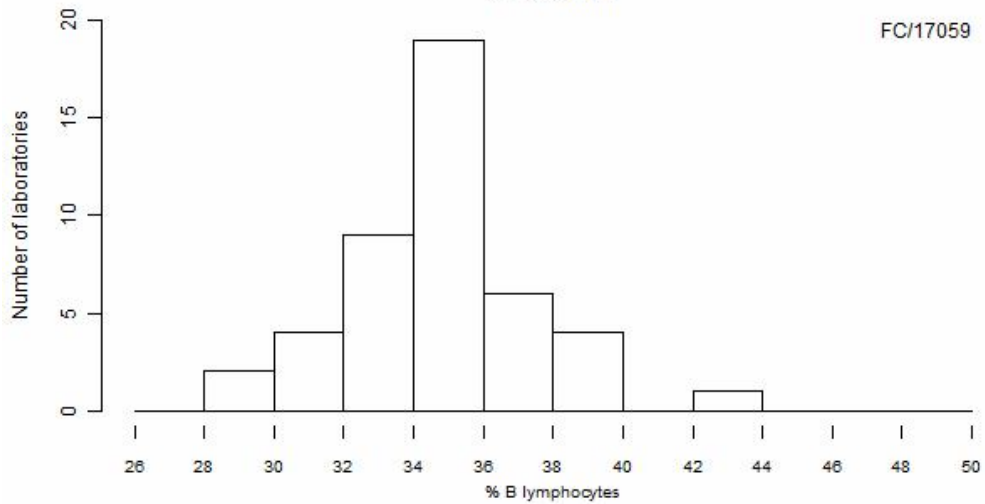
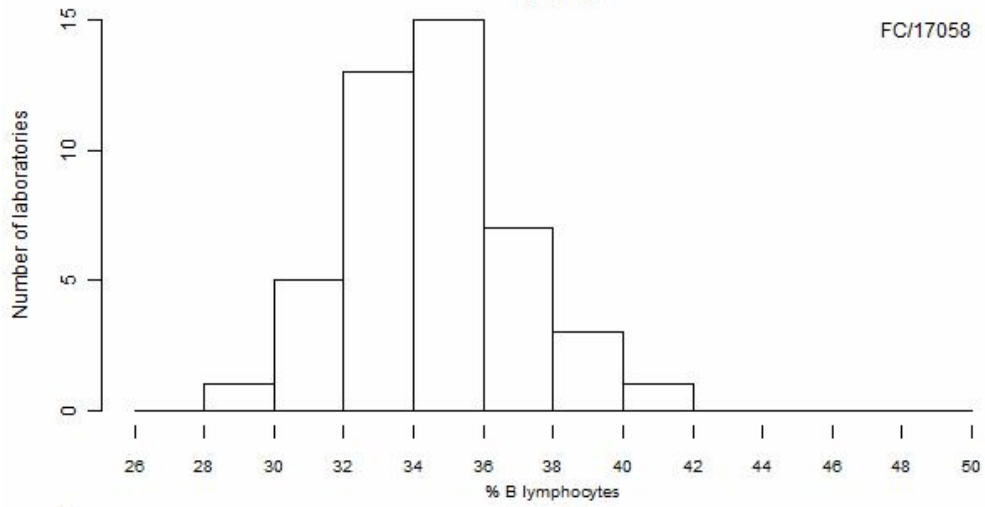
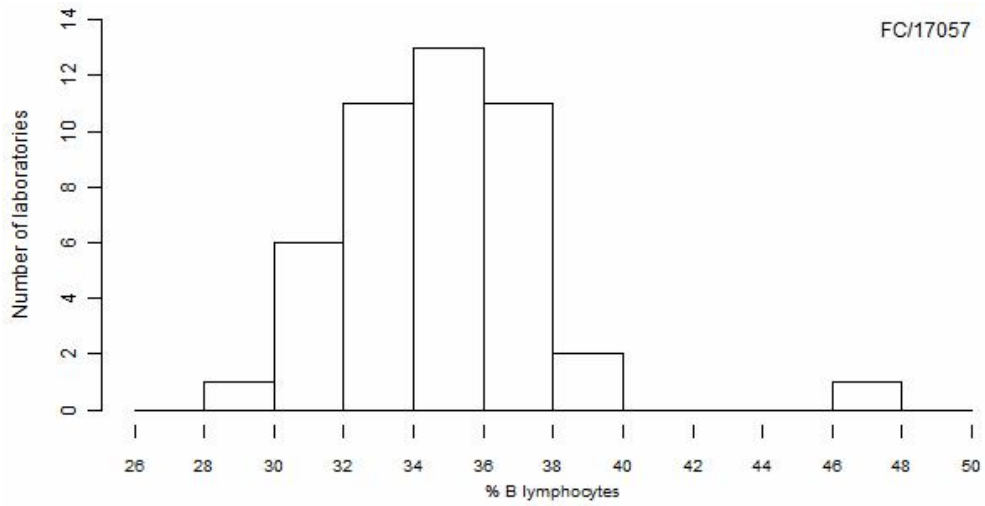
Results not represented on the graph

FC/17057 = 0.098 10e9  
 FC/17057 = 0.19 10e9/L  
 FC/17057 = 0.2 10e9/L  
 FC/17057 = 0.35 10e9/L  
 FC/17057 = 36.48 10e9  
 FC/17057 = 57.45 10e9  
 FC/17058 = 0.22 10e9/L  
 FC/17058 = 0.25 10e9/L  
 FC/17058 = 39.4 10e9/L  
 FC/17058 = 60.23 10e9  
 FC/17059 = 0.2 10e9/L  
 FC/17059 = 0.2 10e9/L  
 FC/17059 = 35.53 10e9  
 FC/17059 = 55.21 10e9

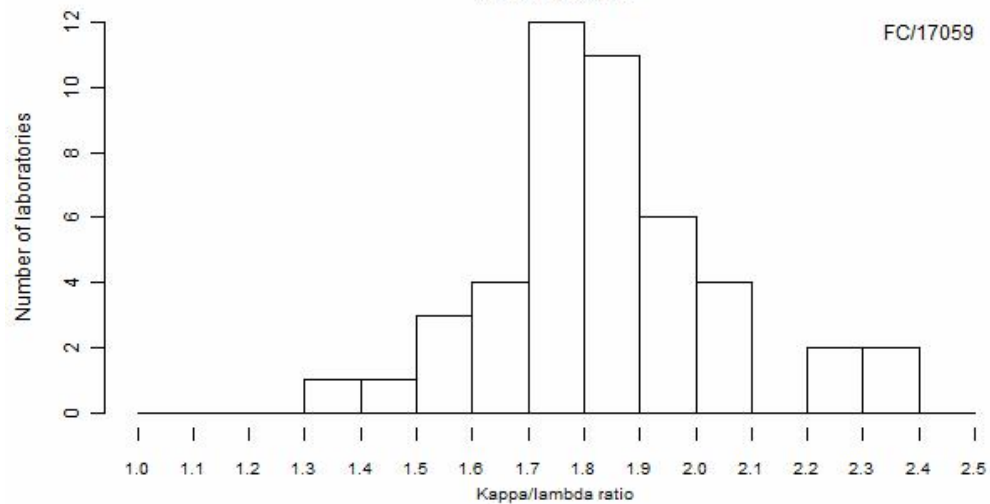
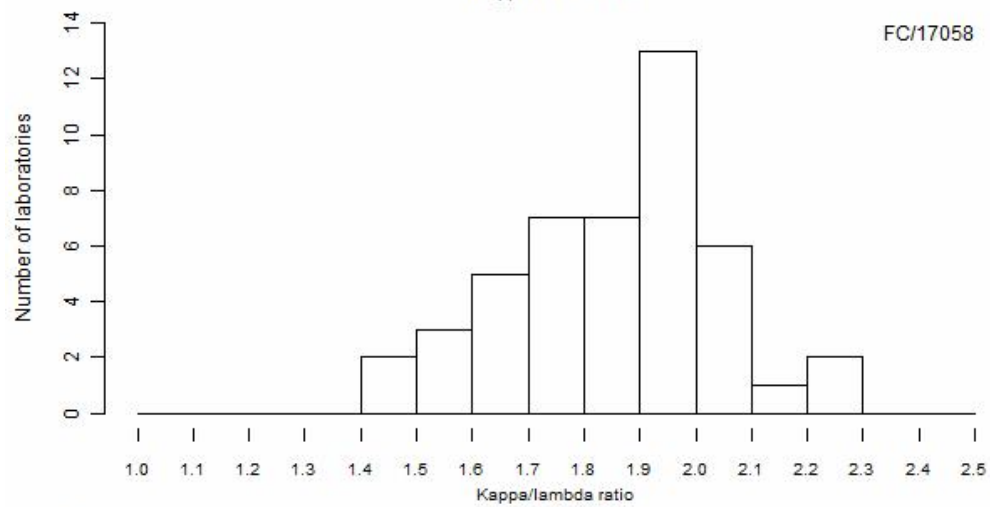
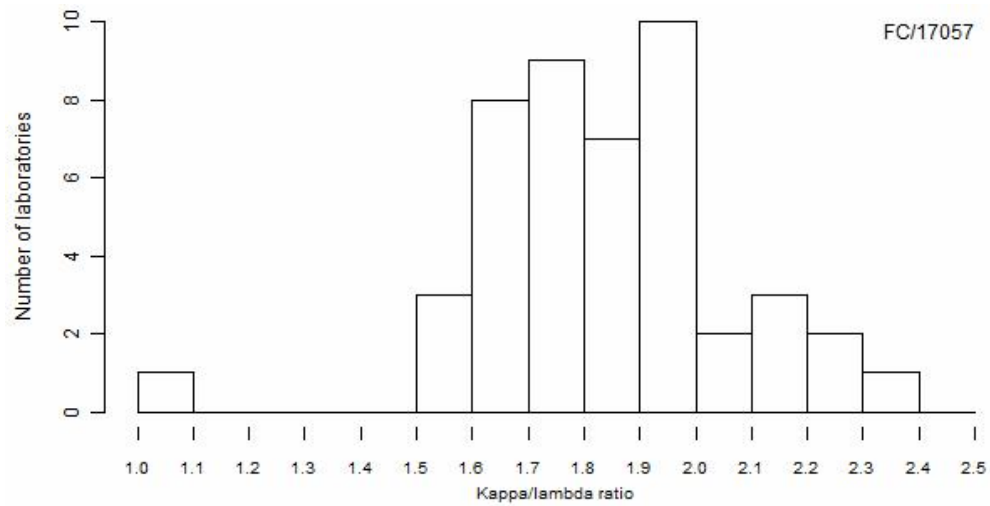
# Kappa % B lymphocytes



# Lambda % B lymphocytes

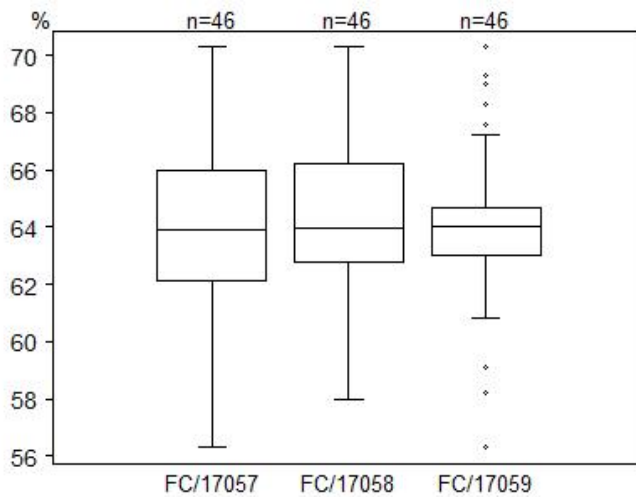


# Kappa/lambda





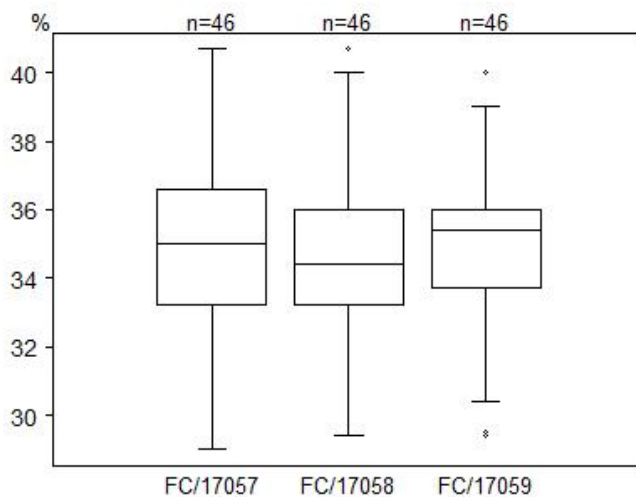
## Kappa % B lymphocytes



Results not represented on the graph

FC/17057 = 0.6 %  
 FC/17057 = 50.6 %  
 FC/17058 = 0.6 %  
 FC/17058 = 54.9 %  
 FC/17059 = 0.6 %

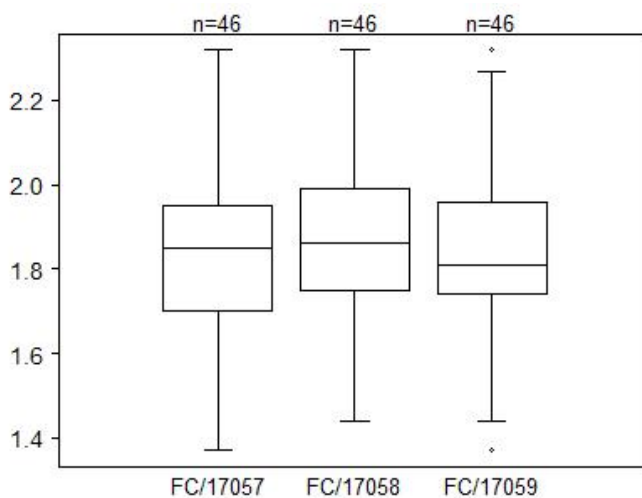
## Lambda % B lymphocytes



Results not represented on the graph

FC/17057 = 0.3 %  
 FC/17057 = 46.2 %  
 FC/17058 = 0.3 %  
 FC/17059 = 0.3 %  
 FC/17059 = 43.2 %

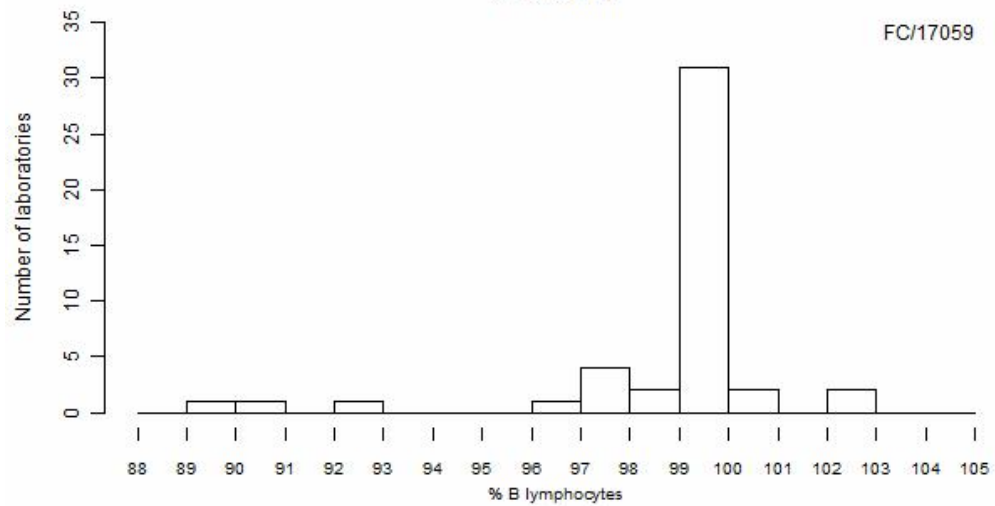
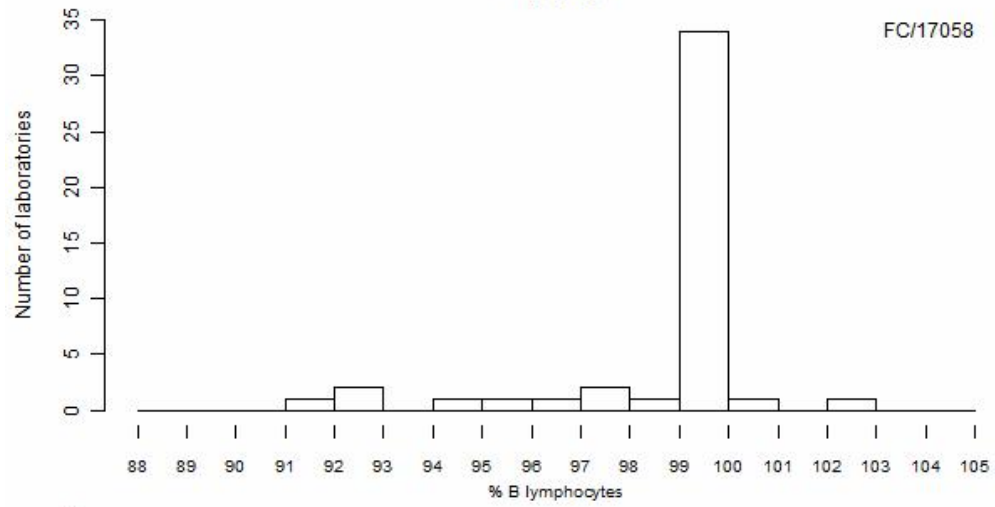
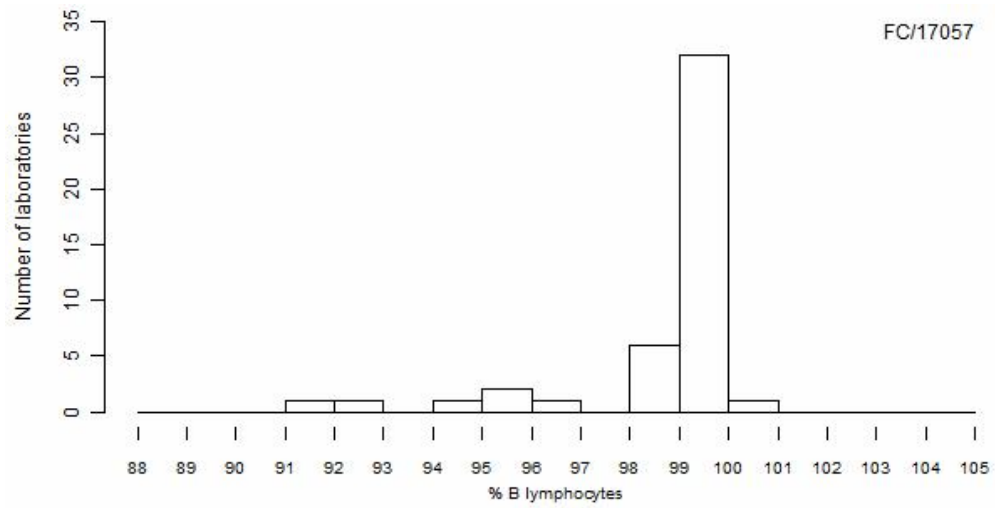
## Kappa/lambda



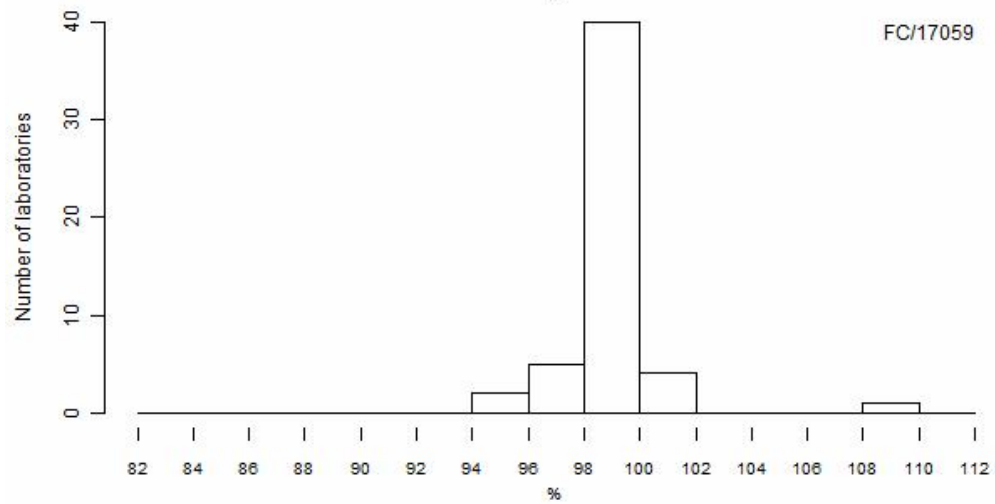
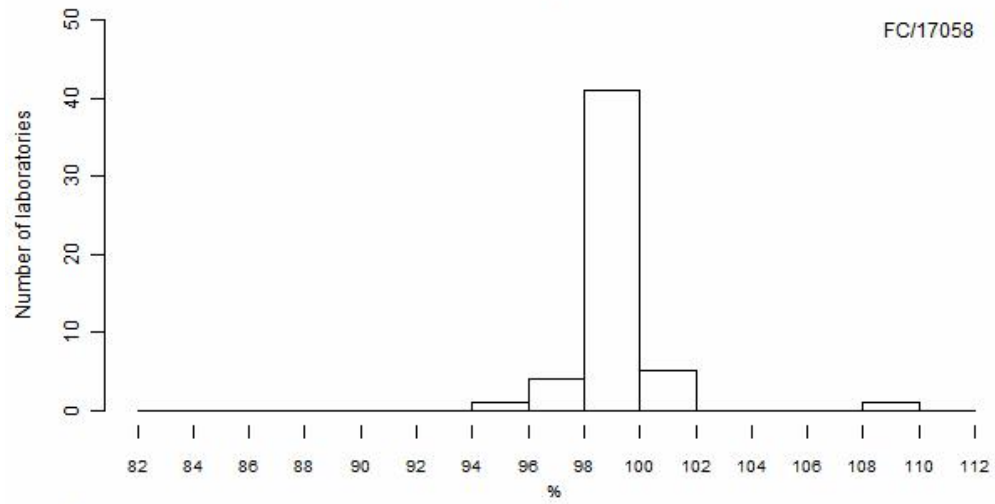
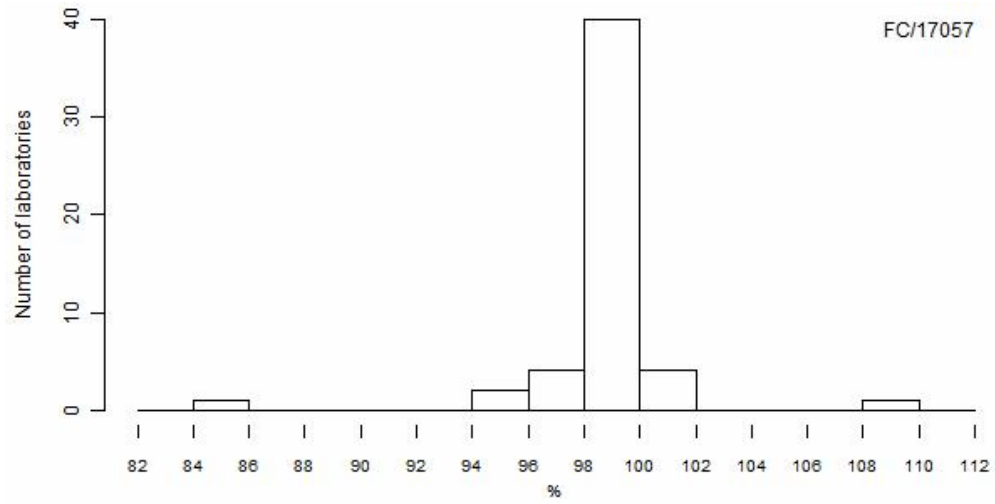
Results not represented on the graph

FC/17057 = 1.1  
 FC/17057 = 2.38  
 FC/17059 = 2.39

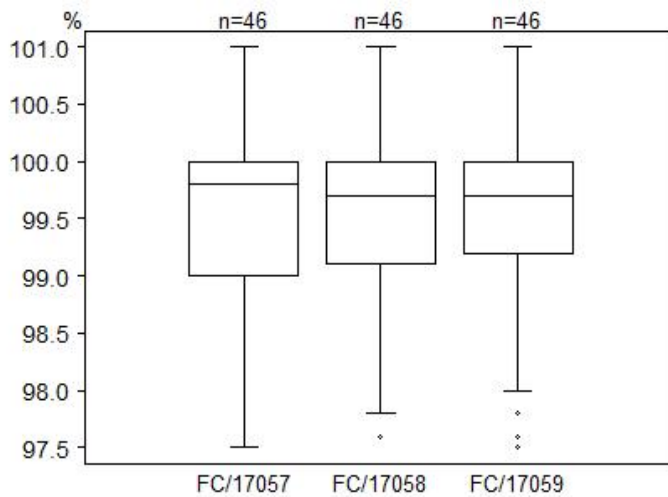
## Sum K+L % B lymphocytes



# Lymphosum %



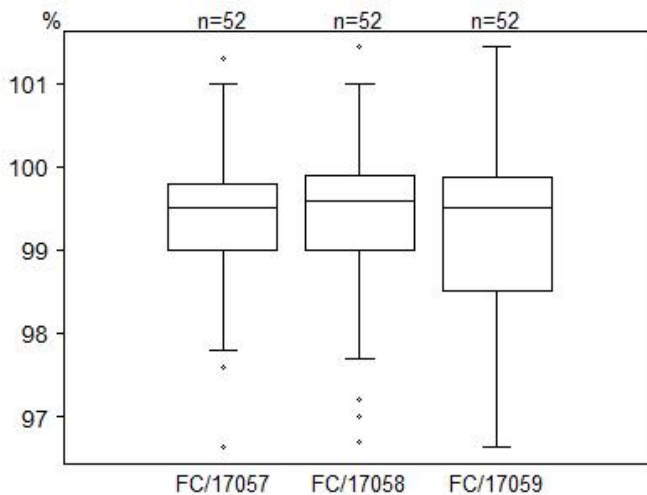
## Sum K+L % B lymphocytes



Results not represented on the graph

FC/17057 = 0.9 %  
 FC/17057 = 91.4 %  
 FC/17057 = 92.4 %  
 FC/17057 = 94.4 %  
 FC/17057 = 95.7 %  
 FC/17057 = 96 %  
 FC/17057 = 96.8 %  
 FC/17058 = 0.9 %  
 FC/17058 = 91.5 %  
 FC/17058 = 92.2 %  
 FC/17058 = 92.5 %  
 FC/17058 = 95 %  
 FC/17058 = 95.5 %  
 FC/17058 = 96.9 %  
 FC/17058 = 103 %  
 FC/17059 = 0.9 %  
 FC/17059 = 89.9 %

## Lymphosum %

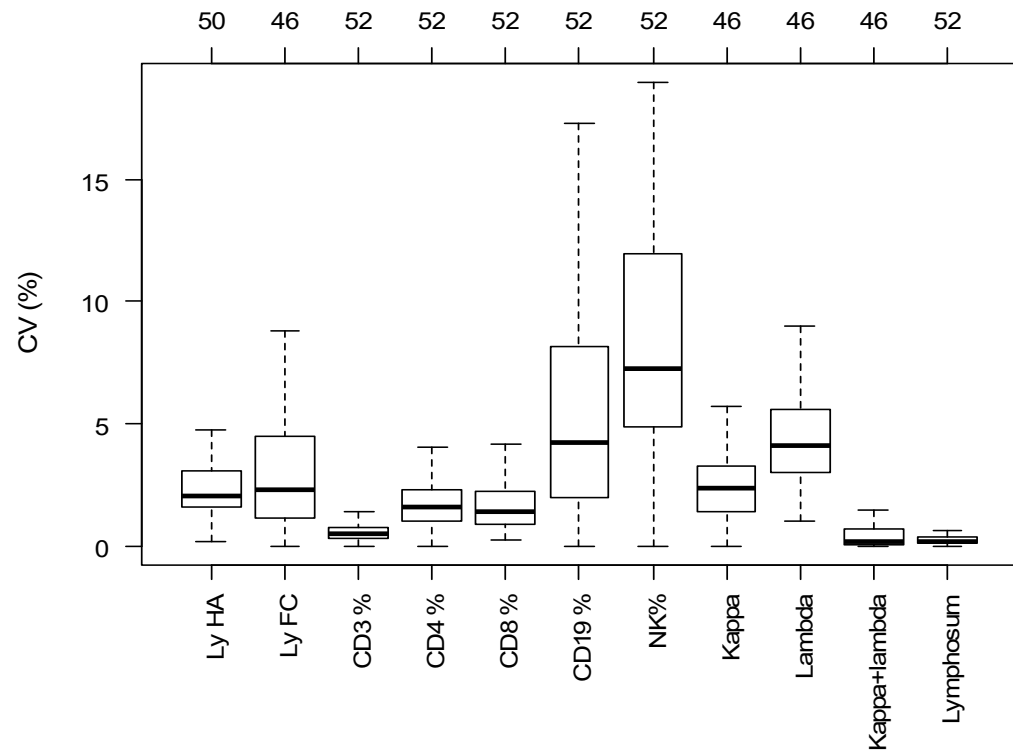
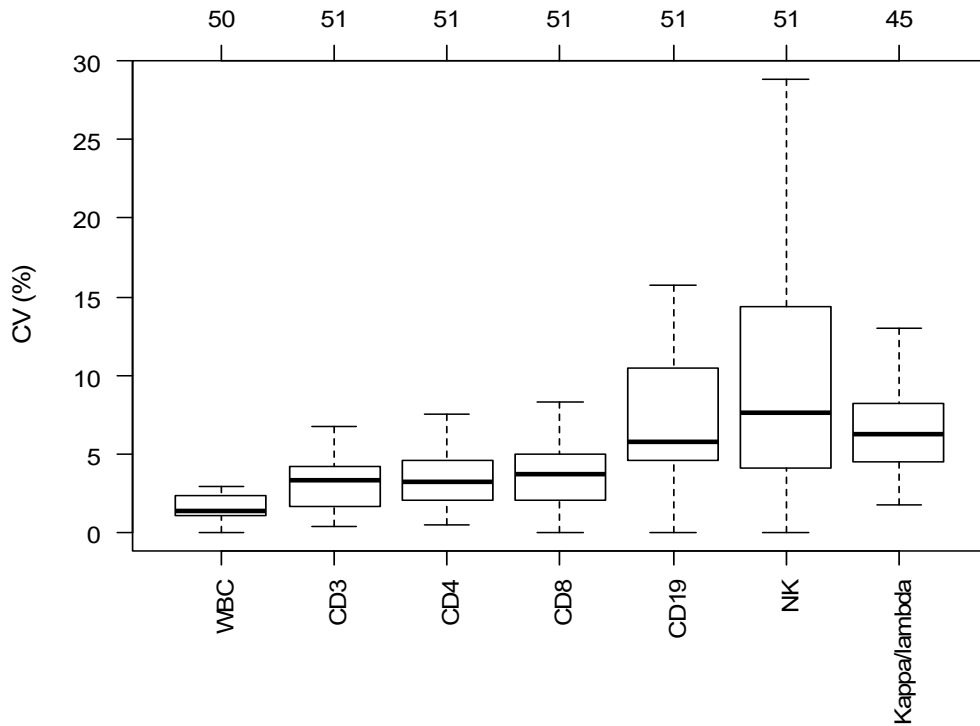


Results not represented on the graph

FC/17057 = 84.5 %  
 FC/17057 = 95.1 %  
 FC/17057 = 95.3 %  
 FC/17057 = 109.1 %  
 FC/17058 = 95.2 %  
 FC/17058 = 109.4 %  
 FC/17059 = 94.6 %  
 FC/17059 = 95.6 %  
 FC/17059 = 96.1 %  
 FC/17059 = 96.3 %  
 FC/17059 = 108.1 %

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<sup>+</sup>CD16<sup>+</sup> and/or CD56<sup>+</sup>%) 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/17057, FC/17058 and FC/17059). The intra-laboratory variability is estimated by the coefficient of variation of the three results calculated for each laboratory and each parameter.



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**END**

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