BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
COMMITTEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

DEFINITIVE GLOBAL REPORT

FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS

SURVEY 2022/2

COMMITTEE OF EXPERTS

Sciensano					
Secretariat		PHONE:	02/642.55.22	FAX:	02/642.56.45
		e-mail:	QL_secretariat@sciensano.be		
Dr. Bouacida L.	Scheme coordinator	PHONE:	02/642.53.83		
		e-mail:	lobna.bouacida@sciensano.be		
Dr. Vernelen K.	Alternate coordinator	PHONE:	02/642.55.29		
		e-mail:	kris.vernelen@sciensano.be		
Experts	Institute				
Dr. Bossuyt X.	UZ Leuven				
Dr. Chatelain B.	UCL Louvain				
Dr. Gothot A.	ULG Liège				
Dr. Heylen E.	ZNA Antwerpen				
Dr. Kornreich A.	Grand Hopital de Charleroi				
Dr. Mullier F.	UCL Louvain				
Dr. Nguyen P.	LHUB-ULB				
Dr. Philippe J.	UZ Gent				
Dr. Saussoy P.	UCL Cliniques St-Luc Bruxelles				

A preliminary version of this report was submitted to the experts EQA Flow Cytometry on: 16/06/2022
This report was discussed at the meeting of the committee of experts EQA Flow Cytometry on: 21/06/2022

Authorization of the report: by Lobna Bouacida, scheme coordinator

Date of publication: 23/06/2022

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 the brochures available on our website (only in Dutch and French):

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

→ select in the proposed menu :

ALGEMENE INFORMATIEBROCHURE EKE

→ select in the proposed menu "Brochures":

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

- Statistische methoden gebruikt voor EKE
- Verwerking van gecensureerde waarden

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

→ select in the proposed menu :

BROCHURE D'INFORMATION GENERALE EEQ

→ select in the proposed menu "Brochures":

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

- Méthodes statistiques appliquées à l'EEQ
- Traitement des valeurs censurées

Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 6 participants:

- a rectangle ranging from percentile 25 (P_{25}) to percentile 75 (P_{75})
- a central line representing the median of the results (P_{50})
- a lower limit showing the smallest value $x > P_{25} - 1.5 * (P_{75} - P_{25})$
- an upper limit representing the largest value $x < P_{75} + 1.5 * (P_{75} - P_{25})$
- all points outside this interval are represented by a dot.
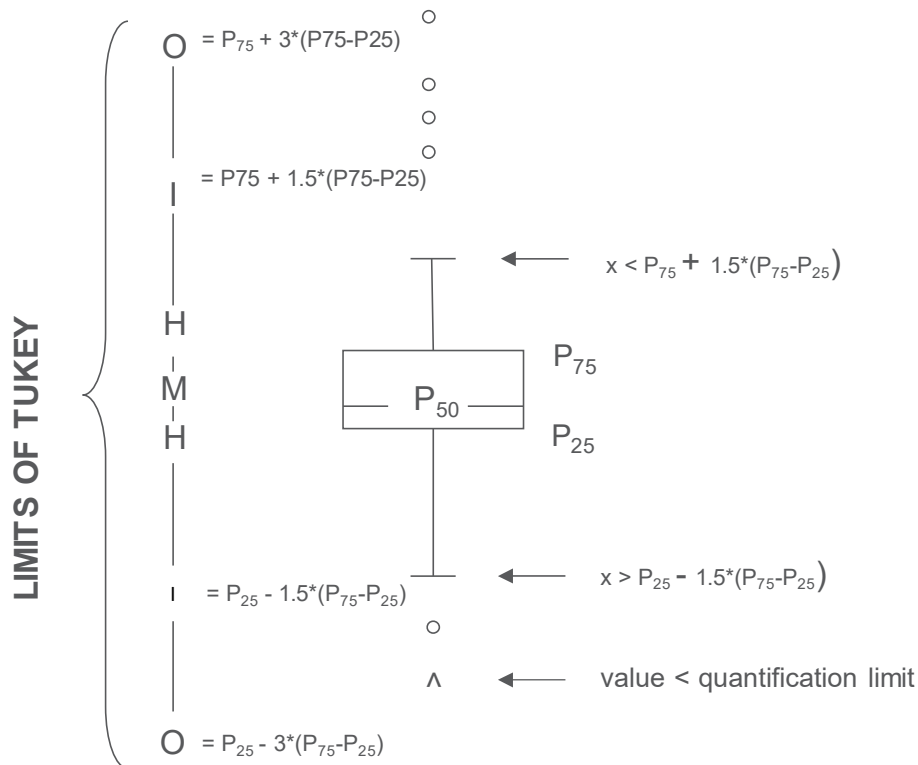
Corresponding limits in case of normal distribution

SAMPLE MATERIAL

Two blood samples (FC/19104 and FC/19105) collected on K2EDTA were sent to the laboratories.

Sample FC/19104 was collected from a healthy and voluntary blood donor, whereas sample FC/19105 was collected from a patient and was kindly provided by Dr Christian Chatelain (CHR Val de Sambre).

The two samples were distributed into aliquots at Sciensano and sent on the day of blood collection by Taxipost 24h. The laboratories were informed by e-mail of the send out of the control material (day 0).

Homogeneity was confirmed based on white blood cells determination.

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

FC19104

	%	10^9/L
Leukocytes		8.3
Lymphocytes	24.4	
CD3$^+$ cells	77.1	1.56
CD4$^+$CD3$^+$ cells	42.4	0.86
CD8$^+$CD3$^+$ cells	23.4	0.47
CD19$^+$ cells	12.0	0.24
NK cells	10.2	0.21
κ % B lymphocytes	64.2	
λ % B lymphocytes	35.8	
κ/λ ratio	1.79	

FC19105

	%	10^9/L
Leukocytes		35.0
Lymphocytes	87.9	
CD3$^+$ cells	8.5	2.62
CD4$^+$CD3$^+$ cells	5.3	1.63
CD8$^+$CD3$^+$ cells	3.4	1.05
CD19$^+$ cells	89.9	27.66
NK cells	1.0	0.31
κ % B lymphocytes	100	
λ % B lymphocytes	0	
κ/λ ratio		

PARTICIPATION

Fifty-one Belgian clinical laboratories participated in the survey 2022/2 (send-out of blood samples on 2nd May, 2022 (day 0)).

RESULTS

98% of the Belgian laboratories received the samples on day 1 or 2. 43 laboratories (84%) received the samples on day 1 and seven (14%) received them on day 2. One laboratory received the samples on day 3.

78% (n=40) of the Belgian laboratories performed the analyses on day 1, 14% (n=7) on day 2 and 8% (n=4) on day 3.

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

Statistics for the evaluation are solely based on the results of the Belgian clinical laboratories (n=51). Statistics for the evaluation of the WBC count, the percentage of lymphocytes by haematology analyser as well as the absolute counts for the different lymphocyte subsets are solely based on the results of the Belgian clinical laboratories that performed the analyses on day 1 or 2 (n=47).

The following tables show the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/19104 and FC/19105:

FC/19104	Median	SD	CV,%	N
WBC 10E9/L	8.41	0.19	2.3	46
Lympho% haematology analyser	23.7	1.0	4.4	45
Lympho% flow cytometer	22.3	2.2	10.0	45
CD3 %	78.1	1.8	2.3	51
CD3 10E9/L	1.577	0.075	4.7	47
CD4 %	41.8	1.6	3.7	51
CD4 10E9/L	0.845	0.054	6.4	47
CD8 %	24.5	1.4	5.6	51
CD8 10E9/L	0.493	0.045	9.2	47
CD19 %	10.0	1.1	11.5	51
CD19 10E9/L	0.202	0.030	14.7	47
NKcells %	10.3	1.3	12.6	51
NKcells 10E9/L	0.206	0.030	14.4	47
Kappa % B lymphocytes	63.2	2.8	4.4	43
Lambda % B lymphocytes	35.5	2.3	6.4	43
Kappa/lambda	1.77	0.18	10.2	43
Sum K+L % B lymphocytes	99.7	1.1	1.1	43
Lymphosum %	99.3	1.1	1.1	51
Other lymphocytes 10E9/L	0.244	0.204	83.7	6

FC/19105	Median	SD	CV,%	N
WBC 10E9/L	37.22	0.81	2.2	44
Lympho% haematology analyser	84.6	3.2	3.8	42
Lympho% flow cytometer	85.6	2.5	2.9	45
CD3 %	7.4	1.0	13.5	51
CD3 10E9/L	2.395	0.333	13.9	45
CD4 %	4.1	0.6	14.4	51
CD4 10E9/L	1.327	0.229	17.3	45
CD8 %	3.0	0.5	16.0	51
CD8 10E9/L	0.942	0.156	16.6	45
CD19 %	91.0	1.8	2.0	51
CD19 10E9/L	28.823	1.758	6.1	43
NKcells %	0.8	0.3	37.0	51
NKcells 10E9/L	0.236	0.079	33.6	45
Kappa % B lymphocytes	99.8	1.7	1.7	43
Lambda % B lymphocytes	0.1	0.1	74.0	43
Kappa/lambda	958.50	498.52	52.0	40
Sum K+L % B lymphocytes	100.0	1.5	1.5	43
Lymphosum %	99.5	0.4	0.4	51
Other lymphocytes 10E9/L	29.427	15.555	52.9	11

FC/19104

Six laboratories reported that the percentage of CD3+/CD4-/CD8- cells was high in sample FC/19104. Two of these laboratories gave percentages of 12.6% and 13% for the CD3+/CD4-/CD8- cells. The median value for this double-negative population was 0.244 x10^9/L (CV= 83.7%, n=6).

FC/19105

For sample FC/19105, 21 laboratories (41%) reported the detection of a monoclonal population. Five laboratories regretted that the quantity of sample was insufficient to carry out the analysis.

The median value for this population was 29.427 x10^9/L (CV= 52.9%, n=11). The percentage of this population has been estimated at 90 to 100% of all lymphocytes.

Eight laboratories responded to the "other lymphocytes" phenotype :

	Phenotype
1	CD19, CD20f, CD5c,Kf
2	CD19+ CD20+ weak Kappa+ weak (LLC-like)
3	CD19+ CD20dim CD5+
4	CD19+CD5+
5	CD5, CD19, CD20 (Weak), CD23, CD43, CD45, CD200, K
6	cd5+ cd10- cd19+ cd20weak cd38- ccd45+
7	CD5+/-,CD20w, CD22w, CD23+, CD200+, CD38-, CD10-,
8	CD19, CD20f, CD5c,Kf

Below are the detailed comments from responding laboratories.

	Comment
1	Large population of CD19+ (B-cells) that express the light chain Kappa weakly
2	Probably monoclonal population which is CD19+CD5+ quantified at **90.1%** of all lymphocytes.
3	Kappa monoclonal CD5+ B cell population
4	The B-cell population is pathological (monoclonal) with the following phenotype: CD5+/CD10-/CD20 weak+ and smIg Kappa weak+ (26,354 10E9/L). Peripheral infiltration by a mature malignancy of B-cell origin, probably of type Chronic Lymphocytic Leukaemia (to be confirmed through further phenotyping, determination of the Catovsky score by additional determination of CD23, FMC7 and CD79b).
5	CD19 10^9/L : 28.173
6	B monoclonal hyperlymphocytosis with low kappa expression equivalent to **91%** of lymphocytes, i.e. 28394/µL. These cells are of phenotype : CD19+ CD5+ CD10neg CD11cneg CD20+dim CD22+dim CD23+ CD38neg CD43+ CD79bneg CD81+dim CD103neg CD200+.
7	The B-Ly are **99.9%** clonal Kappa with weak expression.
8	B cells are CD5+, suggestive of CLL. To further explore.
9	Kappa clonal B cell phenotype: B-CLL (26.6 10E^9/L)
10	Sample FC/19105 contains a B-NHL probably type CLL.
11	Reduced Ig fluorescence. Immunophenotyping in support of B-CLL.
12	Excess of B lymphocytes expressing an isotypic restriction of Kappa type (weak) as well as CD5, CD20 and CD22 weak. Stronger expression of CD200. The phenotype is consistent with chronic lymphoid leukaemia.
13	CLL phenotype
14	B-NHL : CLL
15	B lymphomatous population with a low CD19+ CD20+ low Kappa+ phenotype which is consistent with chronic lymphocytic leukaemia. This tumour entity is quantified at **93.1%** of lymphocytes (29.292 cells/µl).
16	absolute CD19 value: 27260/µL Kappa: low expression

17	Complete phenotype: Positive antigens: CD5, CD19, CD20 (Weak), CD23, CD43, CD45, CD200, Kappa (Weak); Negative antigens: CD10, CD38, CD79b, CD103, Lambda
18	Lymphoproliferative syndrome consistent with chronic lymphocytic leukaemia. Lymphocytosis subject to change. Absolute values subject to change.
19	Monoclonal B-cell lymphocytosis with low Kappa expression at 31738/µL and the following surface markers: low CD45+, CD19+, low CD20+, CD5+, CD38-. Conclusion: phenotype consistent with CLL at 31738/µL. Consider additional immunophenotyping if pathology not described in patient history.
20	**100%** pathological B-cells with kappa restriction.
21	Weak Kappa monoclonal B-cell population, CD20dim, CD5+, CD10-, CD23+, FMC7-, CD79b-, CD200+, CD38- (Catovsky 5/5) quantified at 28410/µL. Phenotype consistent with Chronic Lymphoid Leukaemia

Eight laboratories do not analyse kappa and lambda. Among them, 6 gave no indication as to the presence of an aberrant population.

This is considered an inadequate response. In case of non-analysis of kappa and lambda, a laboratory should still report aberrant populations and either complete the test with kappa and lambda or send the sample to a subcontracted laboratory. In all cases, the biologist should advise the analysis of kappa and lambda.

43 laboratories analyse kappa and lambda. Among them, 13 made no comment as to the presence of an aberrant population, although they reported a high kappa percentage result (>90%). While two participants reported relatively low kappa/lambda ratios (4.97 and 6.53, median = 958.5) without either giving an indication as to the presence of a monoclonal population.

WBC 10E9/L

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

13/39

Lympho% haematology analyser

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

14/39

Lympho% flow cytometer

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

15/39
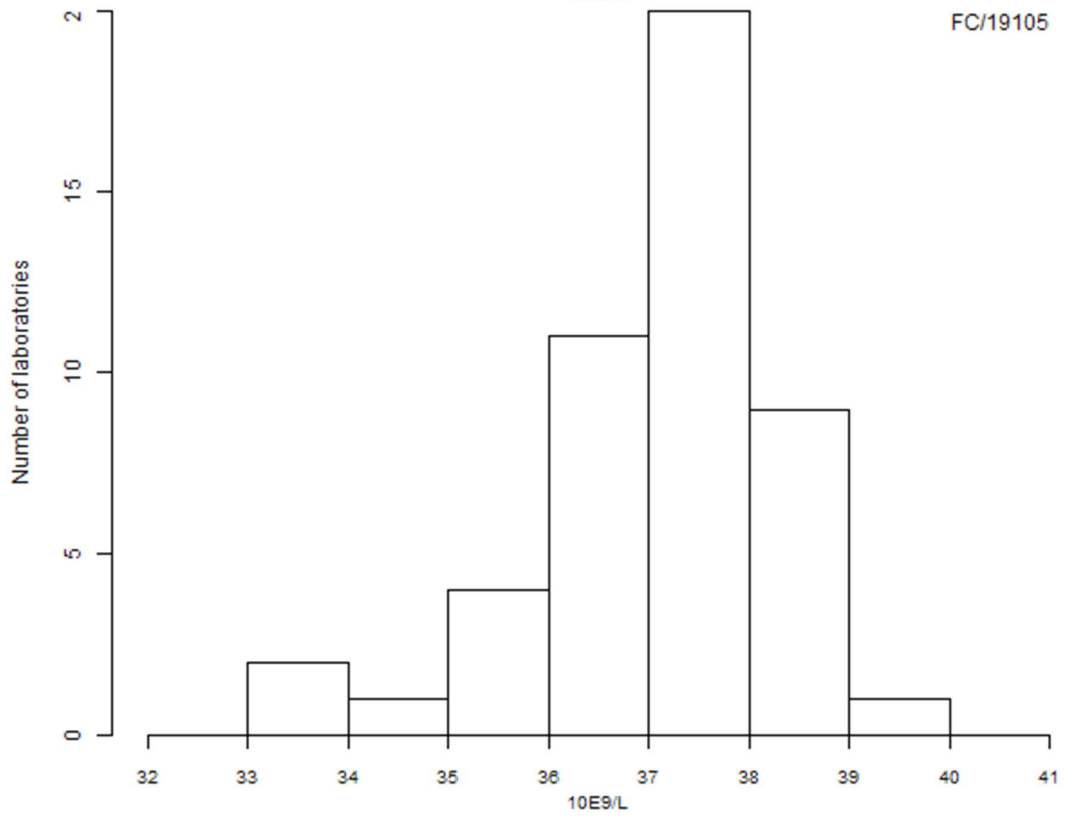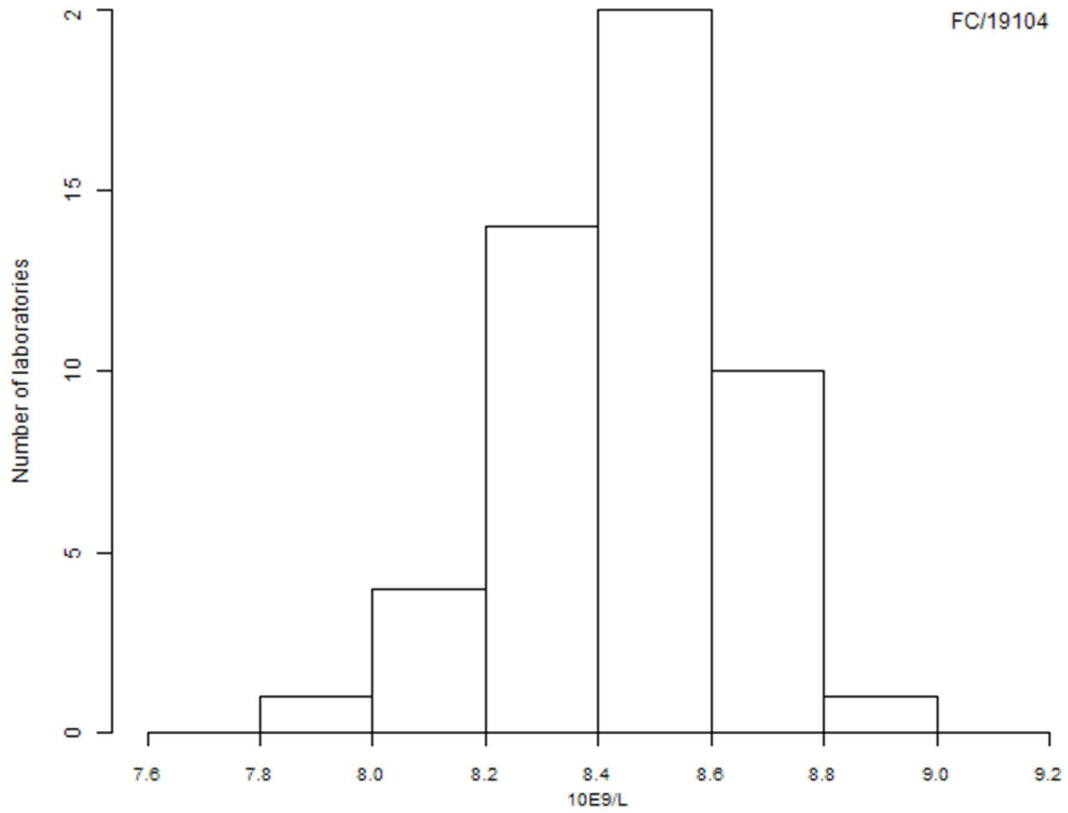
WBC 10E9/L
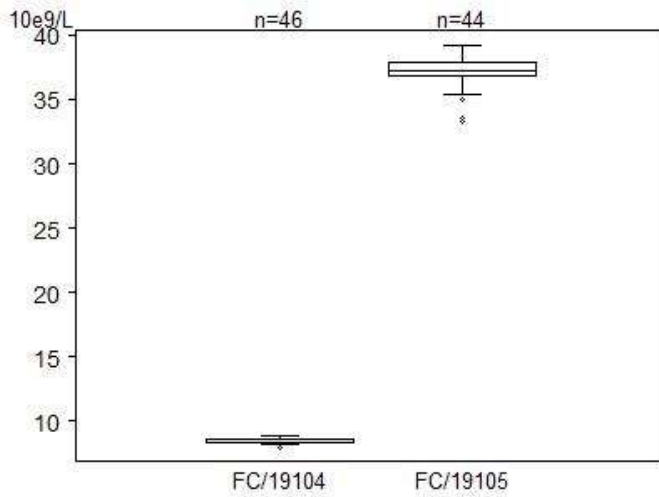
Lympho% haematology analyser
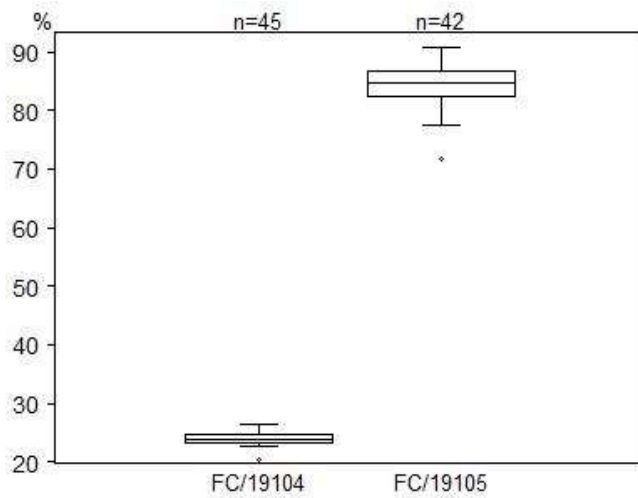
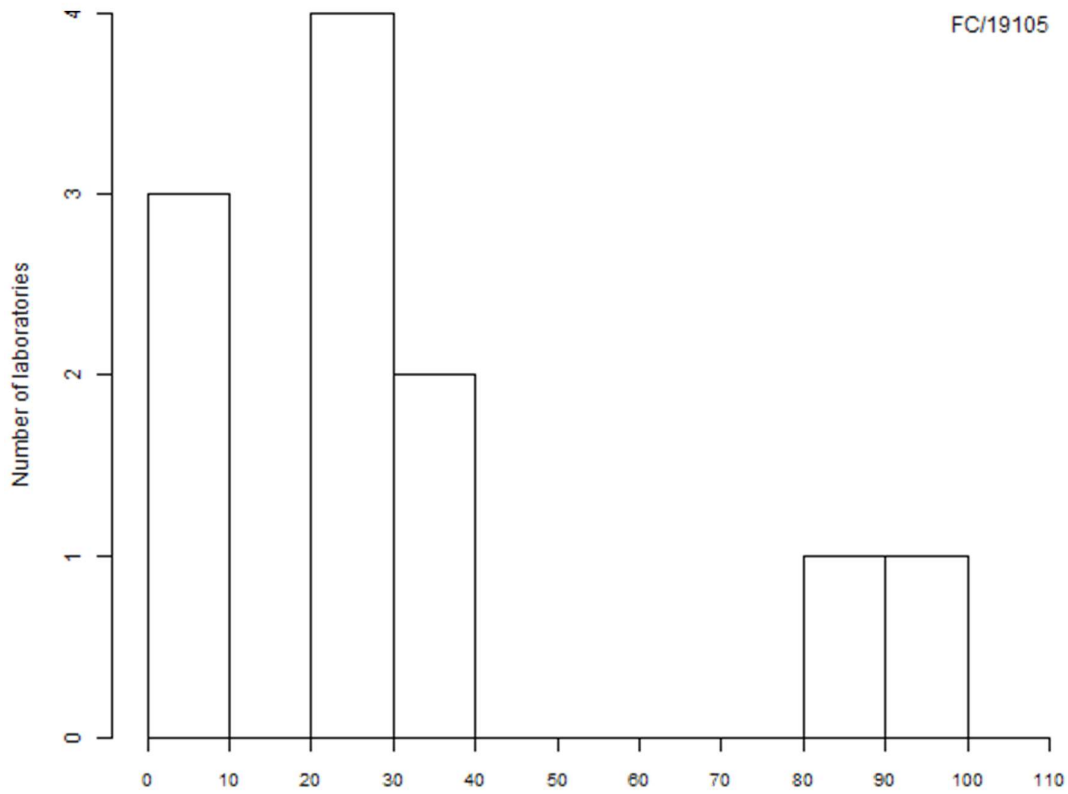
Results not represented
on the graph

FC/19105 = 94 %
FC/19105 = 94.6 %

Lympho% flow cytometer

Results not represented
on the graph

FC/19105 = 8.5 %

CD3 %

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

17/39

CD3 10E9/L

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

18/39
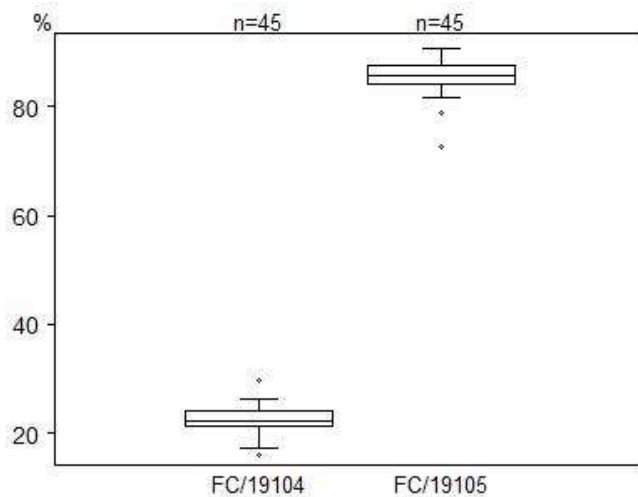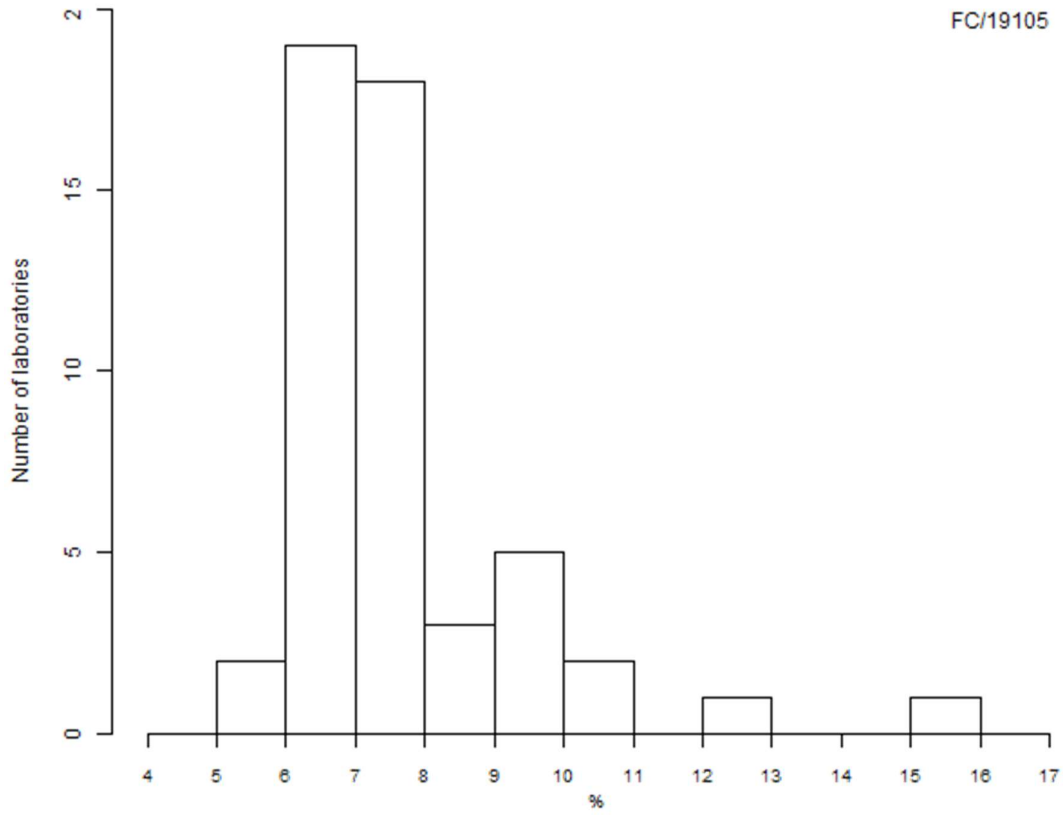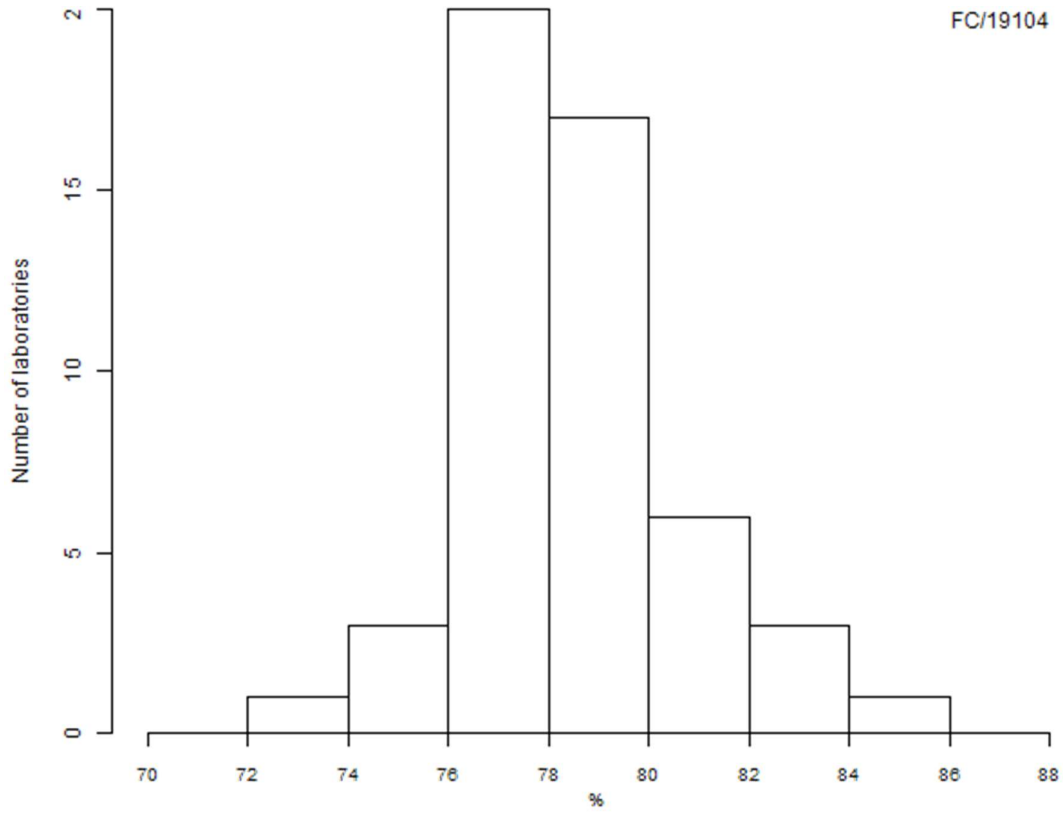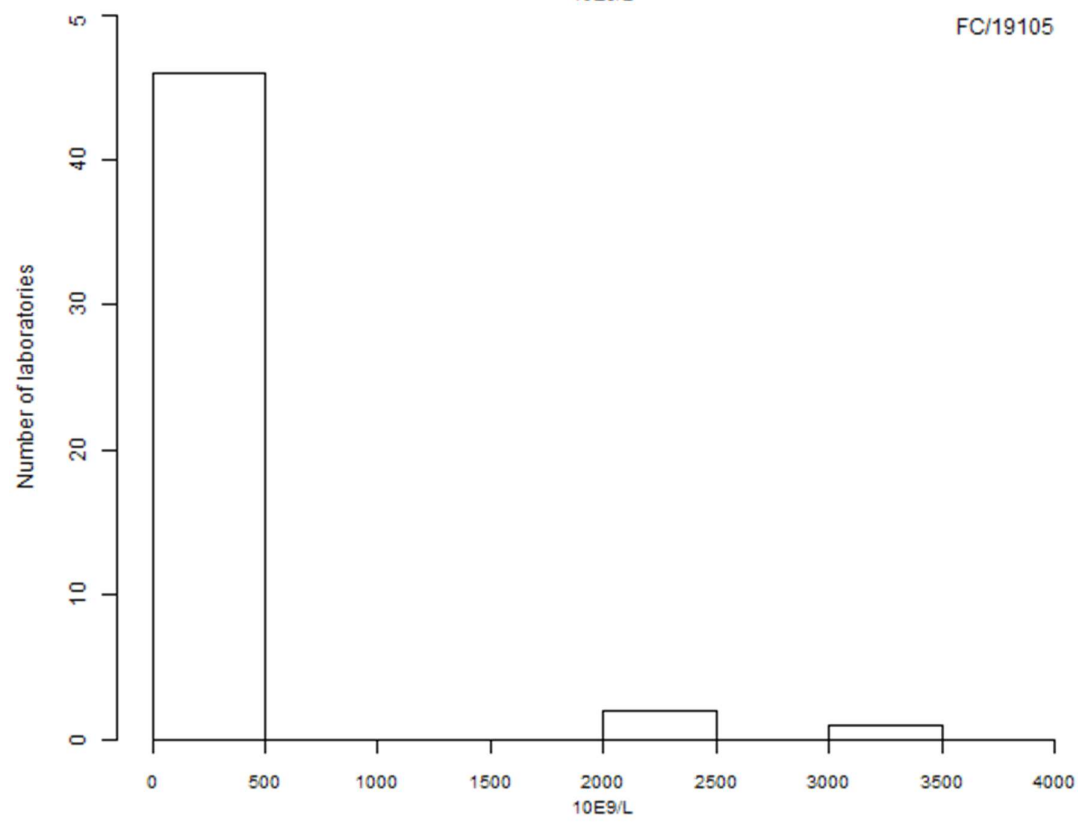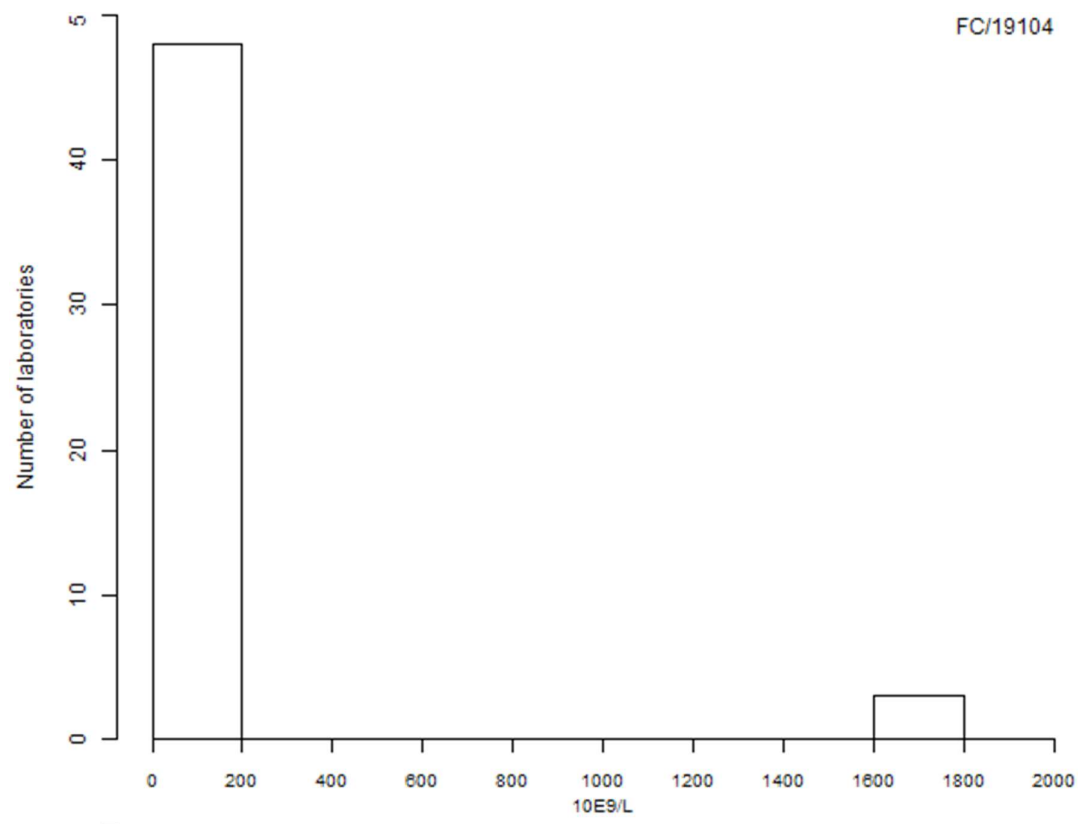
CD3 %
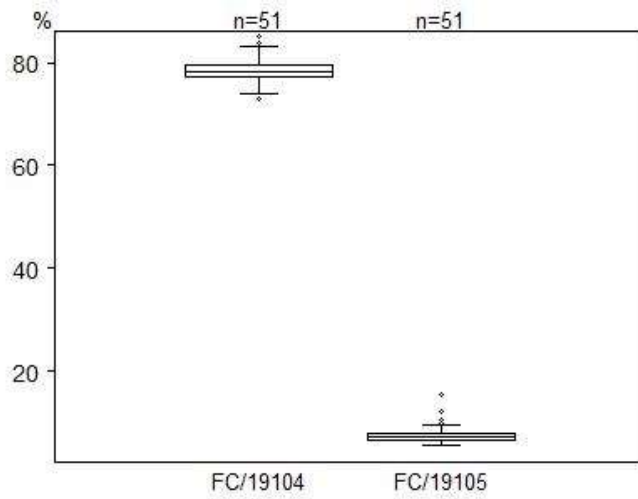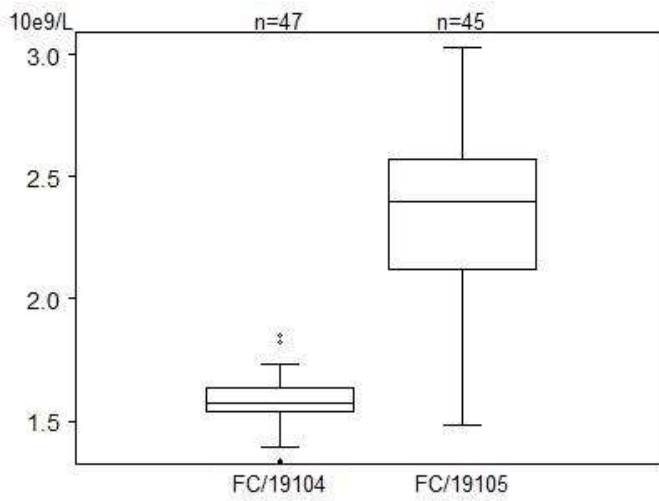
CD3 10E9/L

Results not represented on the graph

FC/19104 = 1607 10e9/
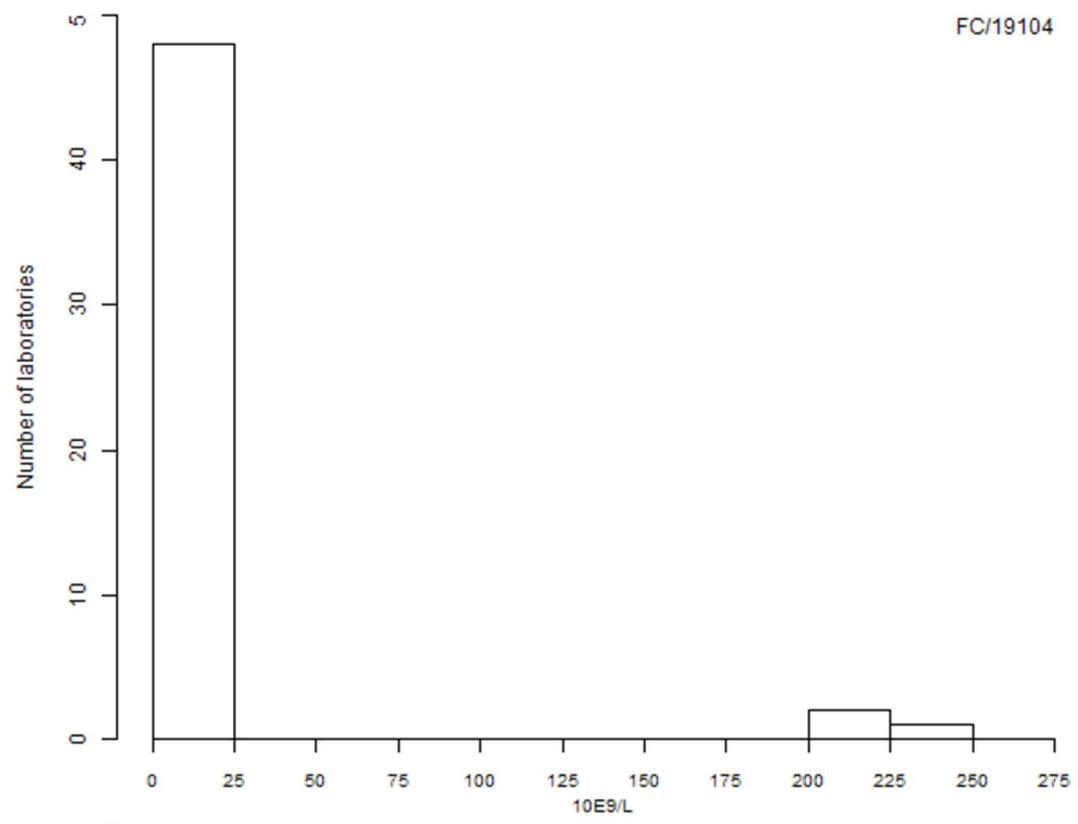FC/19104 = 1620 10e9/
FC/19104 = 1663 10e9/
FC/19105 = 3.252 10e9
FC/19105 = 3.38 10e9/l
FC/19105 = 5.015 10e9
FC/19105 = 2135.88 10
FC/19105 = 2297 10e9/
FC/19105 = 3341 10e9/

CD4 %

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.
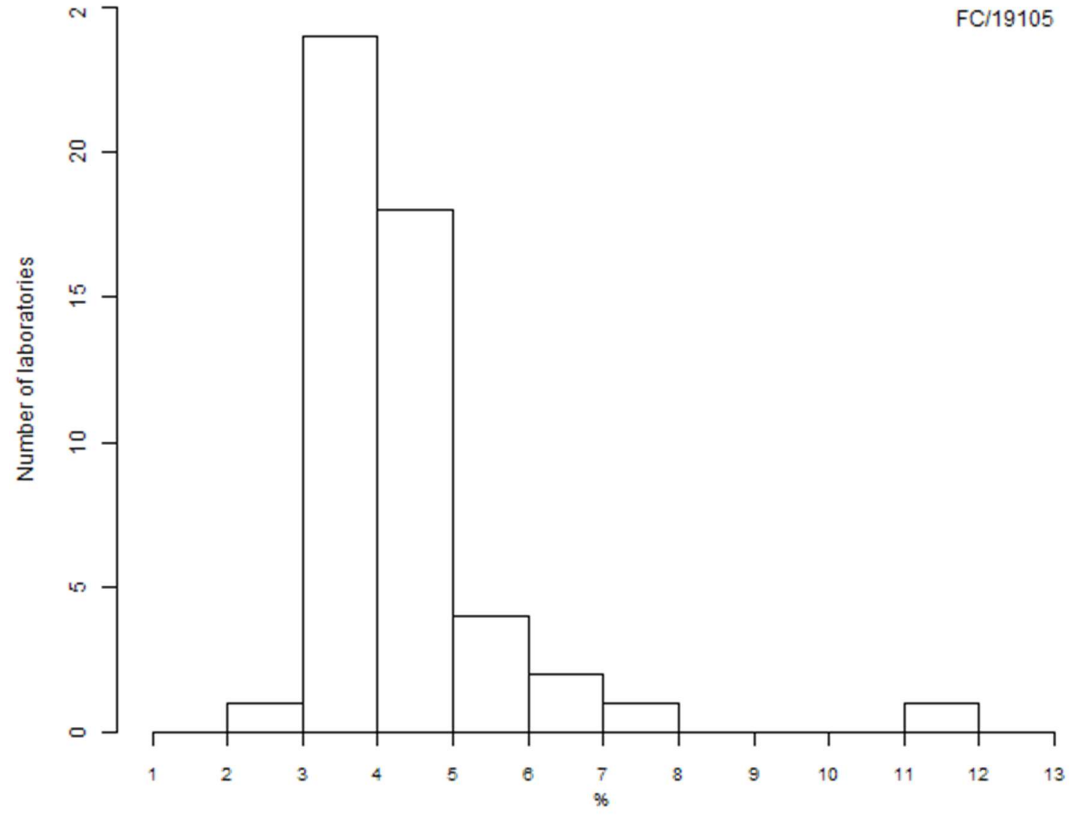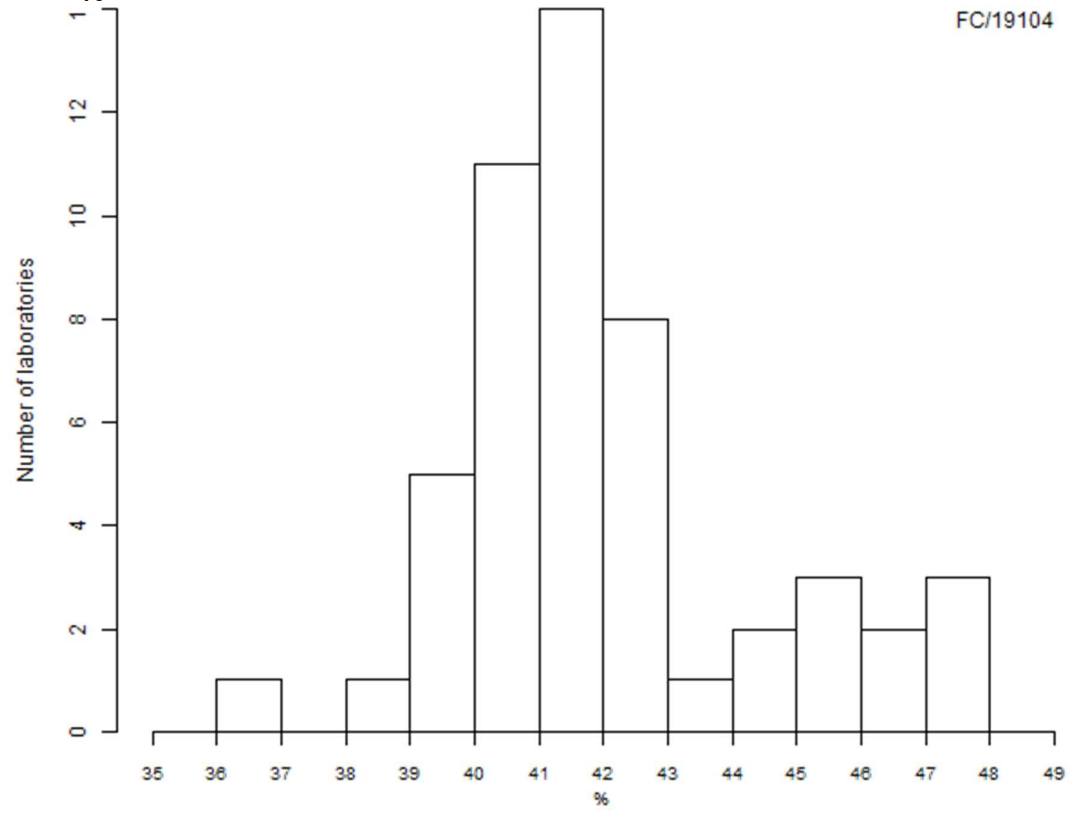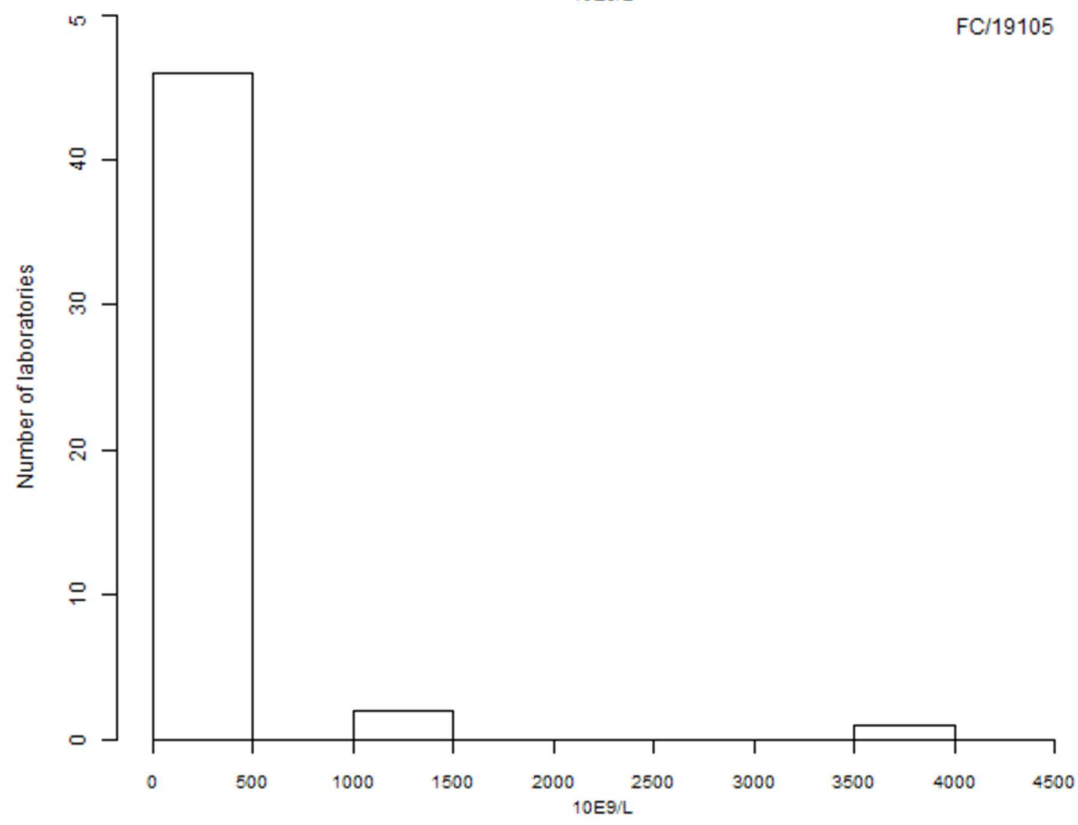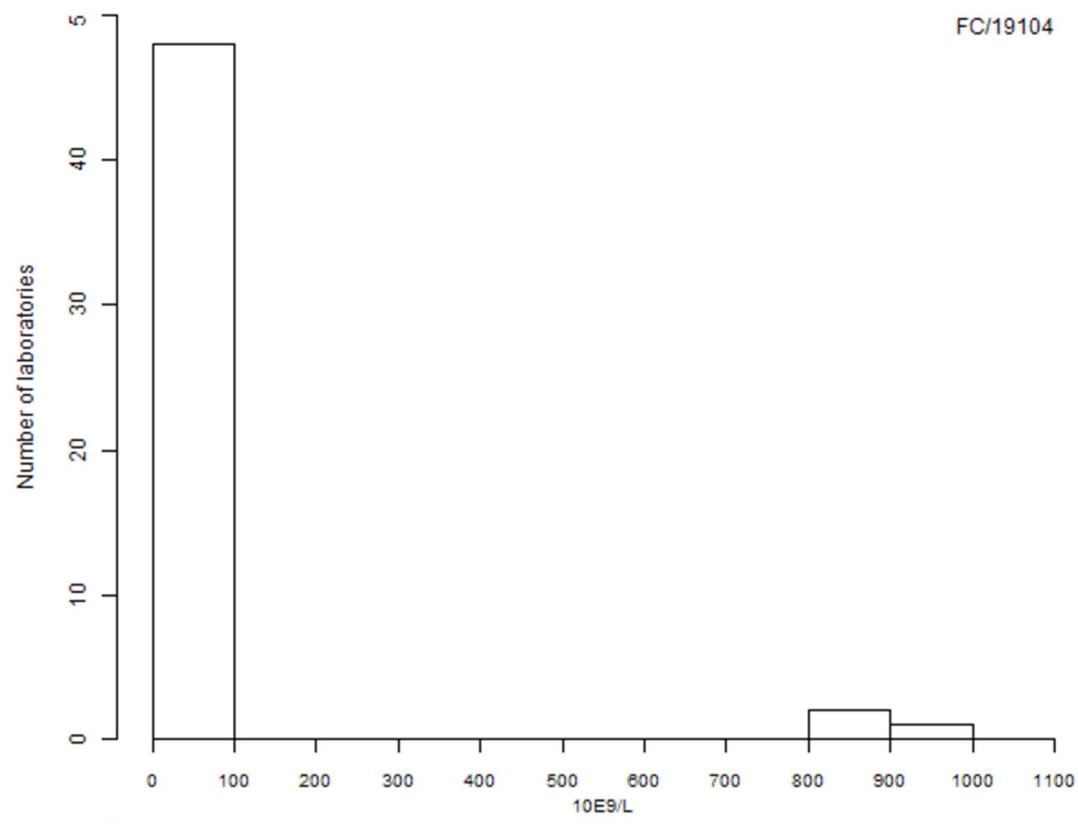
CD4 10E9/L

CD4 %
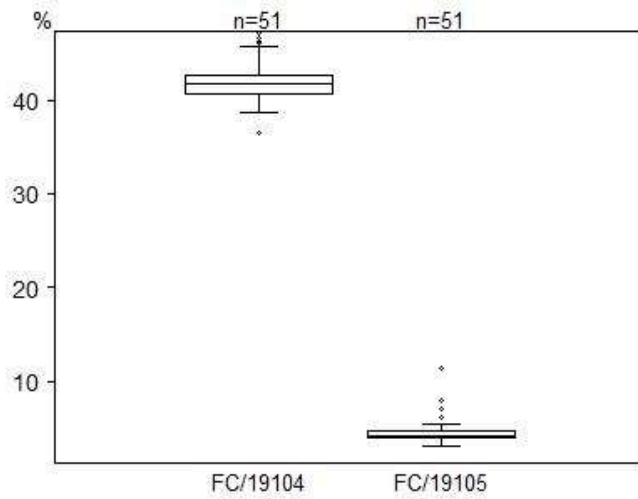
Results not represented on the graph	
FC/19104 = 47.1 %	
FC/19104 = 47.5 %	
FC/19104 = 47.7 %	

CD4 10E9/L
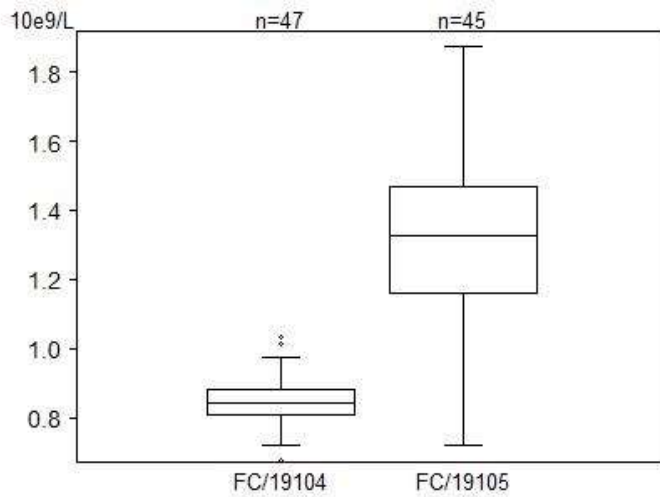
Results not represented on the graph

FC/19104 = 834 10e9/L
FC/19104 = 852.72 10e
FC/19104 = 987 10e9/L
FC/19105 = 1.95 10e9/l
FC/19105 = 2.573 10e9
FC/19105 = 1220.94 10
FC/19105 = 1272 10e9/
FC/19105 = 3771 10e9/

CD8 %

Number of laboratories

18 19 20 21 22 23 24 25 26 27 28 29 30 31

%

Number of laboratories

0 1 2 3 4 5 6 7 8 9

%

CD8 10E9/L

FC/19104

FC/19105

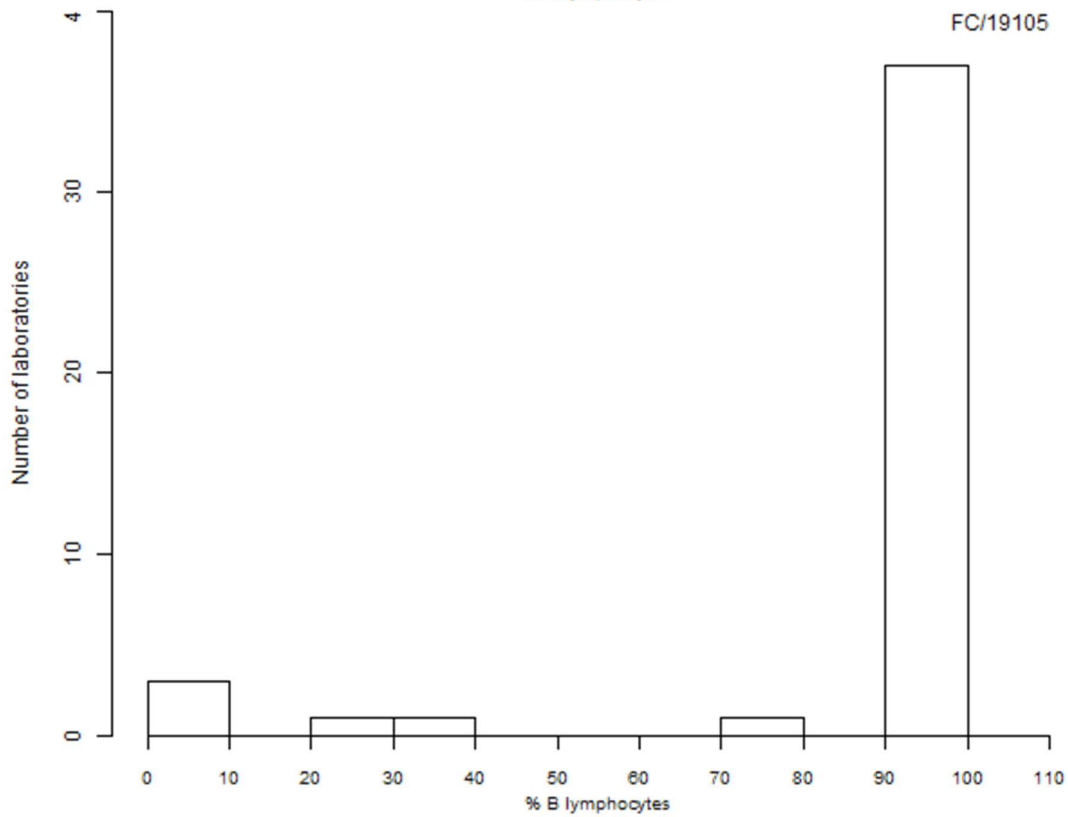Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

24/39

CD8 %
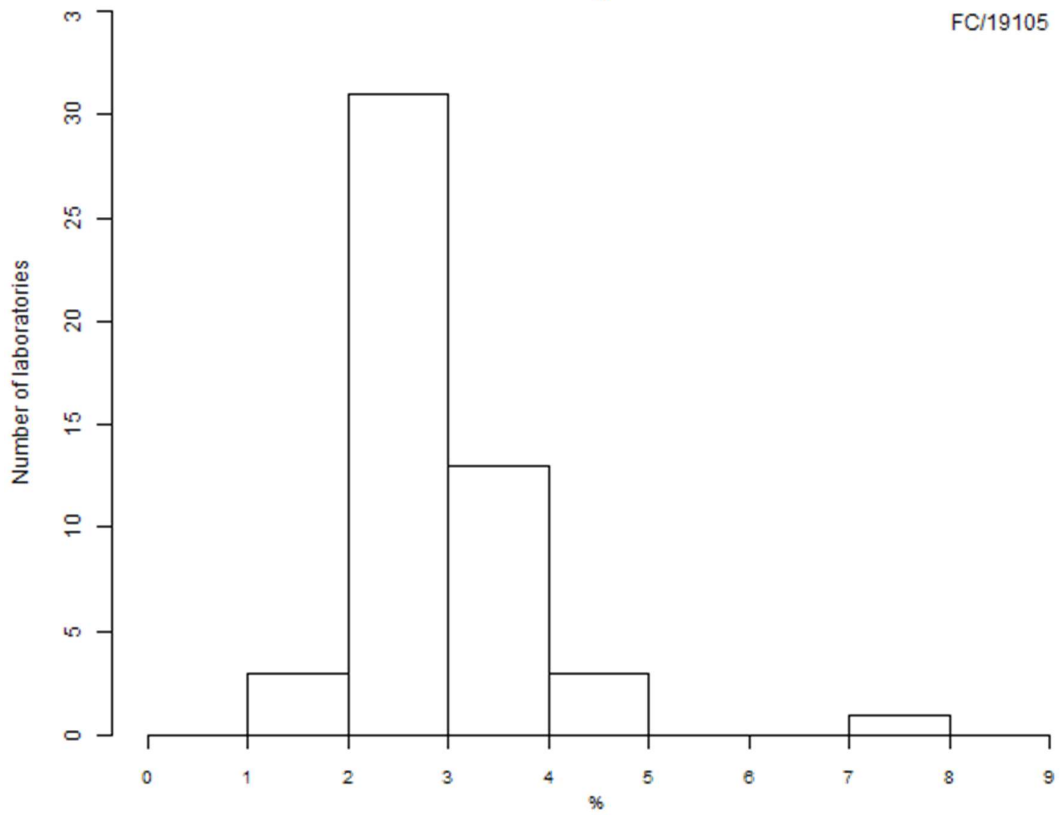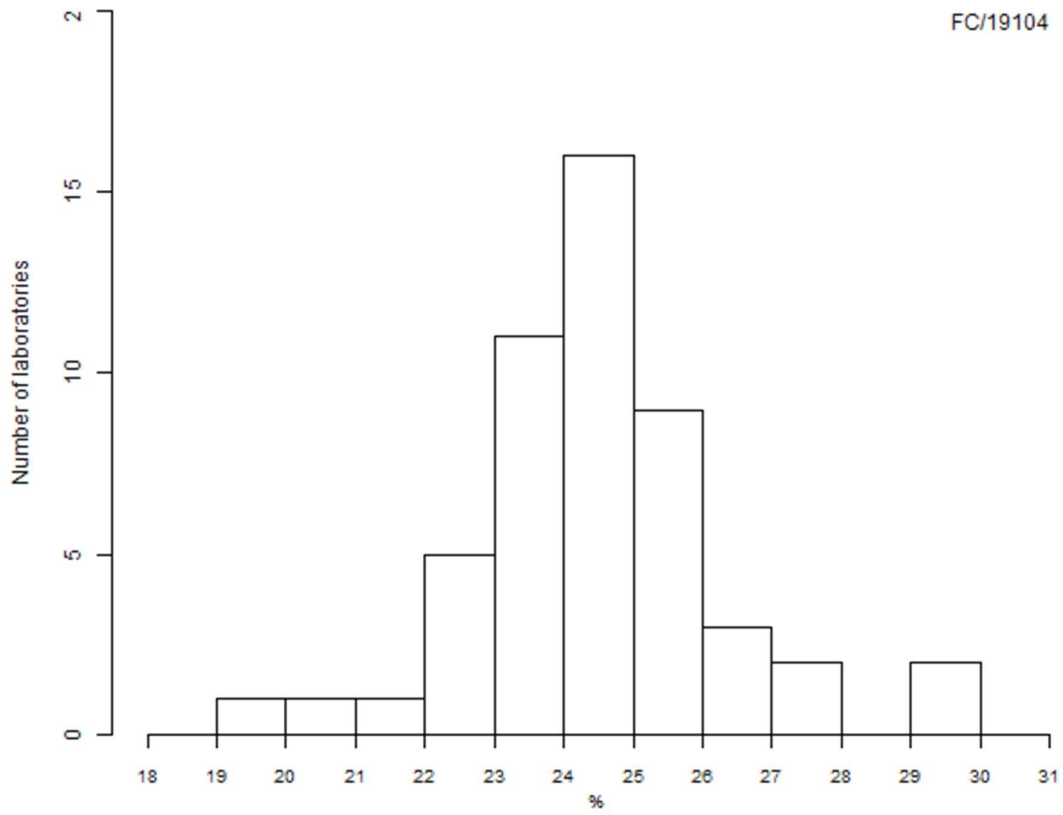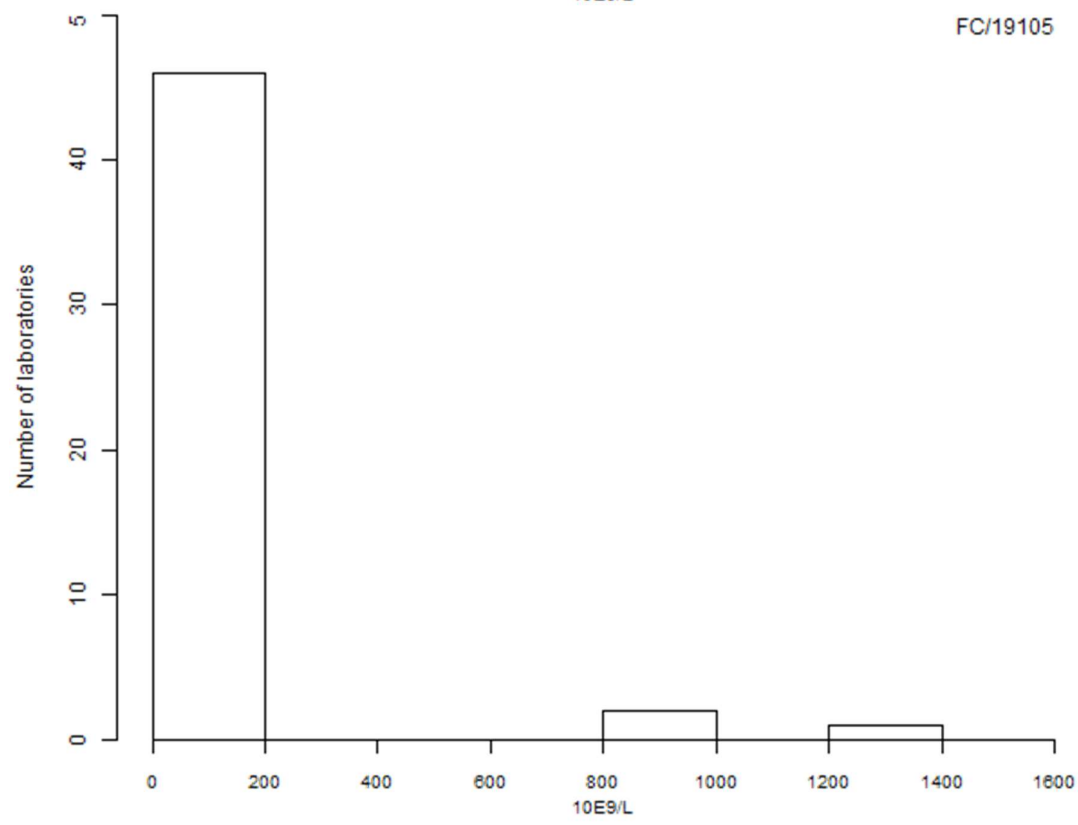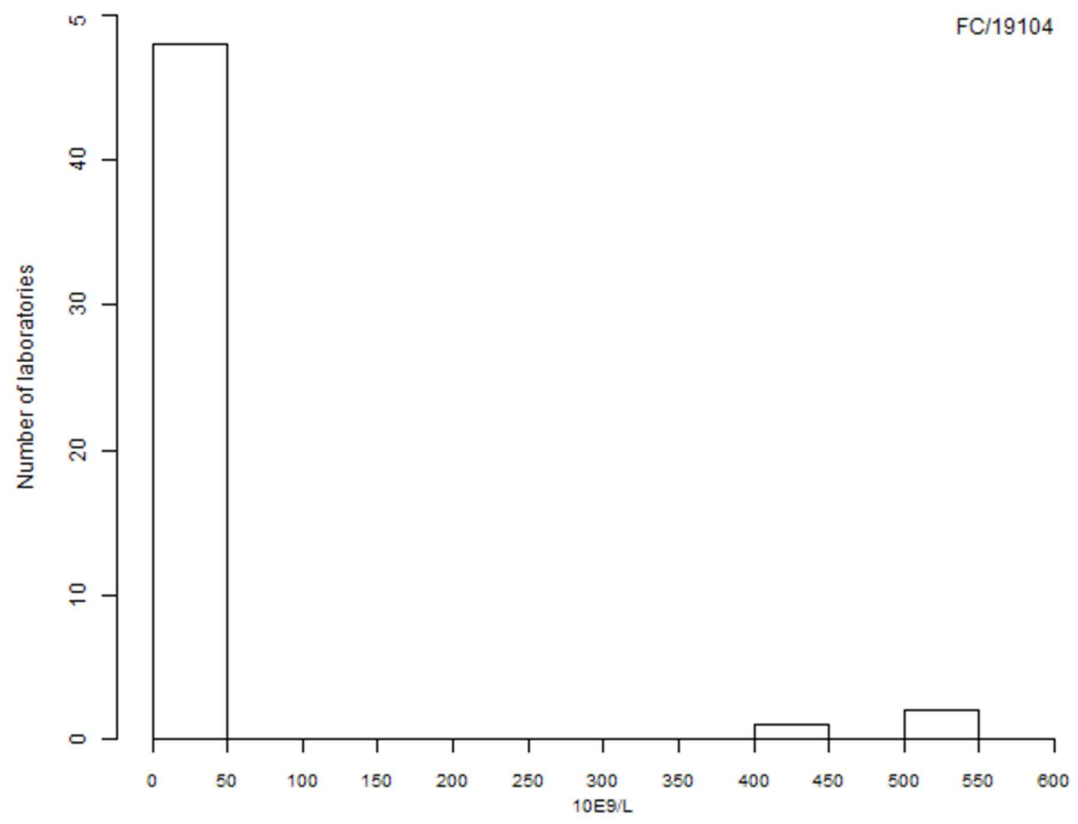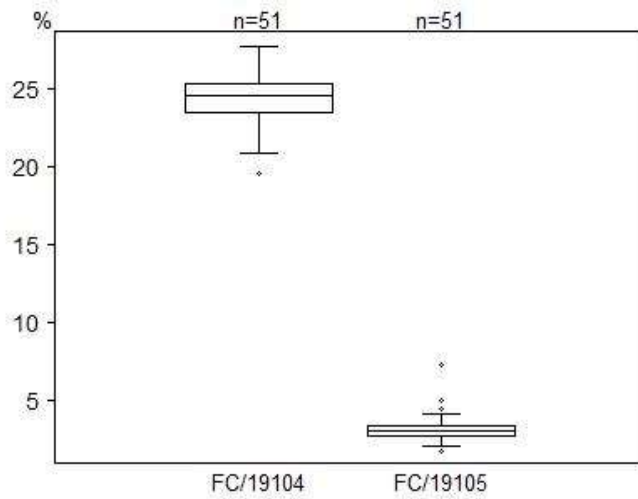
Results not represented
on the graph

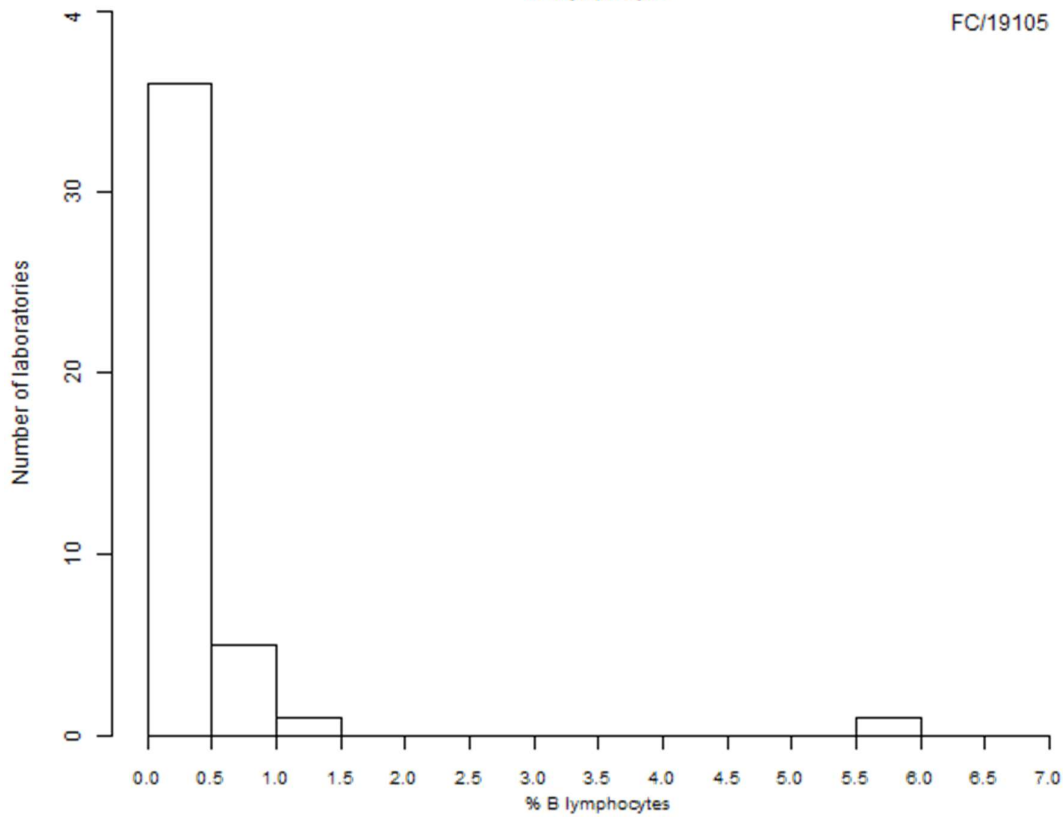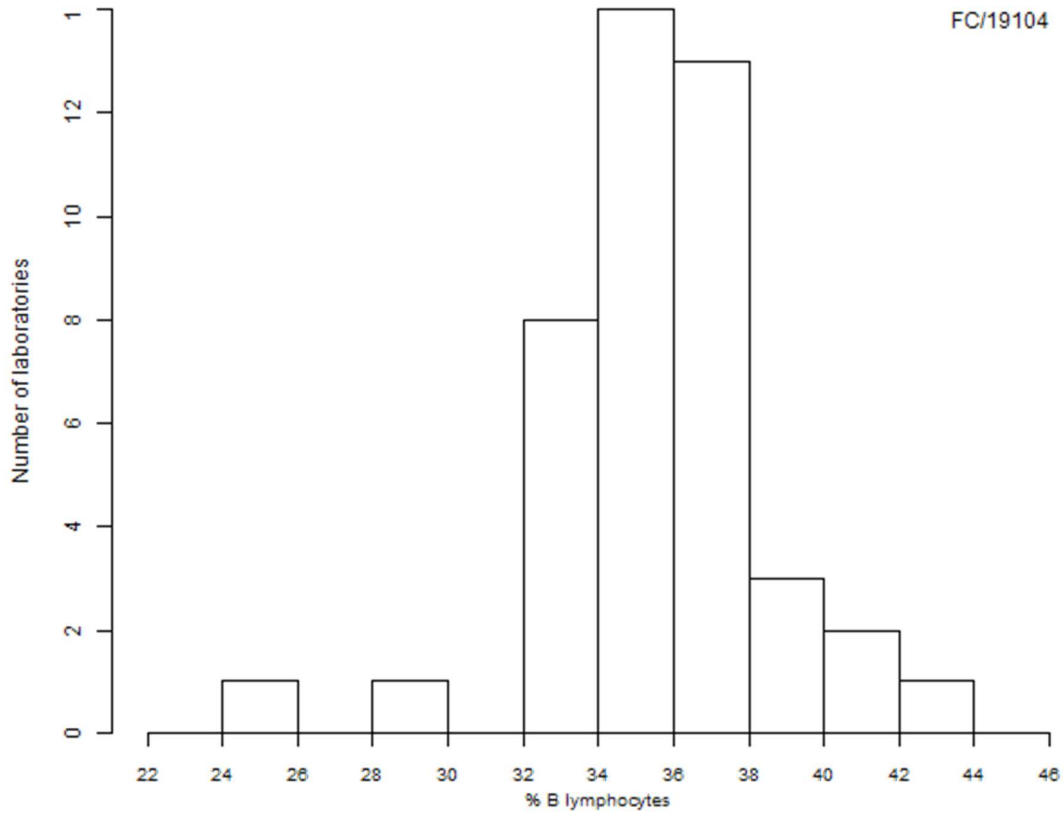FC/19104 = 29.1 %
FC/19104 = 29.9 %

CD8 %
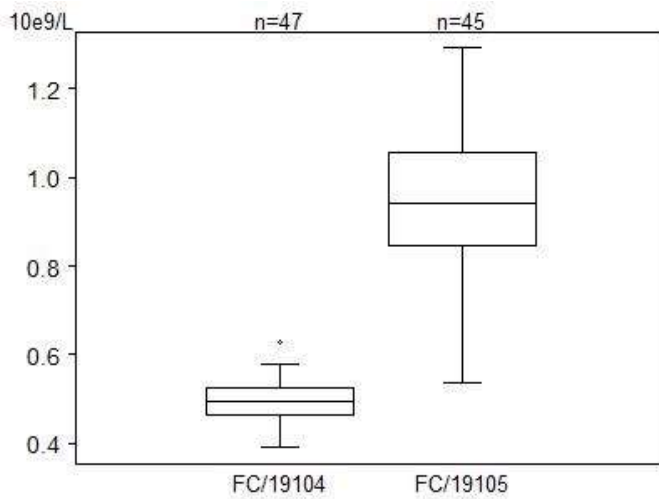
Results not represented
on the graph

FC/19104 = 422 10e9/L
FC/19104 = 508 10e9/L
FC/19104 = 510.378 10
FC/19105 = 1.39 10e9/l
FC/19105 = 2.345 10e9
FC/19105 = 921.06 10e
FC/19105 = 931 10e9/L
FC/19105 = 1290 10e9/

CD19 %
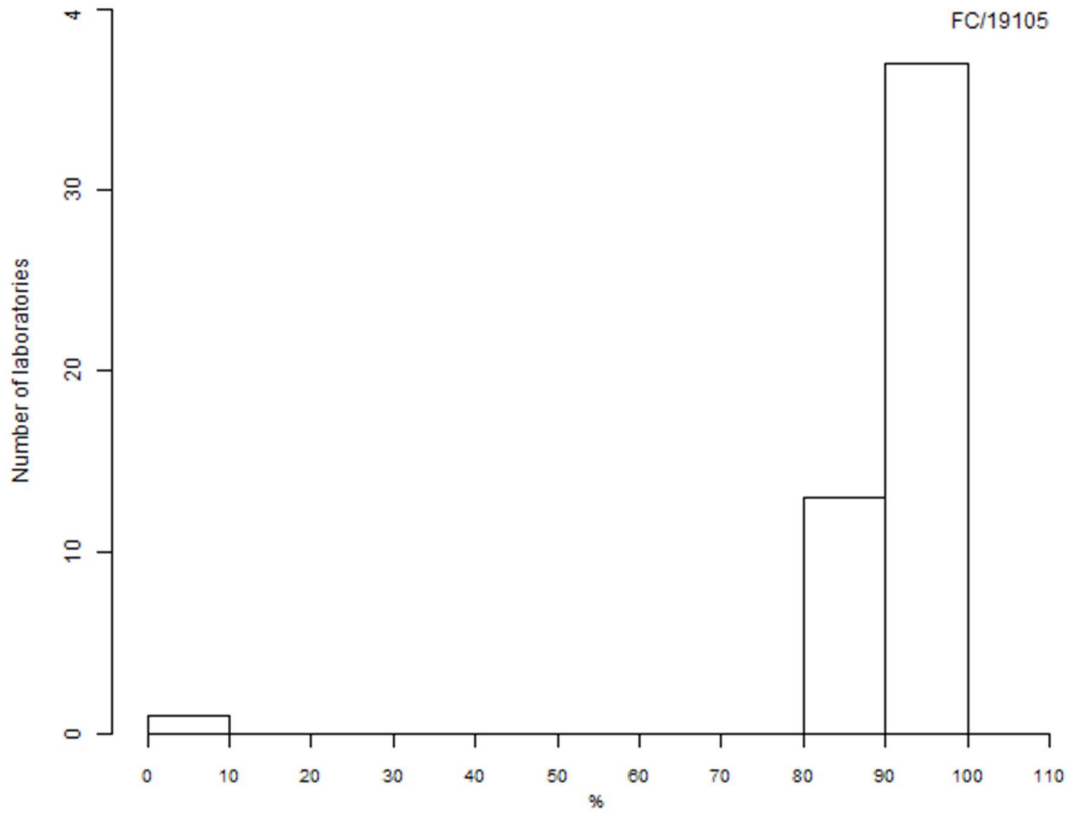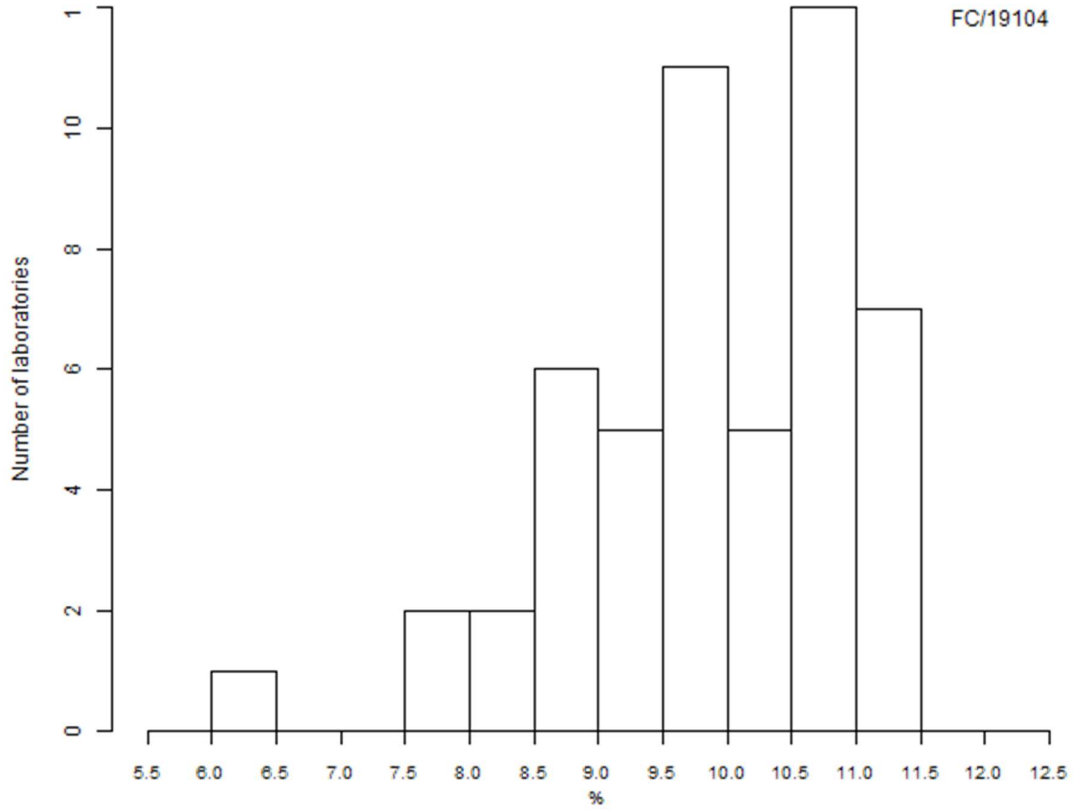
FC/19104
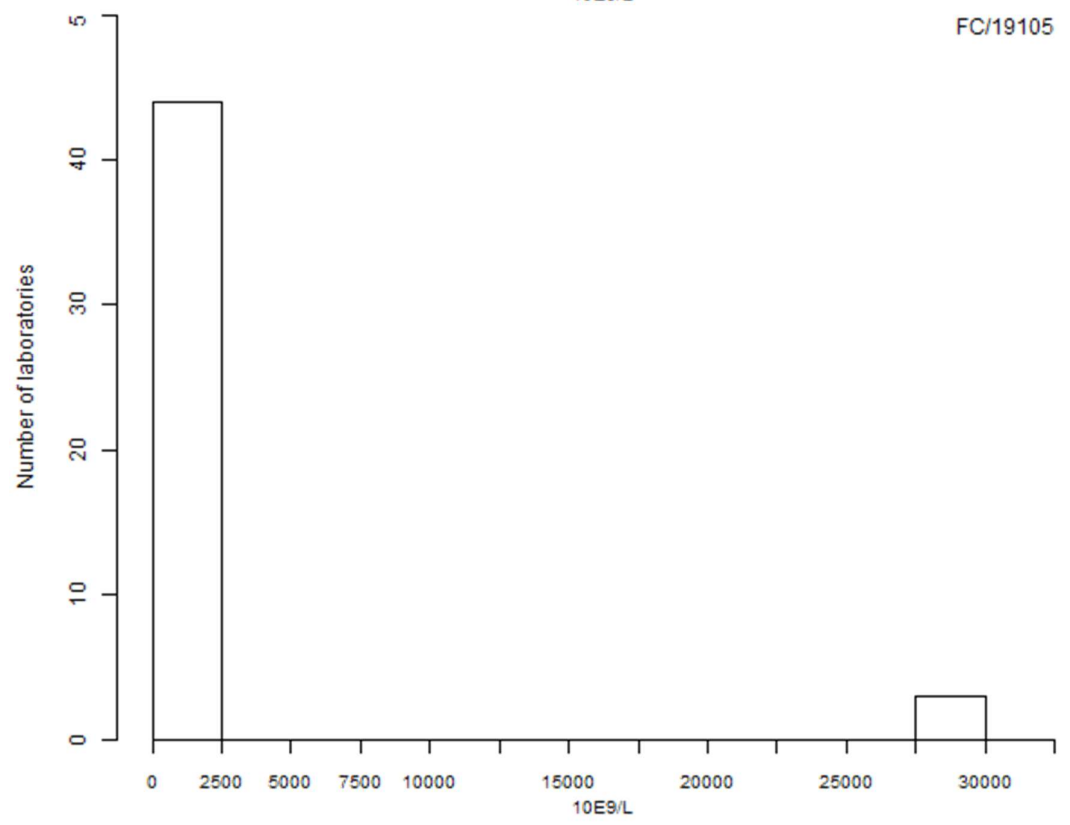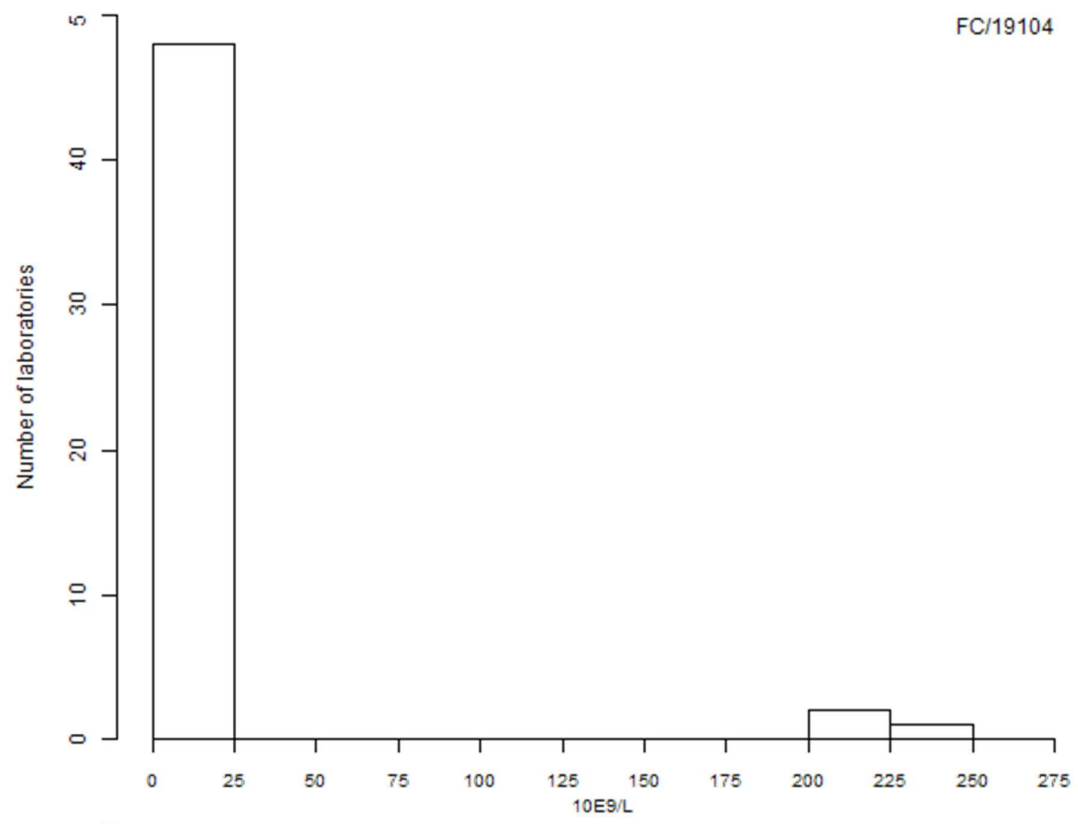
FC/19105
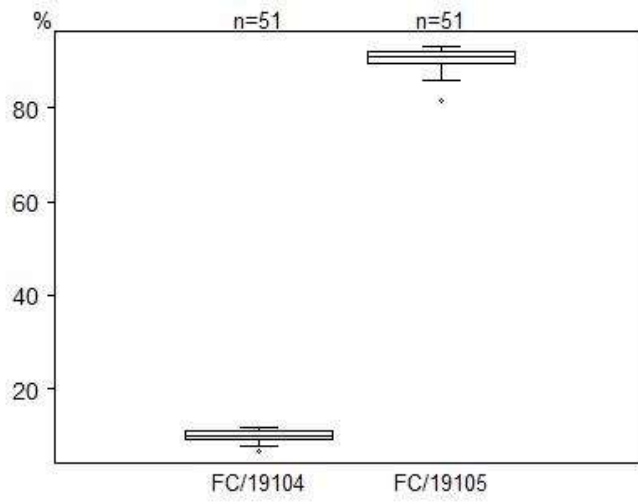
CD19 10E9/L

CD19 %

Results not represented on the graph

FC/19105 = 0 %

CD19 10E9/L
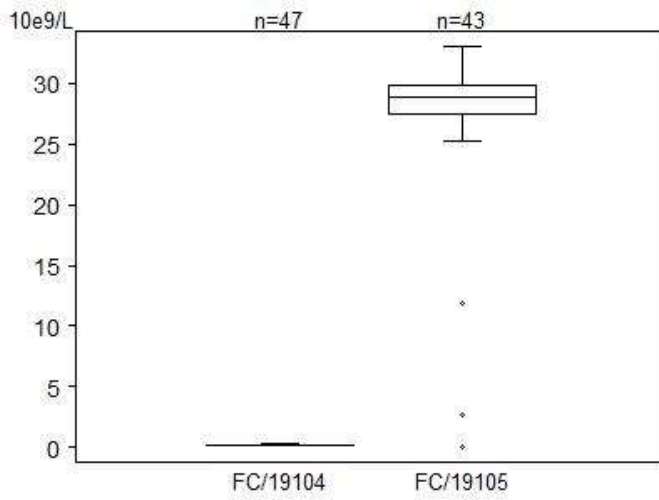
Results not represented on the graph

FC/19104 = 224 10e9/L
FC/19104 = 224.466 10
FC/19104 = 234 10e9/L
FC/19105 = 28008.199
FC/19105 = 28242 10e9
FC/19105 = 29279 10e9

Other lymphocytes (10E9/L)

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

29/39

NKcells 10E9/L

FC/19104

FC/19105

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

30/39

NKcells %

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

31/39
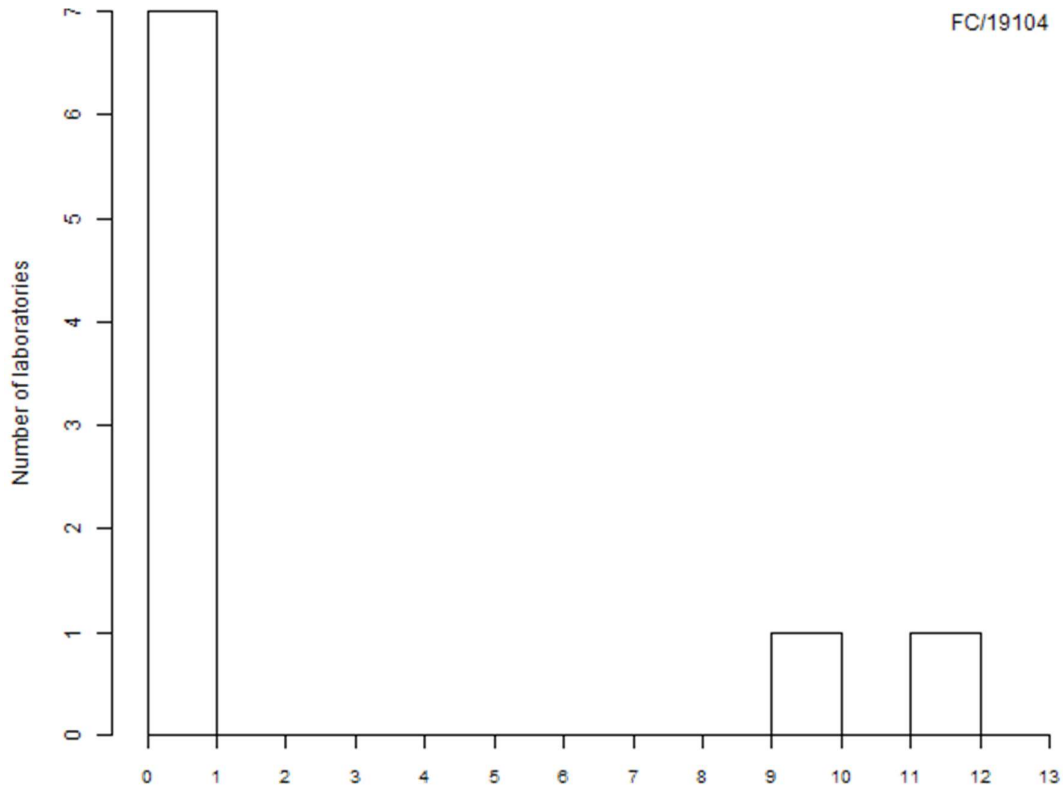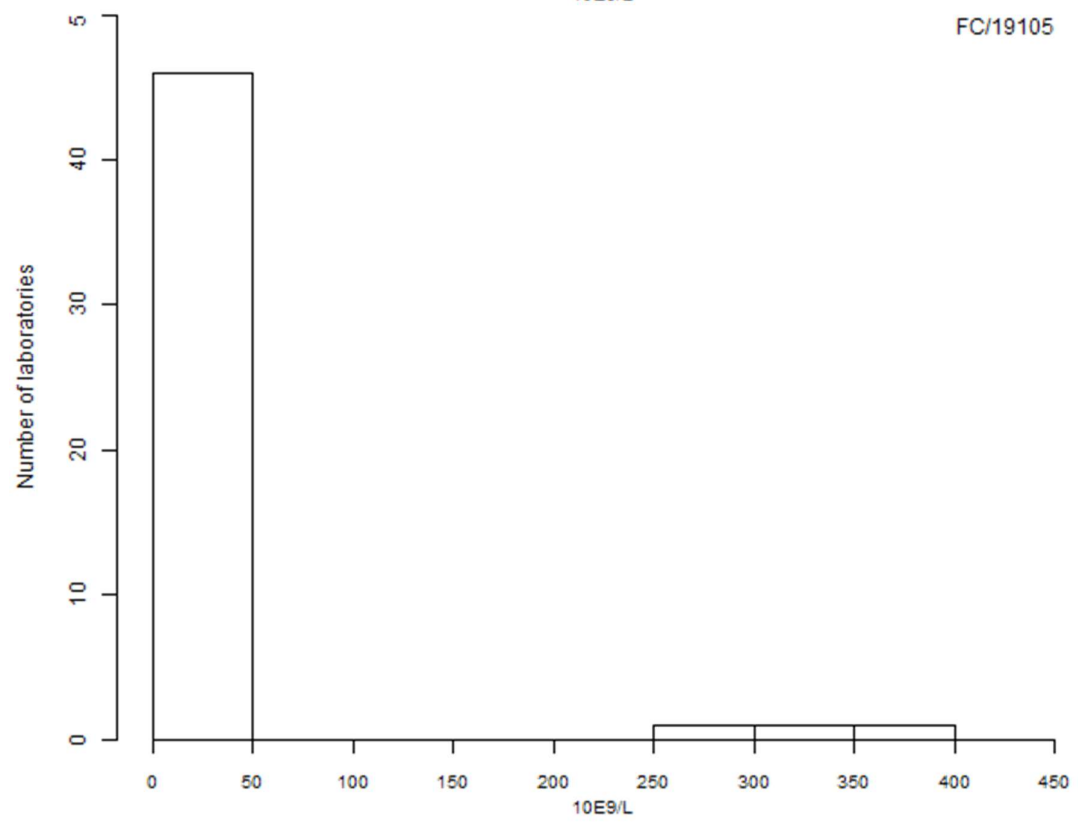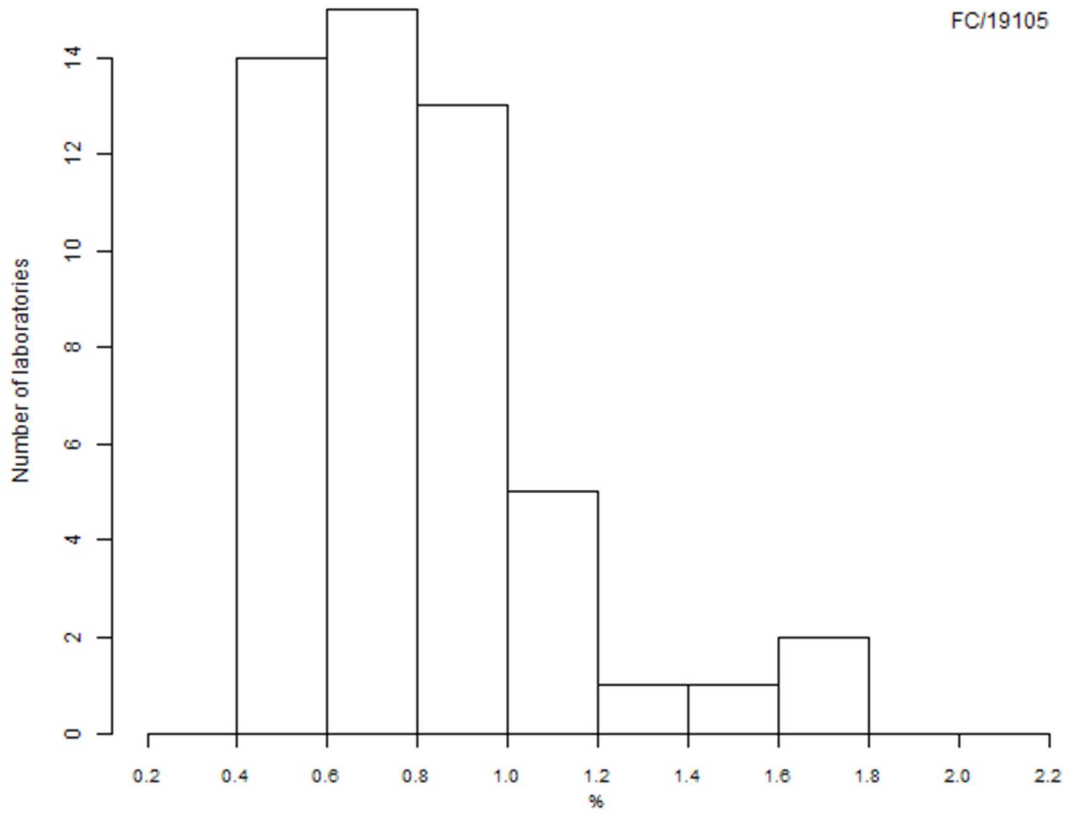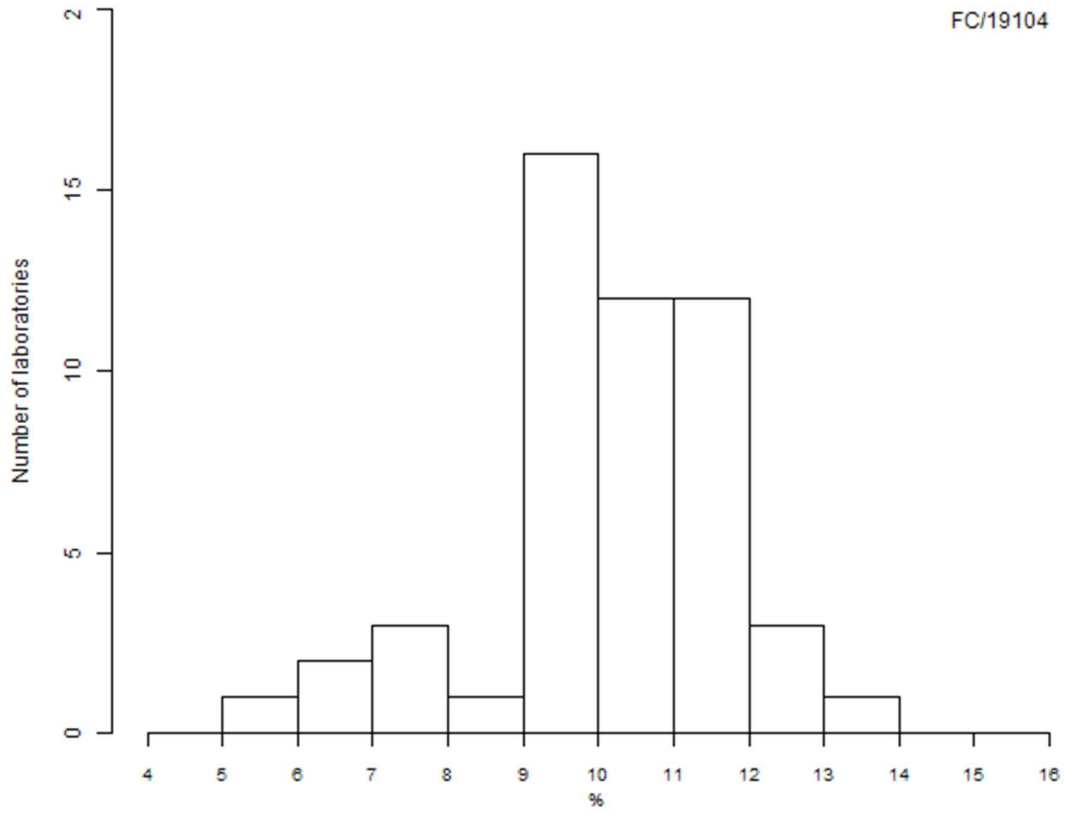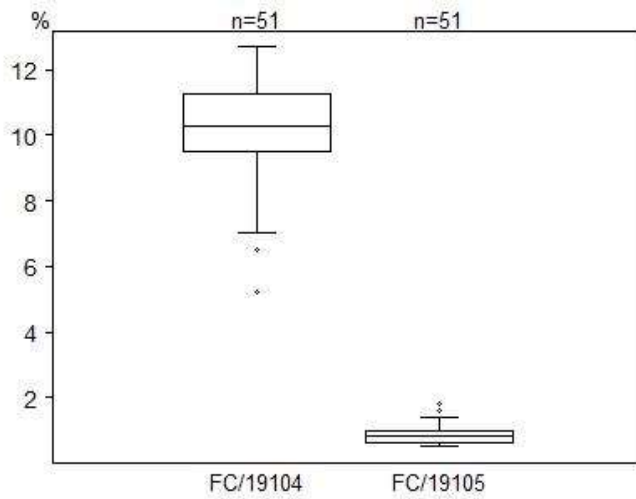
NKcells %

Results not represented
on the graph

FC/19104 = 14 %

NKcells %
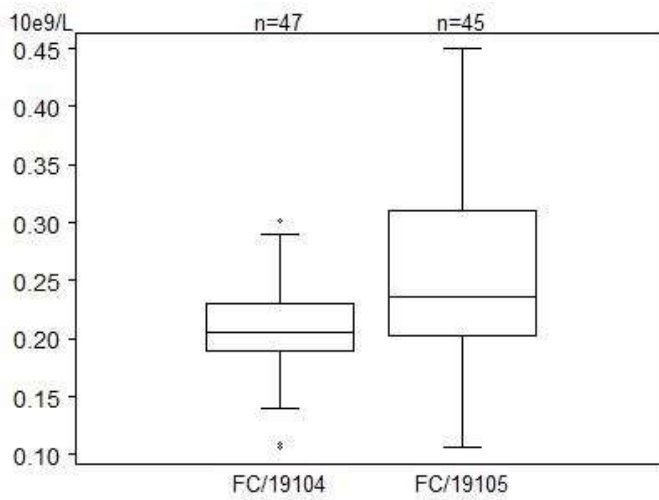
Results not represented
on the graph

FC/19104 = 201 10e9/L
FC/19104 = 223 10e9/L
FC/19104 = 232.826 10
FC/19105 = 0.525 10e9
FC/19105 = 0.56 10e9/l
FC/19105 = 0.586 10e9
FC/19105 = 298 10e9/L
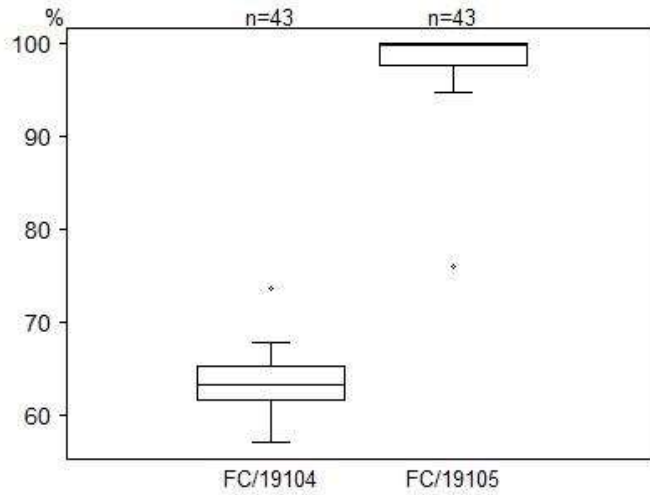FC/19105 = 310 10e9/L
FC/19105 = 373.32 10e

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

32/39

Kappa % B lymphocytes

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

33/39

Lambda % B lymphocytes

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

34/39
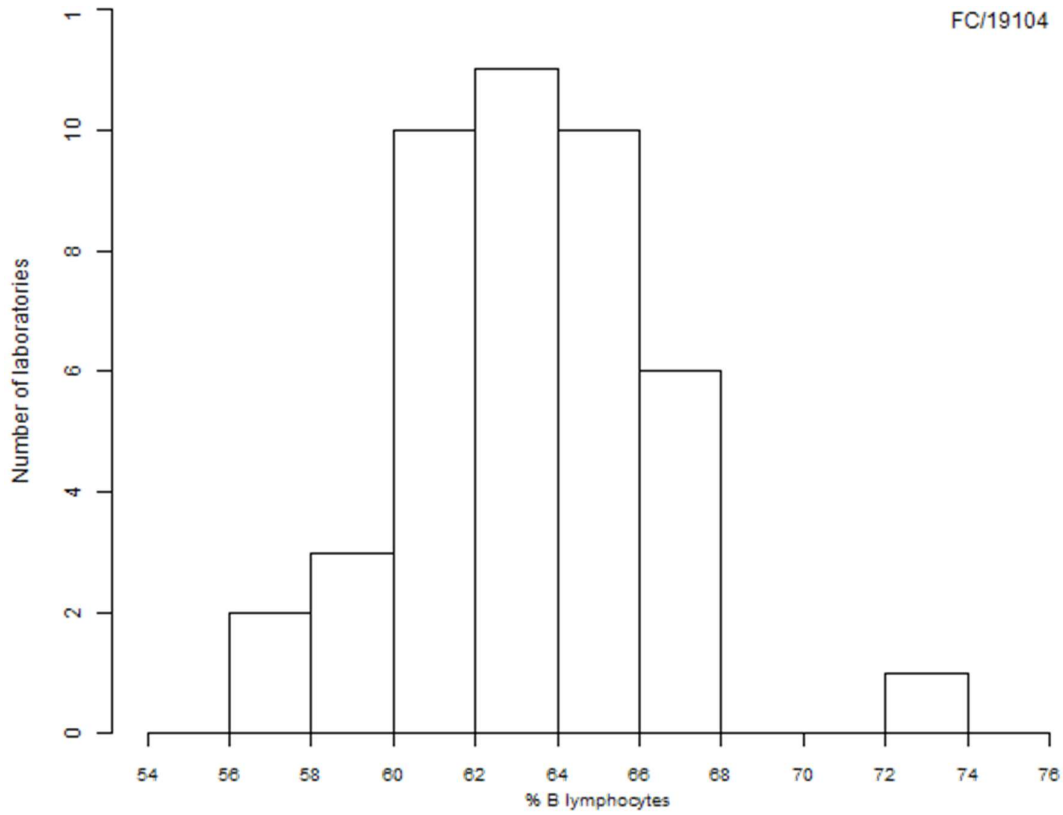
Kappa/lambda
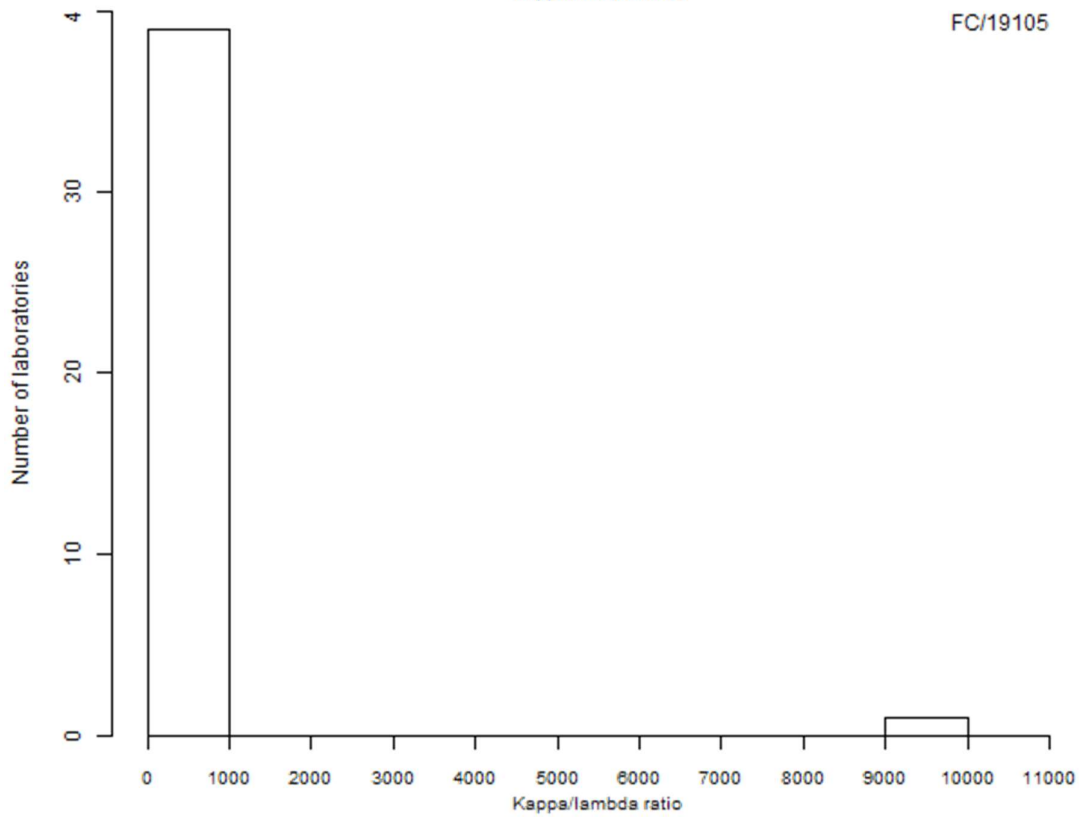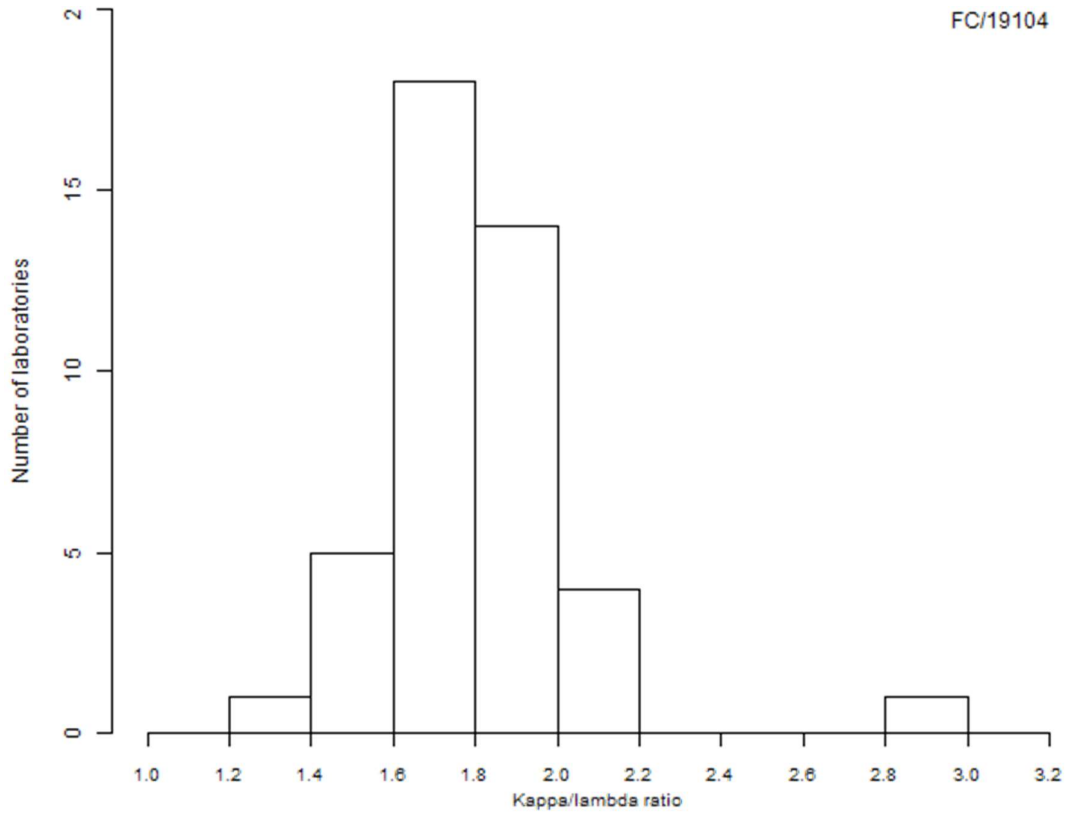
FC/19104

FC/19105

Kappa % B lymphocytes

Results not represented
on the graph

FC/19105 = 0 %
FC/19105 = 1 %
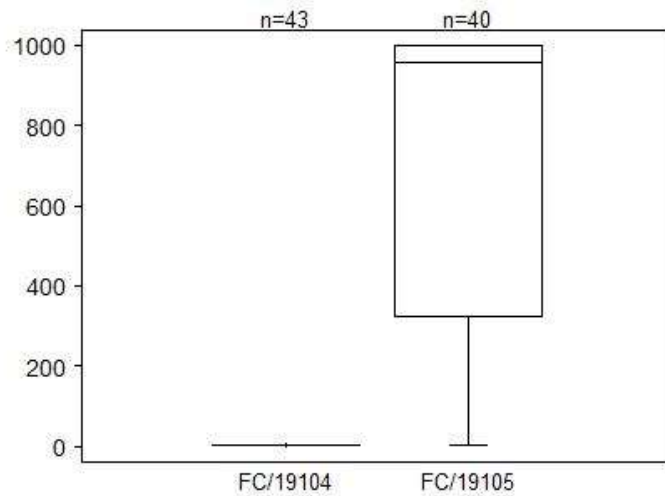FC/19105 = 5.7 %
FC/19105 = 29.8 %
FC/19105 = 34.4 %

Lambda % B lymphocytes
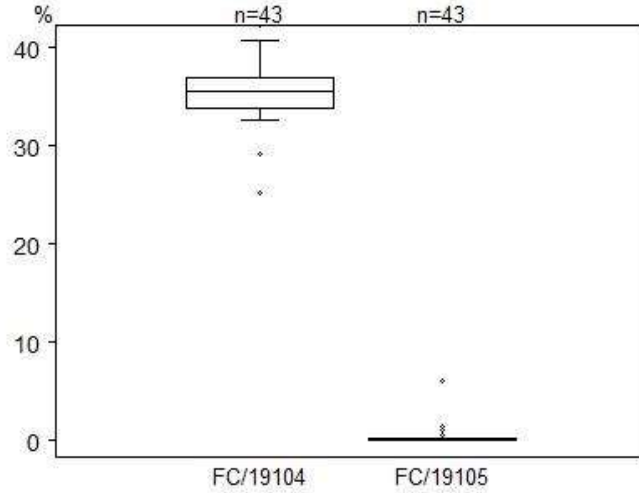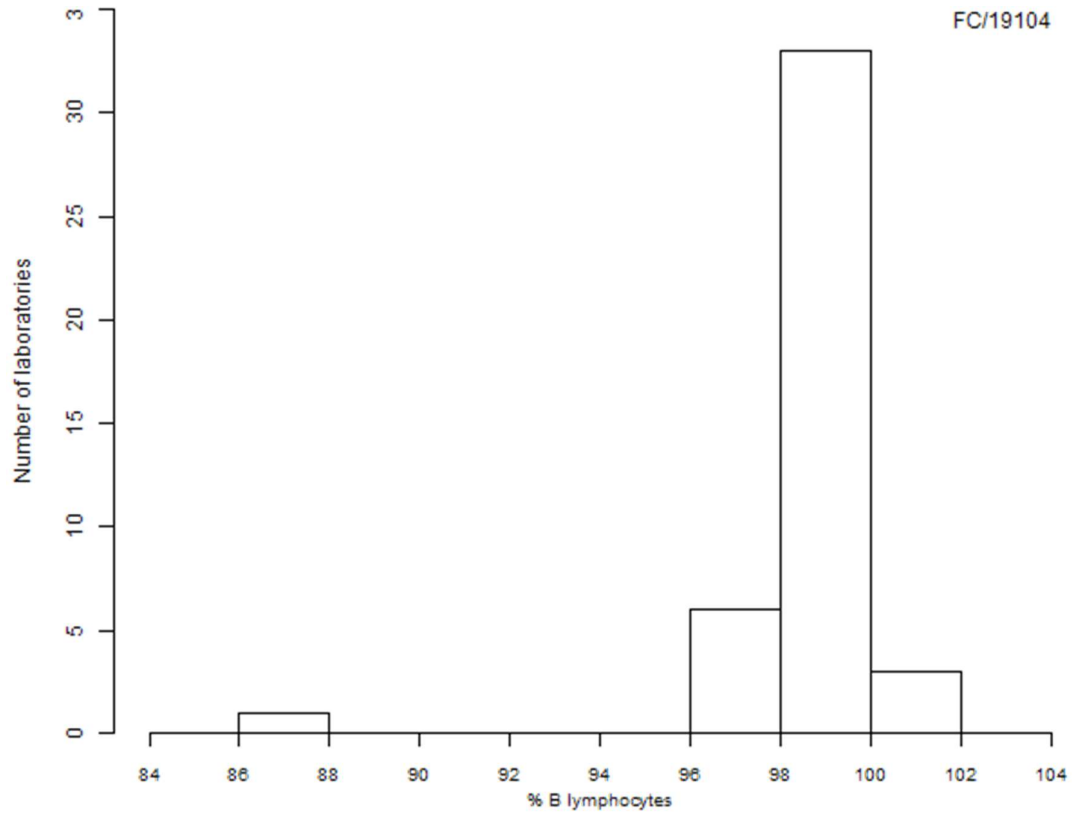

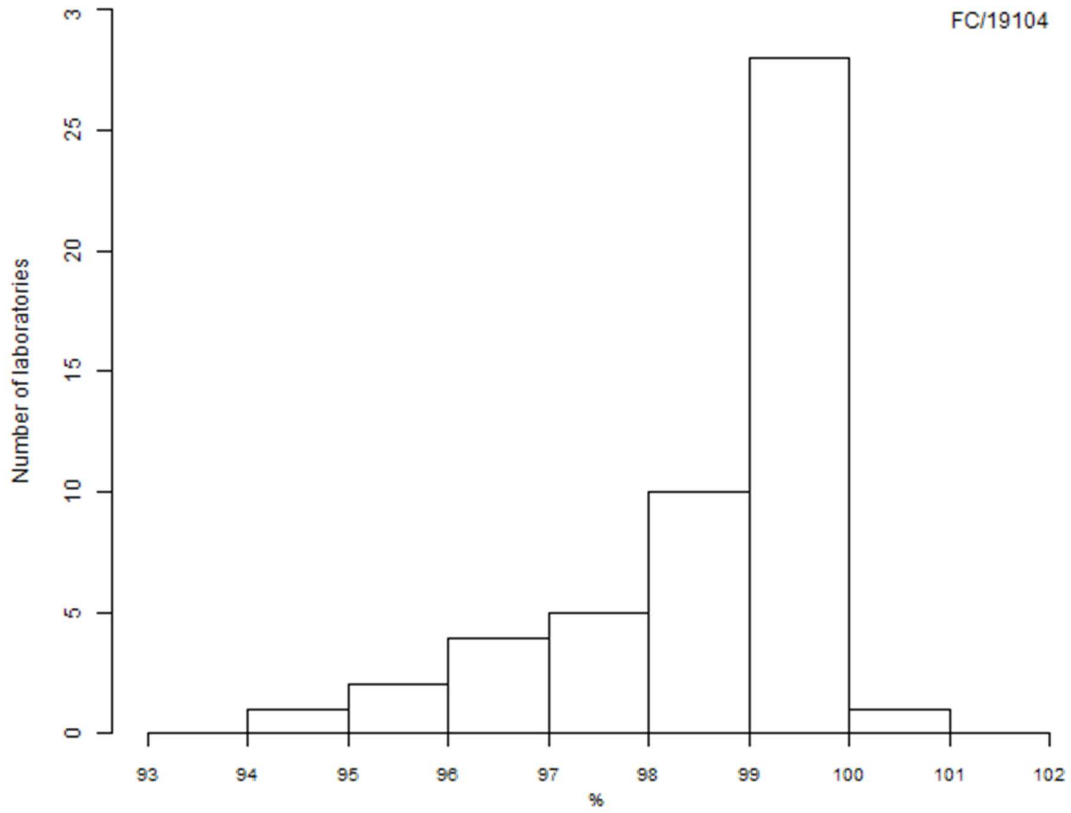Results not represented
on the graph

FC/19104 = 42.2 %

Kappa/lambda
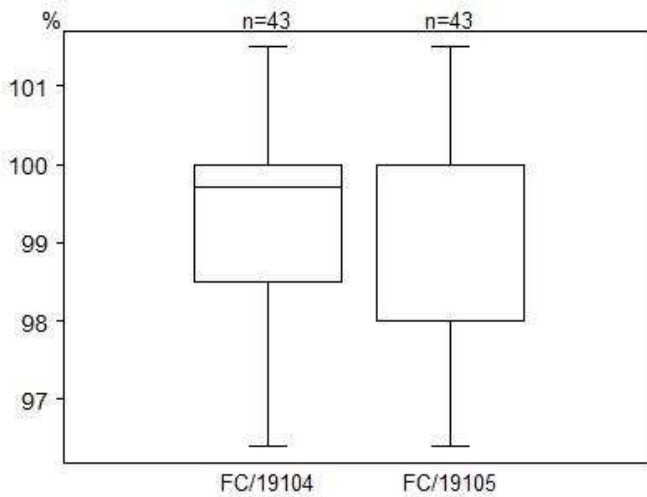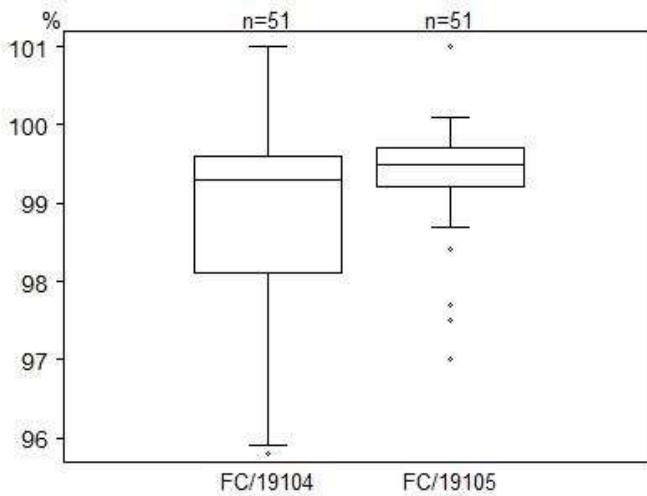

Results not represented
on the graph

FC/19105 = 9999
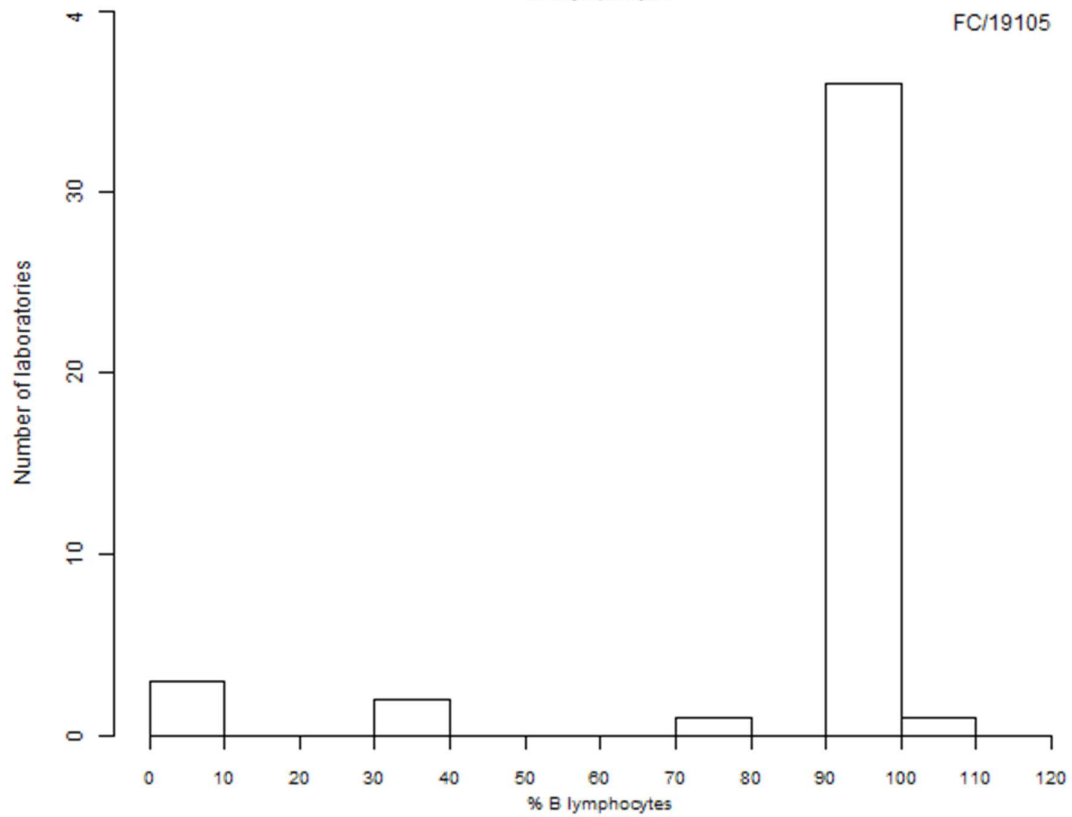
Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

36/39

Sum K+L % B lymphocytes

FC/19104

Number of laboratories

% B lymphocytes

FC/19105

Number of laboratories

% B lymphocytes

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

37/39

Lymphosum %

Flow cytometry: lymphocyte subset analysis, definitive global report 2022/2.
FORM 43/124/E V15.

38/39

Sum K+L % B lymphocytes

Lymphosum %
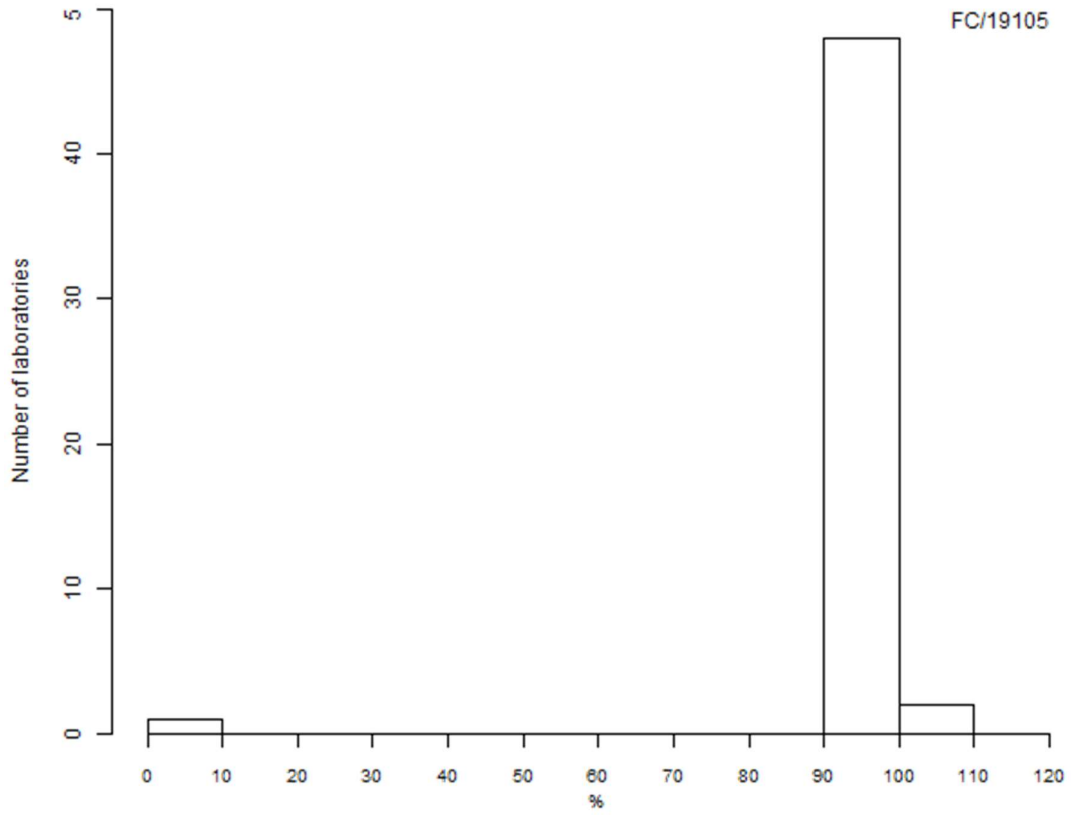
For technical validation purposes it is worth noting that in non-pathological peripheral blood of adults the sum of kappa and lambda (expressed as a % of CD19+ B-cells) should be between 90 and 110. The lymphosum (sum of $CD3^+$% plus $CD19^+$% plus $CD3^-CD16^+$ and/or $CD56^+$%) should equal the purity of the lymphocytes in the gate $\pm$ 5%, with a maximum variability of $\leq$ 10%.

END