

**BIOLOGICAL HEALTH RISKS  
QUALITY OF LABORATORIES**

**CLINICAL BIOLOGY COMMISSION  
COMMITTEE OF EXPERTS**

**EXTERNAL QUALITY ASSESSMENT  
IN CLINICAL BIOLOGY**

**DEFINITIVE GLOBAL REPORT**

**FLOW CYTOMETRY: LYMPHOCYTE SUBSET ANALYSIS**

**SURVEY 2023/1**

**Sciensano/Flow cytometry/85-E**

Biological health risks  
Quality of laboratories  
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 28/03/2023

Authorization of the report: by Lobna Bouacida, scheme coordinator

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All the reports are also available on our webpage:

- NL: <https://www.sciensano.be/nl/kwaliteit-van-laboratoria>
- FR: <https://www.sciensano.be/fr/qualite-des-laboratoires>

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## INTERPRETATION OF THE INDIVIDUAL REPORT

Besides this global report, an individual report is at your disposal via toolkit.

Below you can find information to help you interpreting this report.

The position of your quantitative results is presented on the one hand in comparison with the results from all the participants and on the other hand in comparison with the results of the laboratories using your method.

Following information is provided:

- Your result ( $R$ )
- Your method
- Global median ( $M_G$ ):  
central value of the results obtained by all laboratories (all methods together).
- Global standard deviation ( $SD_G$ ):  
measure of the spread of the results obtained by all the laboratories (all methods together).
- Global median of your method ( $M_M$ ):  
central value of the results obtained by the laboratories using your method.
- Standard deviation of your method ( $SD_M$ ):  
measure of the spread of the results obtained by the laboratories using your method.
- The coefficient of variation  $CV$  (expressed in %) for all laboratories and for the laboratories using your method:  
$$CV_M = (SD_M / M_M) * 100 (\%) \text{ and } CV_g = (SD_G / M_G) * 100 (\%).$$

- 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 %):  
$$U_m = ((R - M_M) / M_M) * 100 (\%) \text{ and } U_G = ((R - M_G) / M_G) * 100 (\%).$$
- 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 ( $M \pm 2.7 SD$ )

**O<sub>M/G</sub>** : external limits ( $M \pm 4.7 SD$ )

The global graph and the one of your method are presented on the same scale, which allows you to compare them. These graphs give you a rough estimation of the position of your result (R) with respect to the medians ( $M_{M/G}$ ).

More information can be found in the brochures available on our website (only in Dutch and French):

Klinische gezondheid | EKE klinische biologie | sciensano.be

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

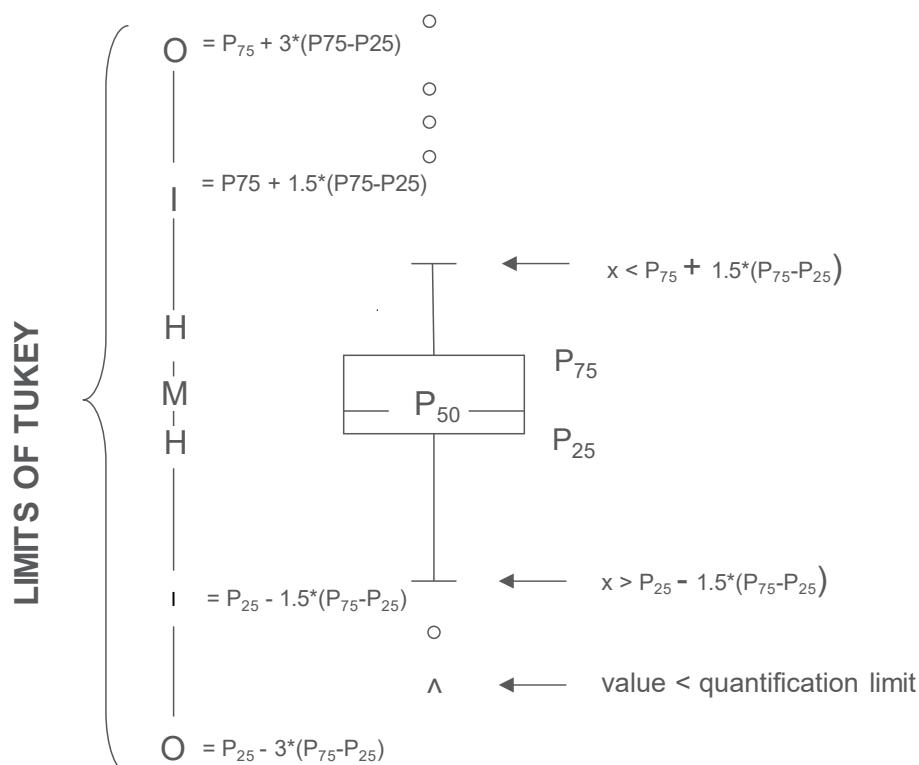
Santé clinique | EEQ biologie clinique | sciensano.be

- Brochure d'information générale EEQ
- Méthodes statistiques appliquées à l'EEQ
- Traitement des valeurs censurées

## Graphical representation

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

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



Corresponding limits in case of normal distribution

## SAMPLE MATERIAL

Two blood samples (FC/19753 and FC/19754) collected on K2EDTA were sent to the laboratories.

These two samples were collected from two healthy and voluntary blood donors and distributed into aliquots at Sciensano.

The samples were sent by Taxipost 24h and the laboratories were informed by e mail of the send out of the control material (day 0).

Homogeneity was confirmed based on white blood cells determination.

At Sciensano's request, the UZ Brussel laboratory, friendly, carried out control analysis not only on the day of collection and distribution (day 0), but also on days 1, 3 and 4.

### FC/19753

FC/19753	Day 0	Day 1	Day 3	Day 4
<b>WBC 10E9/L</b>	5.1	5.2	4.5	4.5
<b>Lympho% haematology analyser</b>	35.8	34.5	37.5	37.2
<b>Lympho% flow cytometer</b>	32.9	31.6	30.1	33
<b>CD3 %</b>	78.5	79	77.8	77.6
<b>CD3 10E9/L</b>	1.43	1.42	1.31	1.30
<b>CD4 %</b>	52.6	53.5	53.3	53.3
<b>CD4 10E9/L</b>	0.96	0.96	0.90	0.89
<b>CD8 %</b>	24.4	24.8	23.2	22.8
<b>CD8 10E9/L</b>	0.45	0.44	0.39	0.38
<b>CD19 %</b>	8.6	7.7	7.8	8.8
<b>CD19 10E9/L</b>	0.16	0.14	0.13	0.15
<b>NKcells %</b>	12.7	12.9	13.5	13.1
<b>NKcells 10E9/L</b>	0.23	0.23	0.23	0.22
<b>Kappa % B lymphocytes</b>	56.5	55.8	56.1	56.2
<b>Lambda % B lymphocytes</b>	43.4	44.2	43.8	43.8
<b>Kappa/lambda</b>	1.30	1.26	1.28	1.28
<b>Sum K+L % B lymphocytes</b>	99.9	100	99.9	100
<b>Lymphosum %</b>	99.8	99.6	99.1	99.5

**FC/19754**

FC/19754	Day 0	Day 1	Day 3	Day 4
<b>WBC 10E9/L</b>	9.3	9.3	8.9	9.0
<b>Lympho% haematology analyser</b>	24.5	24	24.3	25.1
<b>Lympho% flow cytometer</b>	24.6	23.9	24.6	24.5
<b>CD3 %</b>	59.1	57.9	59.3	58.4
<b>CD3 10E9/L</b>	1.35	1.29	1.28	1.32
<b>CD4 %</b>	46.2	44.1	45.8	44.7
<b>CD4 10E9/L</b>	1.05	0.98	0.99	1.01
<b>CD8 %</b>	11.7	13	12.6	13
<b>CD8 10E9/L</b>	0.27	0.29	0.27	0.29
<b>CD19 %</b>	9.6	11.4	10.5	10.5
<b>CD19 10E9/L</b>	0.22	0.25	0.23	0.24
<b>NKcells %</b>	31.2	30.4	29.5	31.9
<b>NKcells 10E9/L</b>	0.71	0.68	0.64	0.72
<b>Kappa % B lymphocytes</b>	56.2	54.4	56.1	56.5
<b>Lambda % B lymphocytes</b>	43.7	45.6	43.9	43.5
<b>Kappa/lambda</b>	1.29	1.19	1.28	1.30
<b>Sum K+L % B lymphocytes</b>	99.9	100	100	100
<b>Lymphosum %</b>	99.9	99.7	99.3	100.8

A first statistical analysis of these results did not show a significant stability problem. On the other hand, the analysis of the results of the survey showed a lack of stability for the majority of the parameters. This discrepancy is likely due to some difference in the transport and storage conditions of the samples.

## PARTICIPATION

Forty-eight Belgian clinical laboratories participated in the survey 2023/1 (send-out of blood samples on 27 February 2023 (day 0)).

## RESULTS

Due to a postal strike, the laboratories did not receive the samples within 48 hours of collection.

85% of the Belgian laboratories having taken part in the survey received the samples on day 3 and 15% received them on day 4. Laboratories that received the samples after that did not submit results. In fact, the laboratories were asked to submit their results until day 4.

69% of the Belgian laboratories having taken part in the survey performed the analyses on day 3 and 31% on day 4.

Statistics for the evaluation were solely based on the results of the Belgian clinical laboratories. Since there were no results within 48 hours, statistics were exceptionally calculated on all results, obtained up to day 4 (n=48).

A retrospective analysis of the stability based on the results of this survey allowed to validate the stability of certain parameters: WBC, CD19%, CD19 10E9/L, Kappa %, Lambda %, Kappa/lambda, Sum K+L. Only these parameters were retained for the evaluation of the laboratories in the unvalidated individual report.

The committee of experts has chosen not to evaluate the laboratories for any parameter. The final individual report has been adapted accordingly.

The details of the stability assessment are available on page 38.

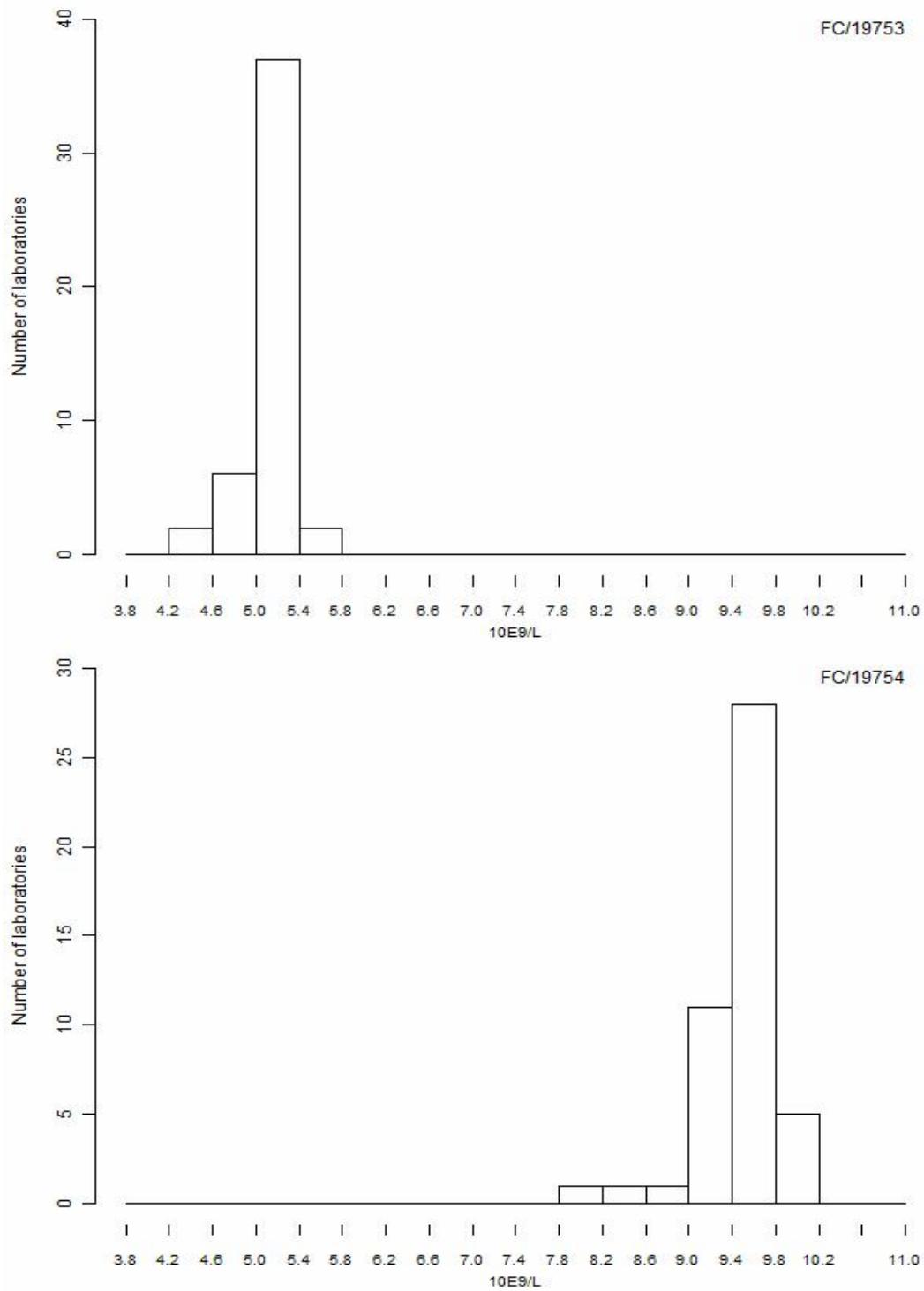
The following tables show the medians and coefficients of variation obtained by the Belgian clinical laboratories for the samples FC/19753 and FC/19754:

<b>FC/19753</b>	<b>Median</b>	<b>SD</b>	<b>CV, %</b>	<b>N</b>
<b>WBC 10E9/L</b>	5.18	0.11	2.1	47
<b>Lympho% haematology analyser</b>	37.0	1.8	4.8	44
<b>Lympho% flow cytometer</b>	34.2	3.6	10.4	41
<b>CD3 %</b>	82.3	4.3	5.2	48
<b>CD3 10E9/L</b>	1.521	0.135	8.9	47
<b>CD4 %</b>	57.0	4.7	8.2	48
<b>CD4 10E9/L</b>	1.062	0.107	10.0	47
<b>CD8 %</b>	23.0	1.6	6.8	48
<b>CD8 10E9/L</b>	0.423	0.041	9.8	47
<b>CD19 %</b>	7.0	0.9	12.7	48
<b>CD19 10E9/L</b>	0.135	0.022	16.4	48
<b>NKcells %</b>	10.9	4.7	43.4	48
<b>NKcells 10E9/L</b>	0.190	0.082	43.0	47
<b>Kappa % B lymphocytes</b>	54.6	2.7	4.9	39
<b>Lambda % B lymphocytes</b>	44.7	3.2	7.1	39
<b>Kappa/lambda</b>	1.20	0.14	11.4	39
<b>Sum K+L % B lymphocytes</b>	99.7	1.0	1.0	39
<b>Lymphosum %</b>	99.5	0.6	0.6	48

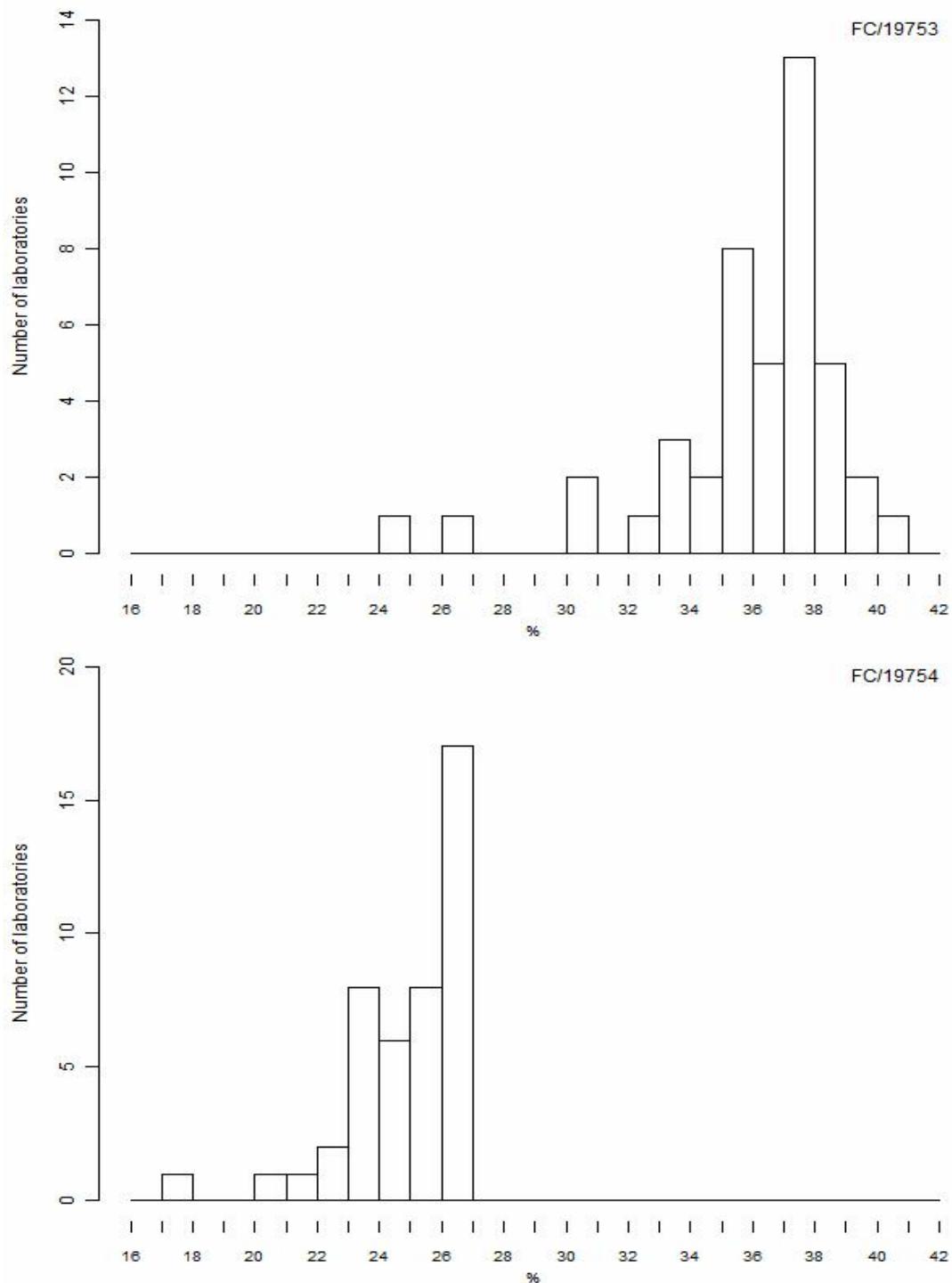
<b>FC/19754</b>	<b>Median</b>	<b>SD</b>	<b>CV, %</b>	<b>N</b>
<b>WBC 10E9/L</b>	9.52	0.23	2.4	47
<b>Lympho% haematology analyser</b>	25.5	1.6	6.4	44
<b>Lympho% flow cytometer</b>	24.4	2.6	10.8	41
<b>CD3 %</b>	65.6	8.3	12.6	48
<b>CD3 10E9/L</b>	1.598	0.269	16.8	47
<b>CD4 %</b>	49.2	6.4	13.1	48
<b>CD4 10E9/L</b>	1.221	0.194	15.9	47
<b>CD8 %</b>	13.7	1.8	13.0	48
<b>CD8 10E9/L</b>	0.345	0.062	18.0	47
<b>CD19 %</b>	10.1	1.1	11.0	48
<b>CD19 10E9/L</b>	0.250	0.041	16.3	48
<b>NKcells %</b>	22.4	8.6	38.3	48
<b>NKcells 10E9/L</b>	0.540	0.200	37.0	47
<b>Kappa % B lymphocytes</b>	56.0	1.6	2.9	40
<b>Lambda % B lymphocytes</b>	43.9	1.5	3.4	40
<b>Kappa/lambda</b>	1.28	0.08	6.4	40
<b>Sum K+L % B lymphocytes</b>	99.8	0.4	0.4	40
<b>Lymphosum %</b>	99.4	1.3	1.3	48

The coefficients of variation appear higher than those of previous surveys, in particular for the parameters: NK cells % and NK cells 10E9/L.

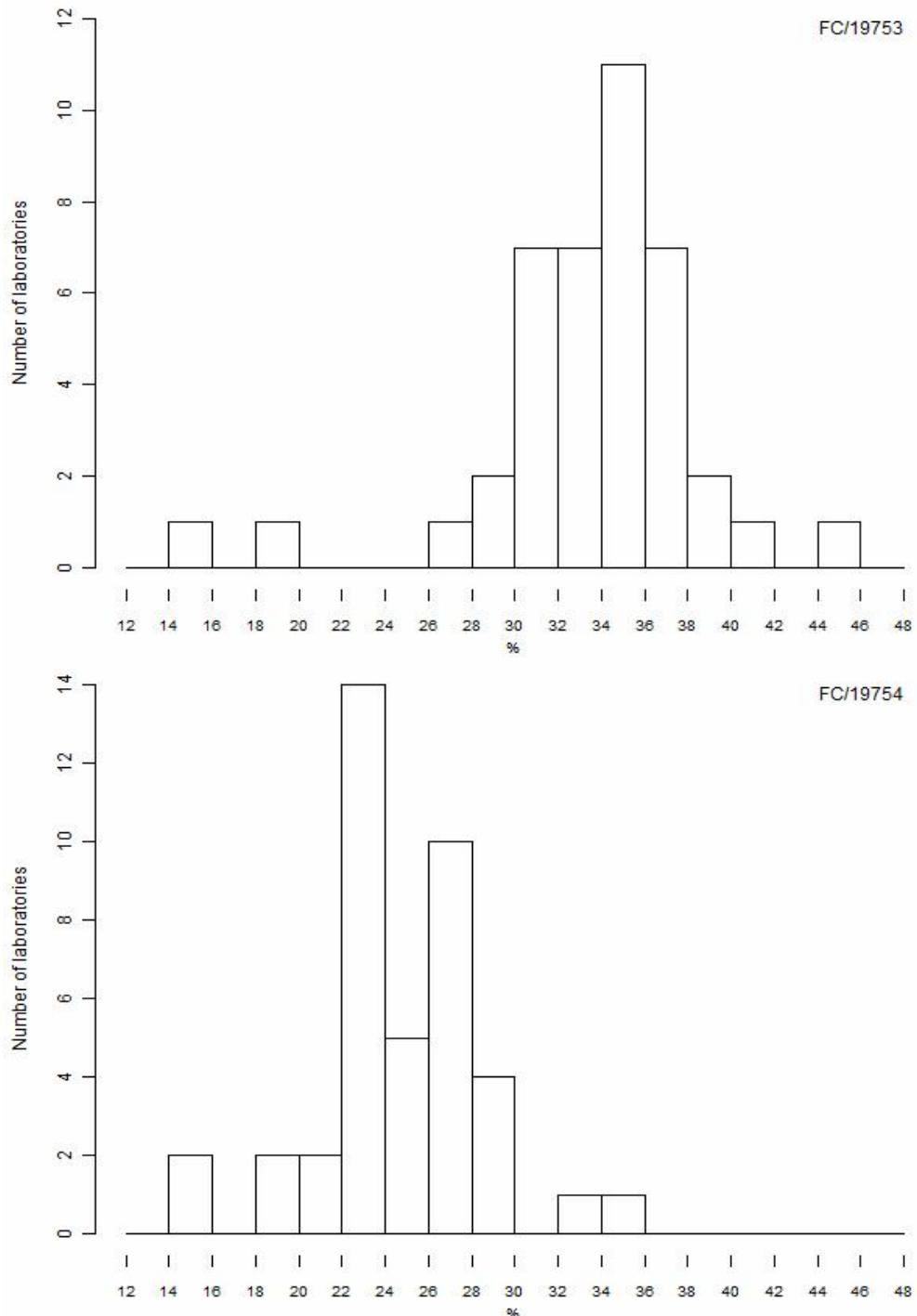
## WBC 10E9/L



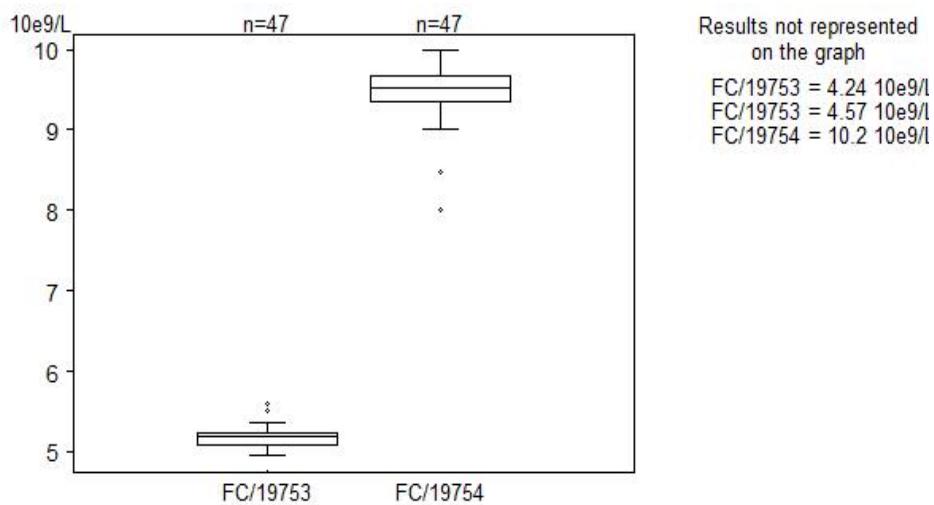
## Lympho% haematology analyser



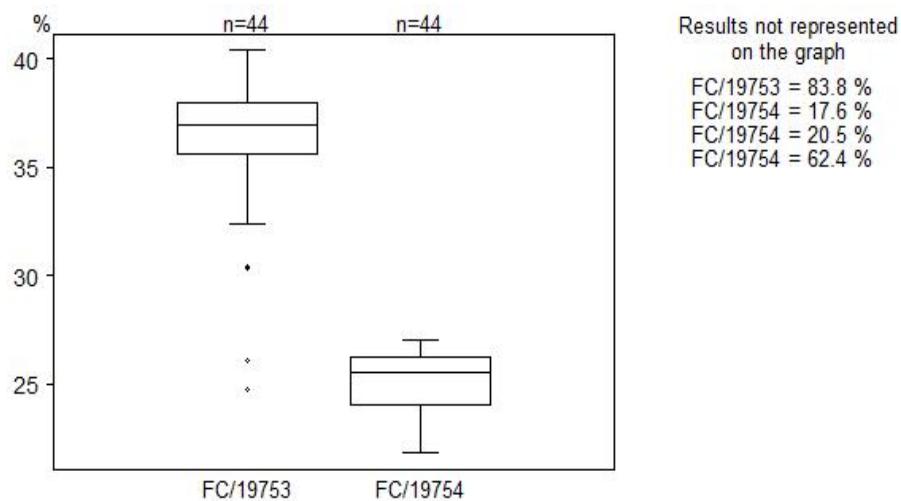
## Lympho% flow cytometer



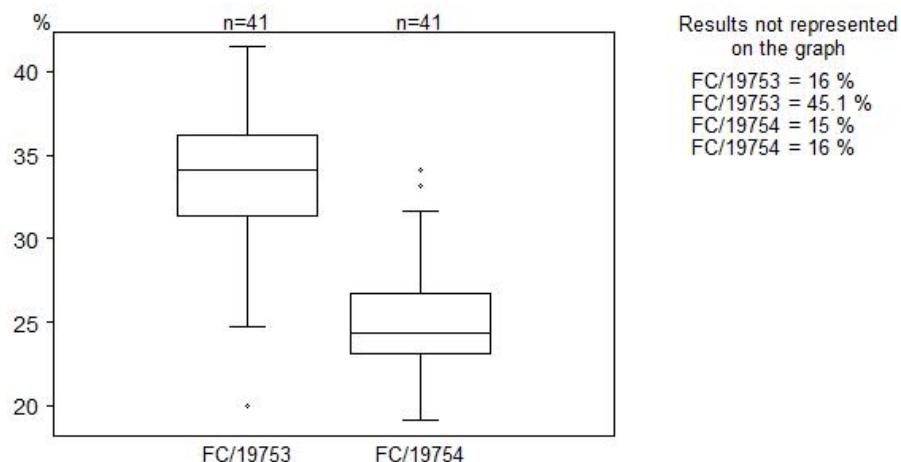
## WBC 10E9/L



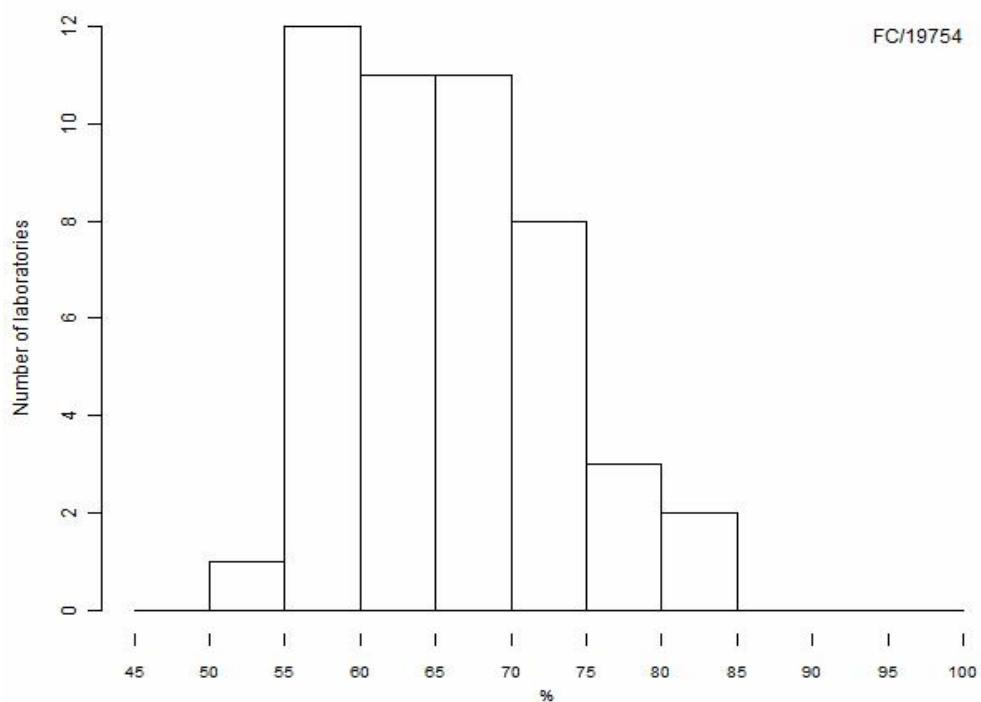
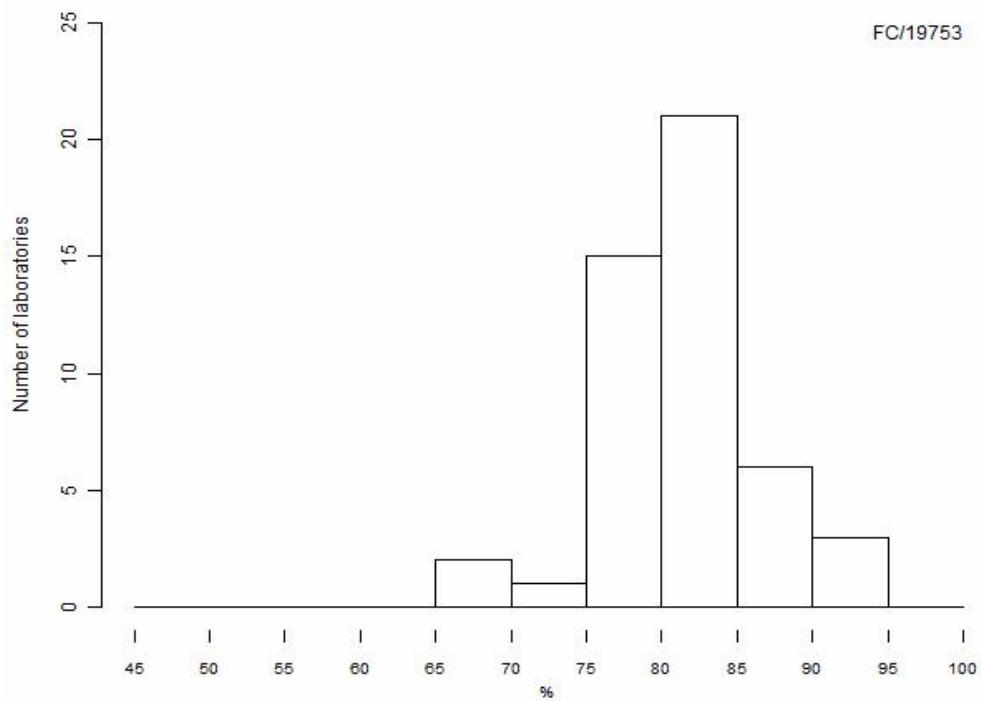
## Lympho% haematology analyser



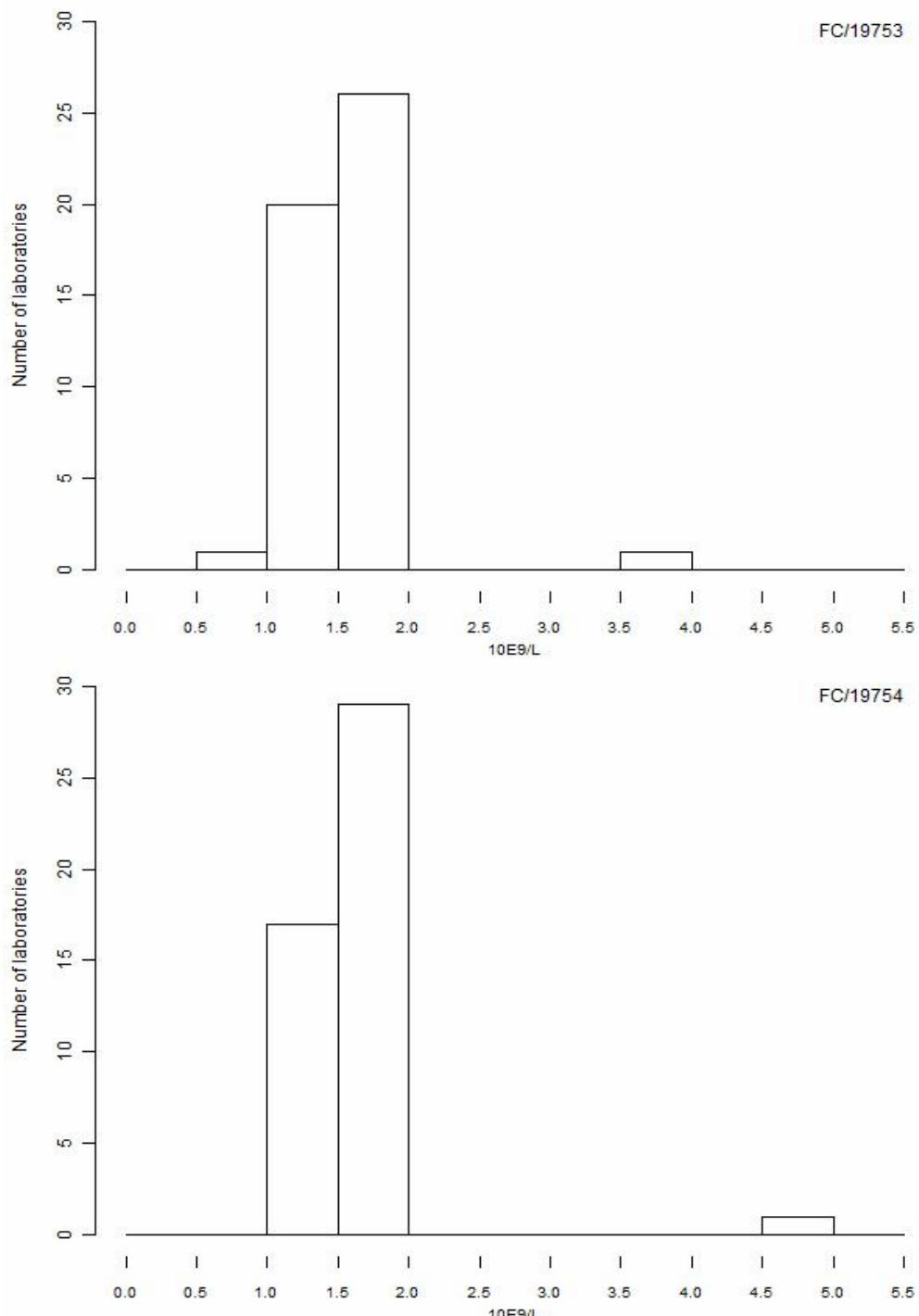
## Lympho% flow cytometer



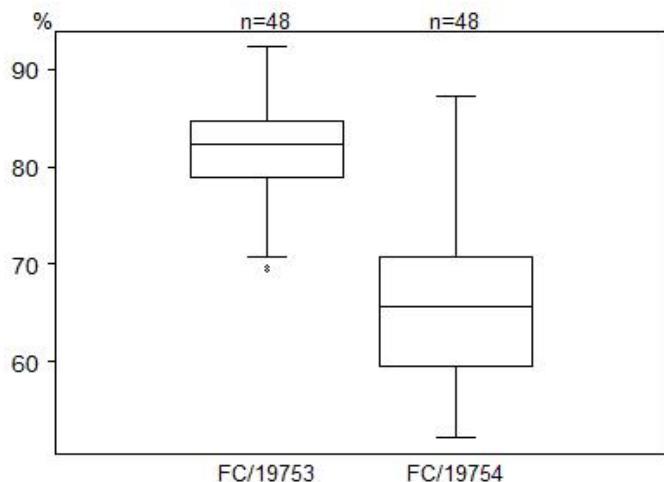
## CD3 %



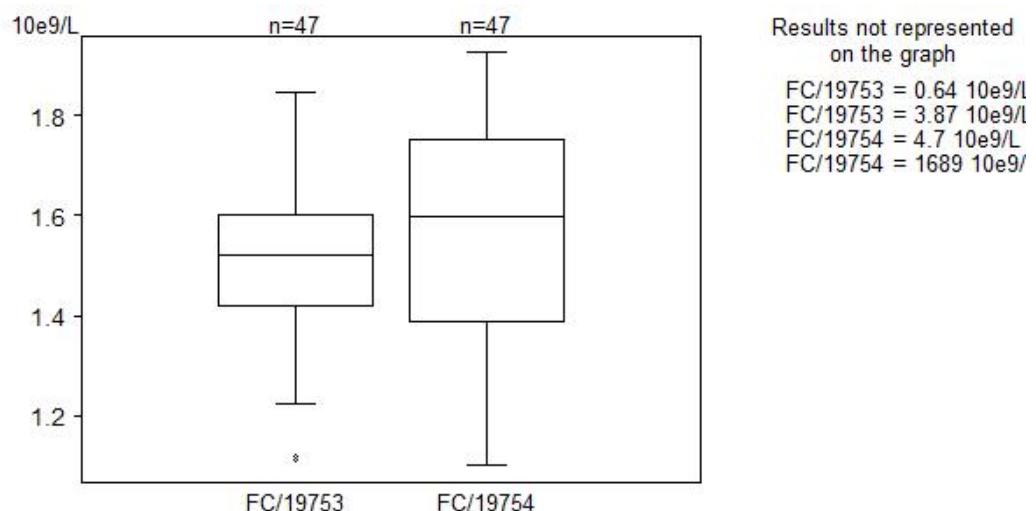
## CD3 10E9/L



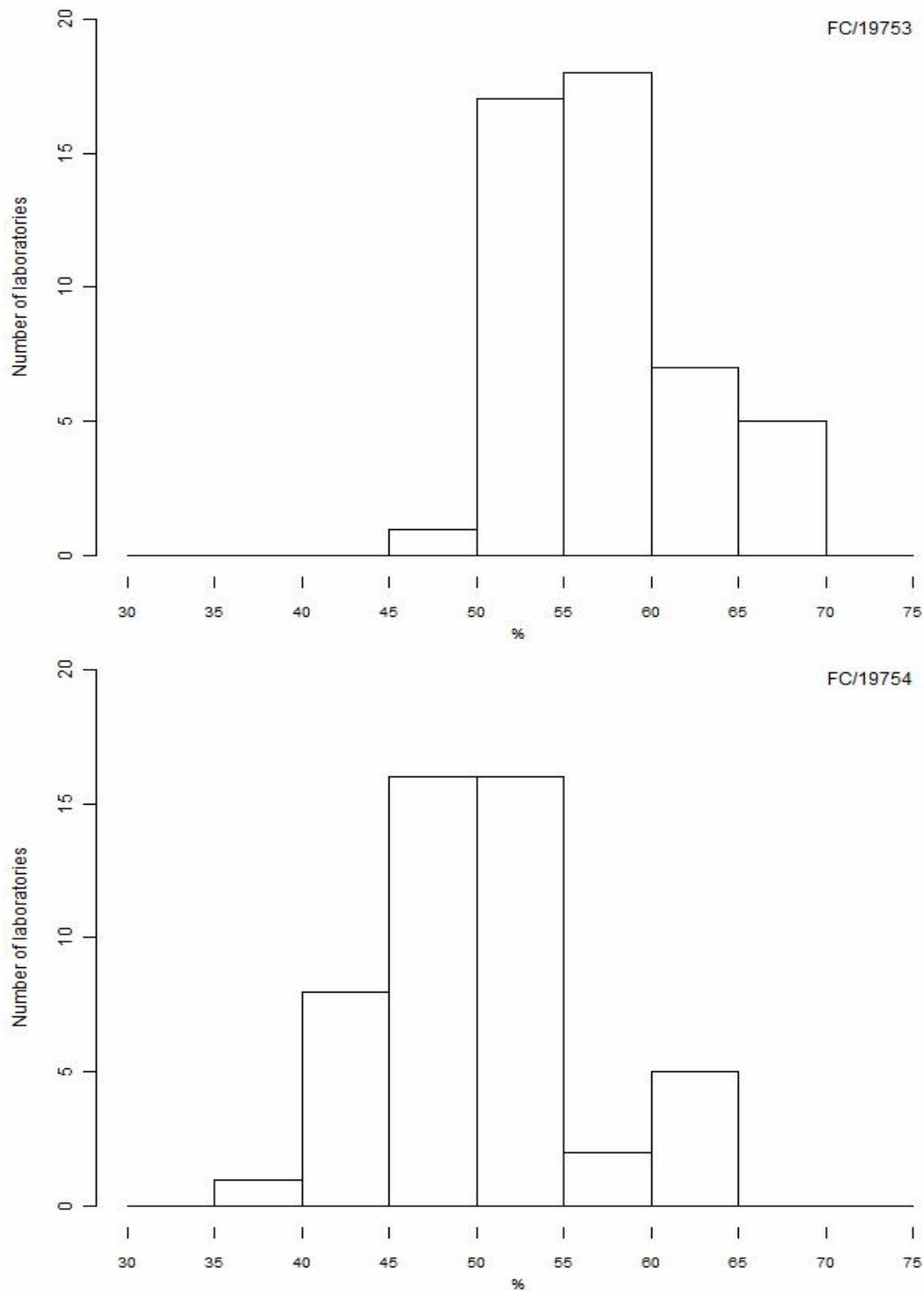
### CD3 %



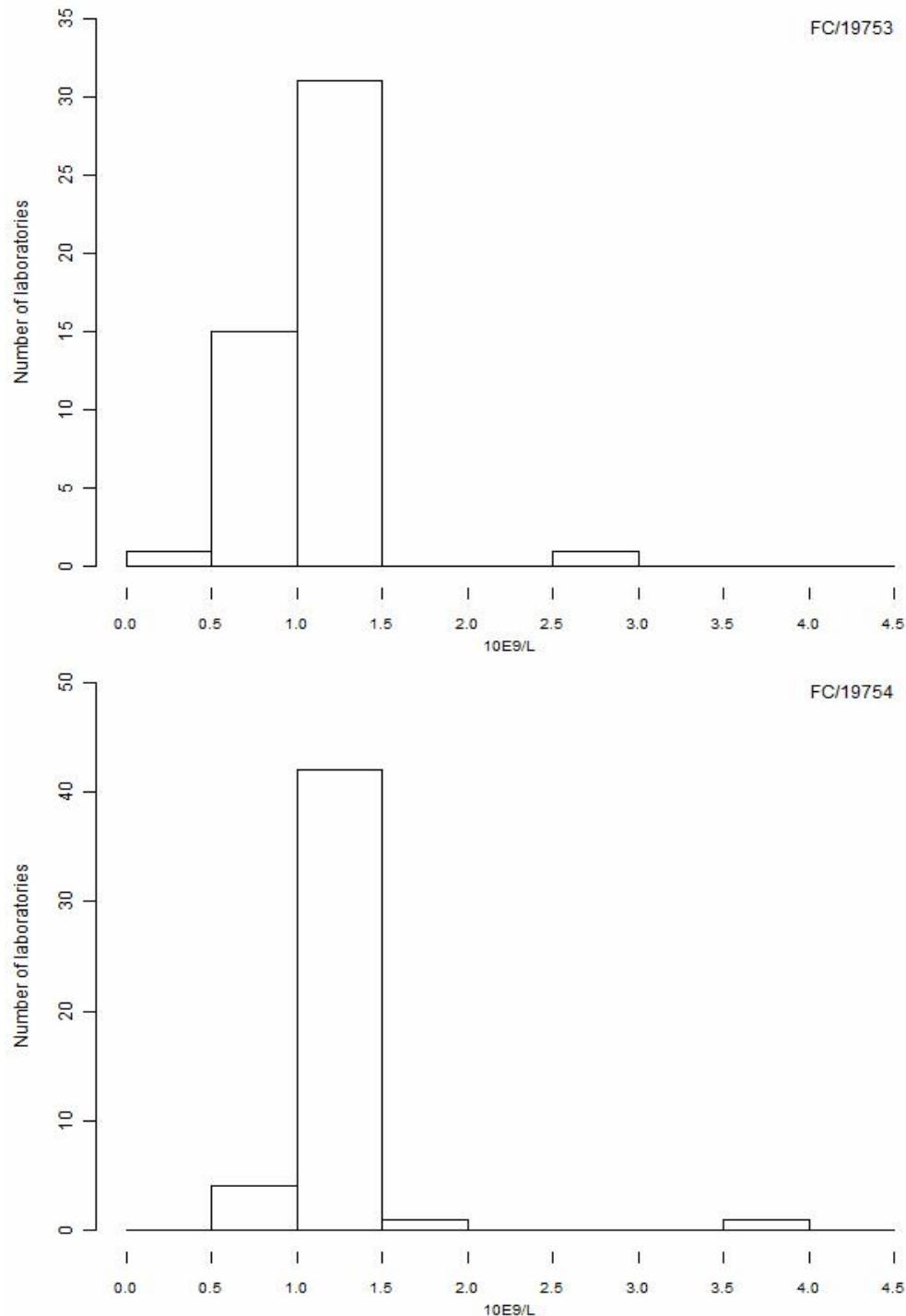
### CD3 10E9/L



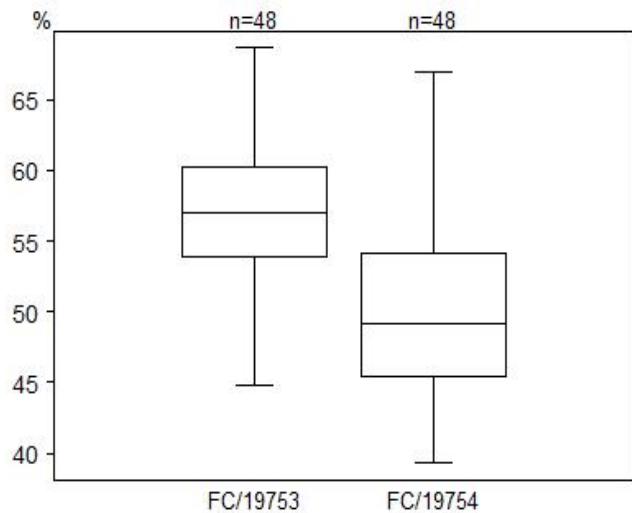
## CD4 %



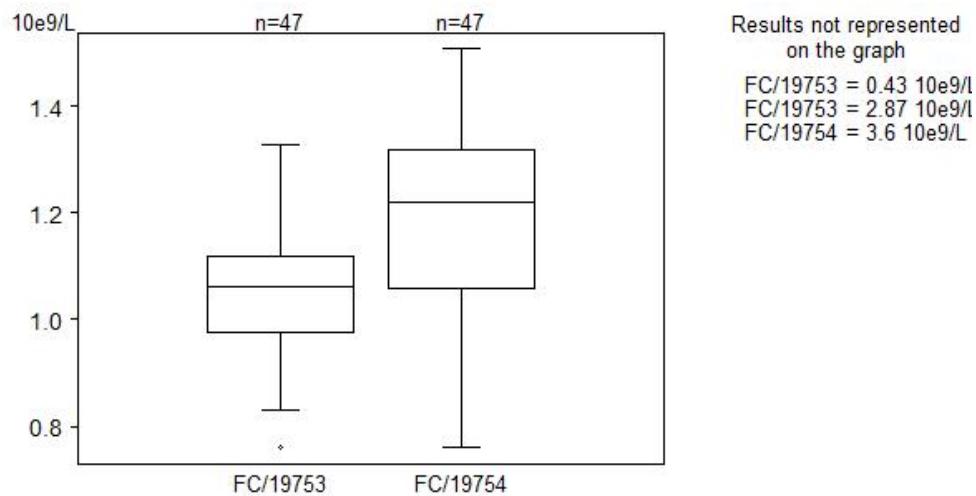
## CD4 10E9/L



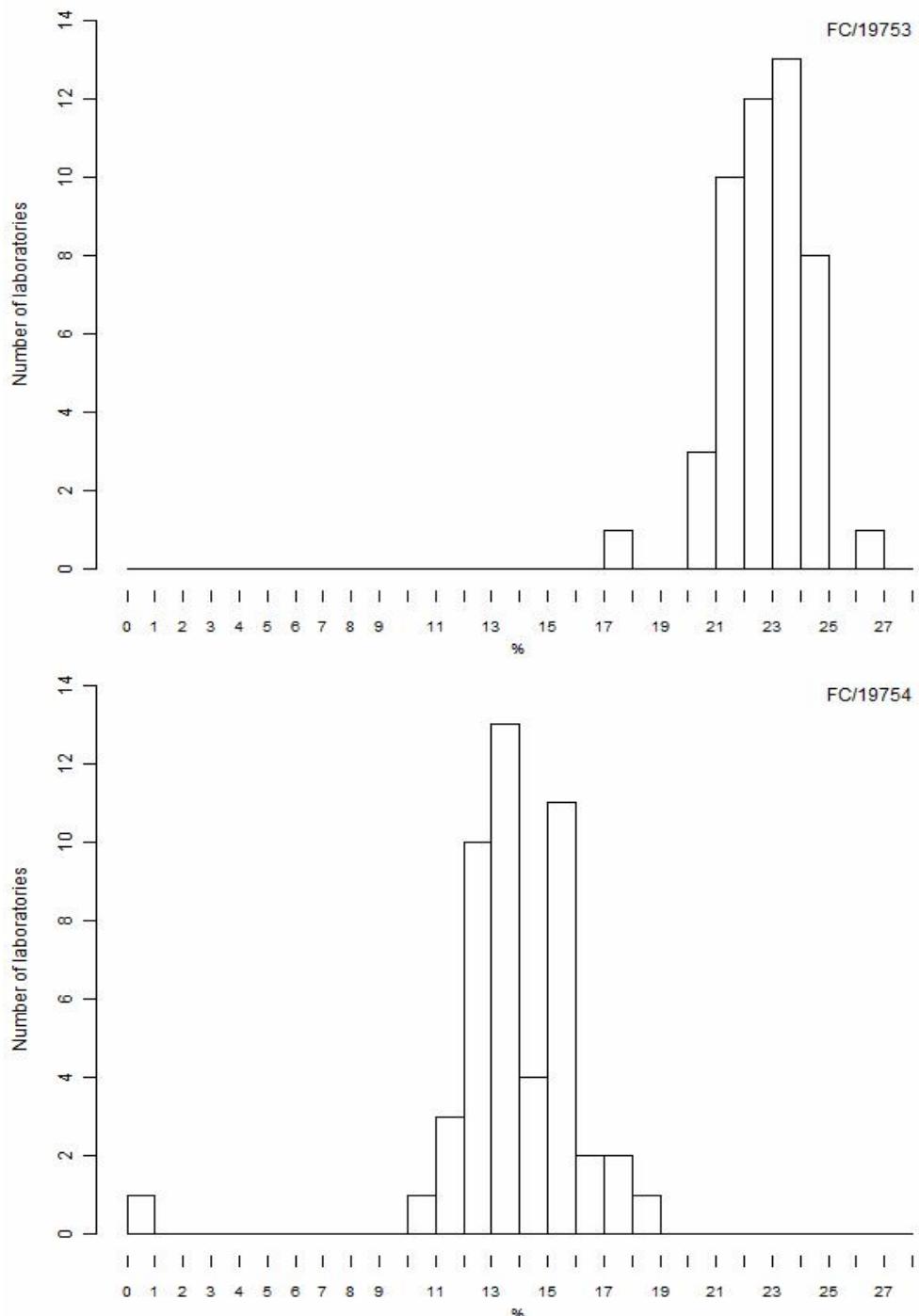
## CD4 %



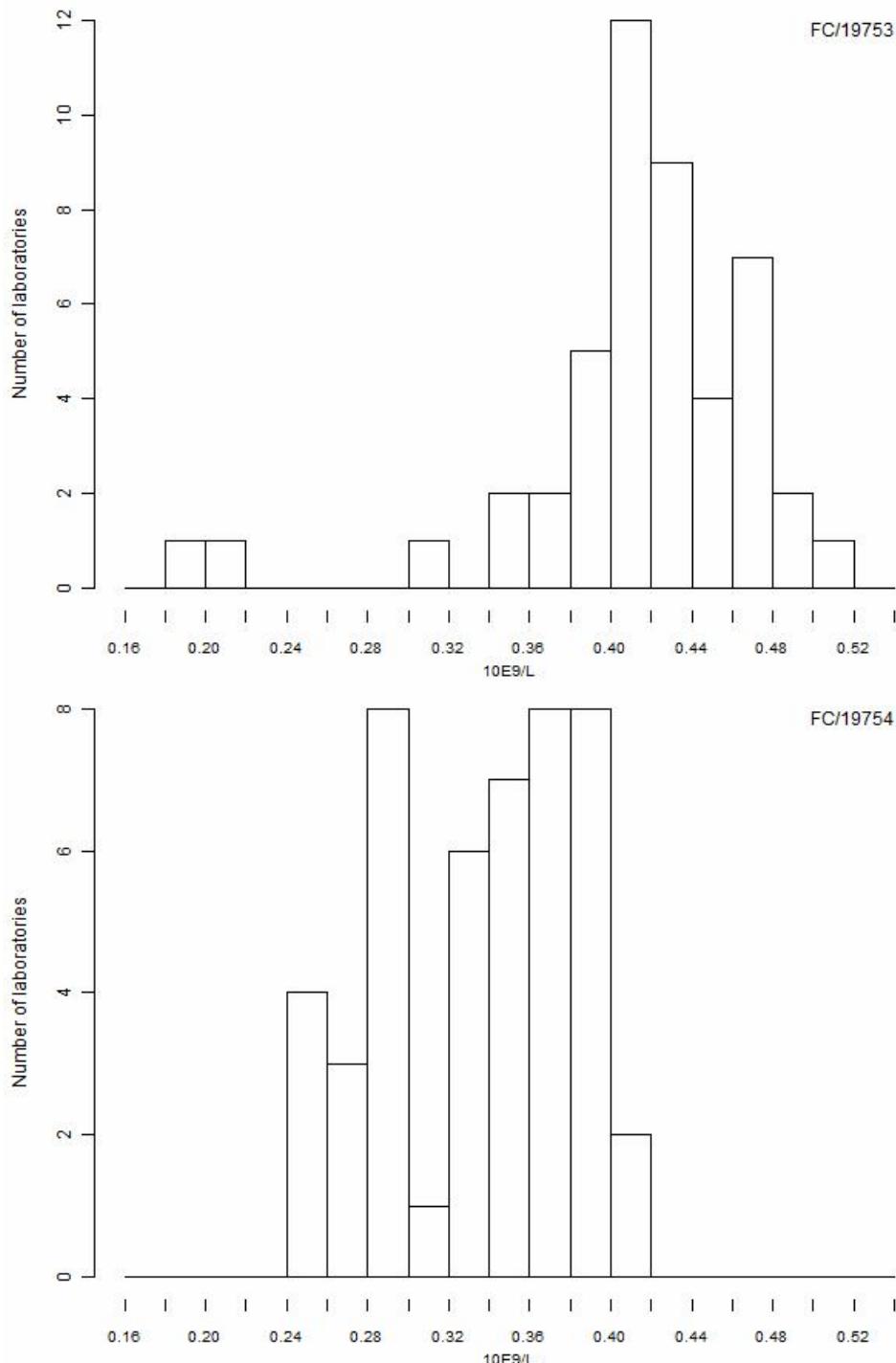
## CD4 10E9/L



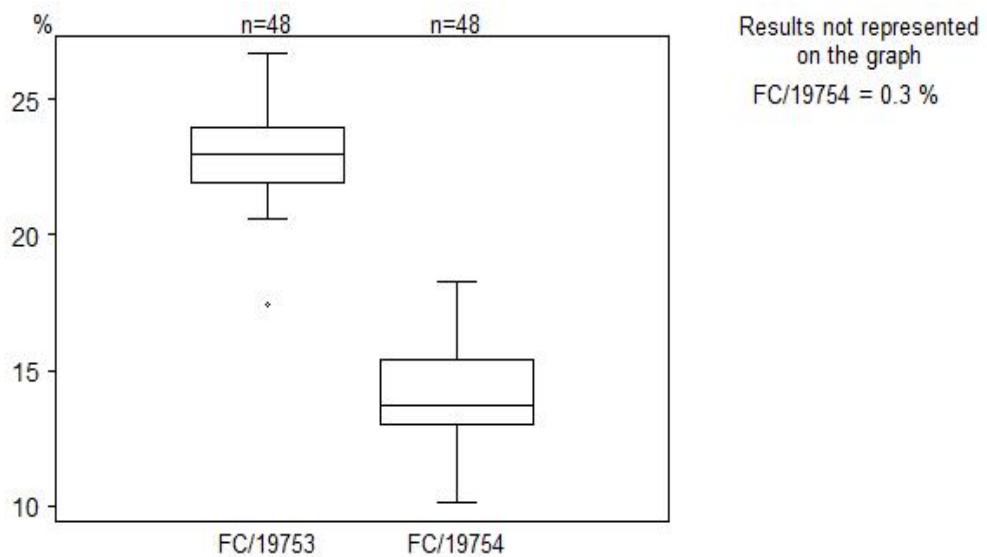
## CD8 %



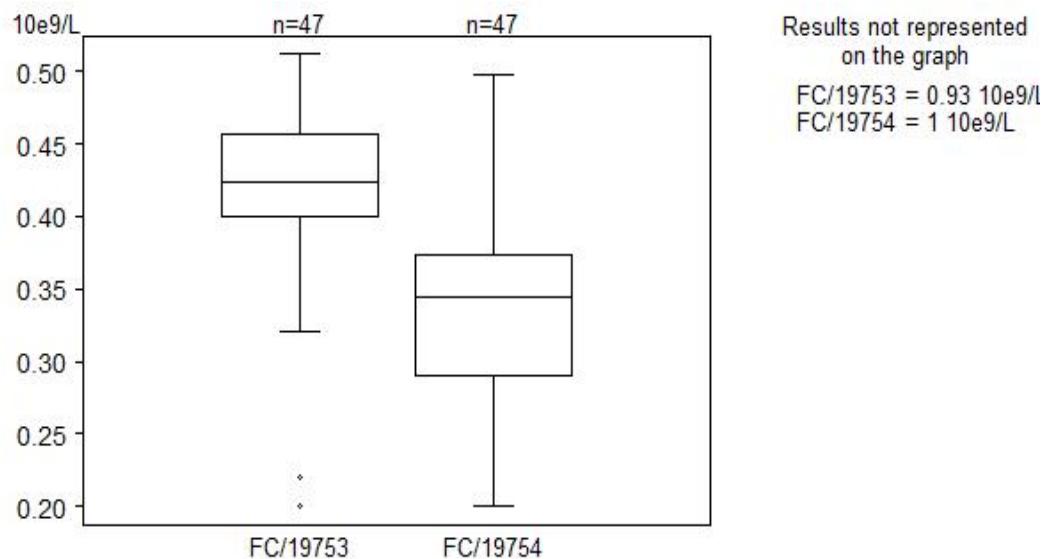
## CD8 10E9/L



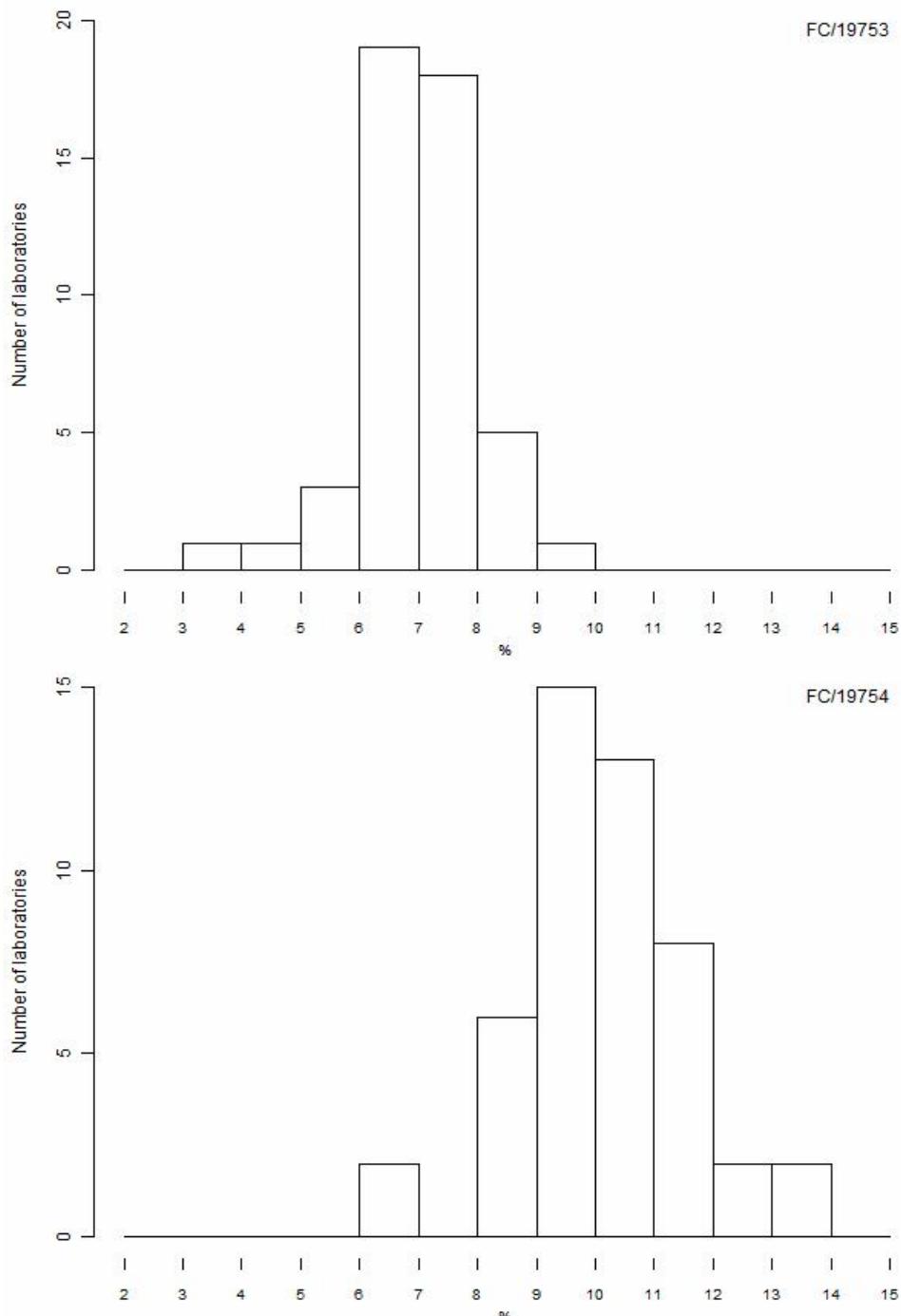
## CD8 %



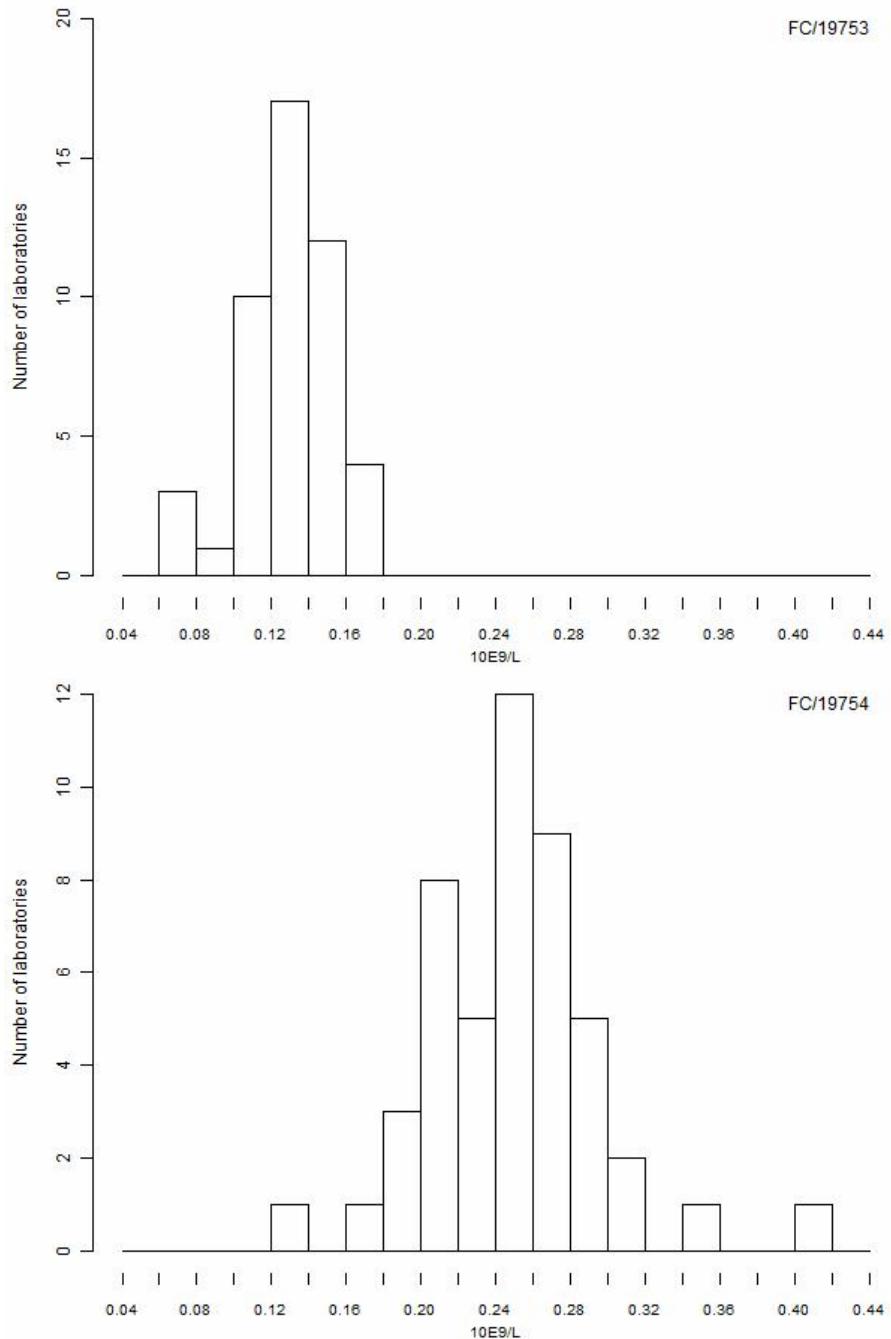
## CD8 10E9/L



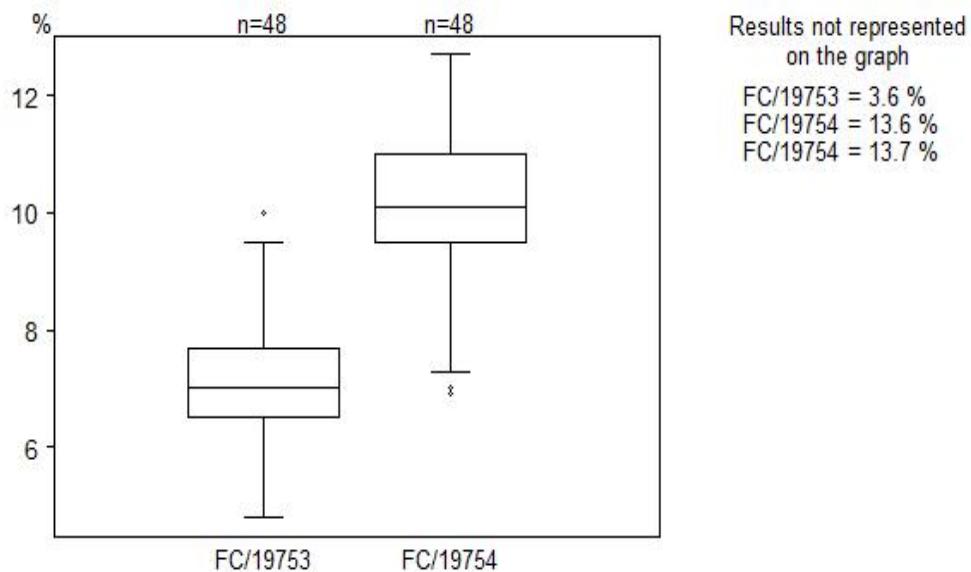
## CD19 %



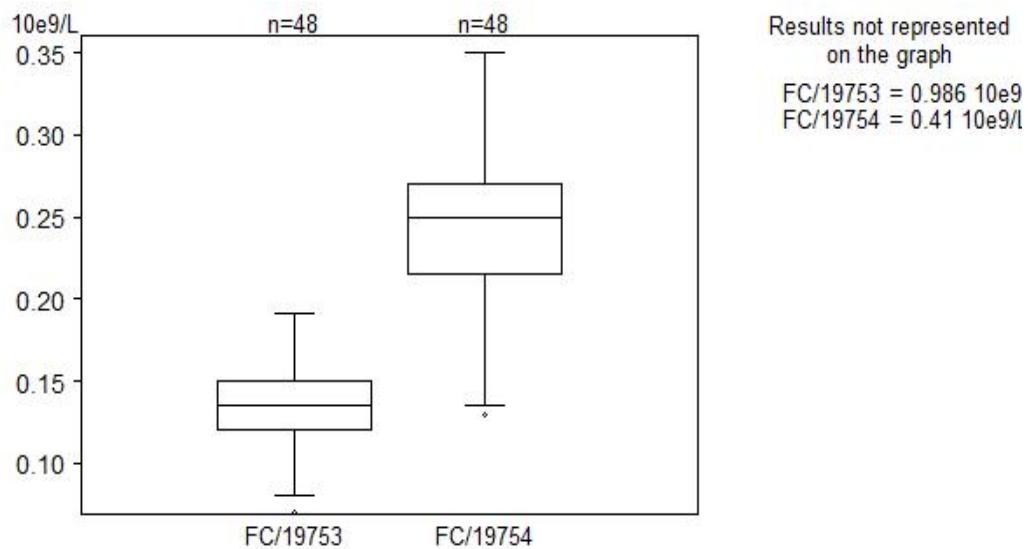
## CD19 10E9/L



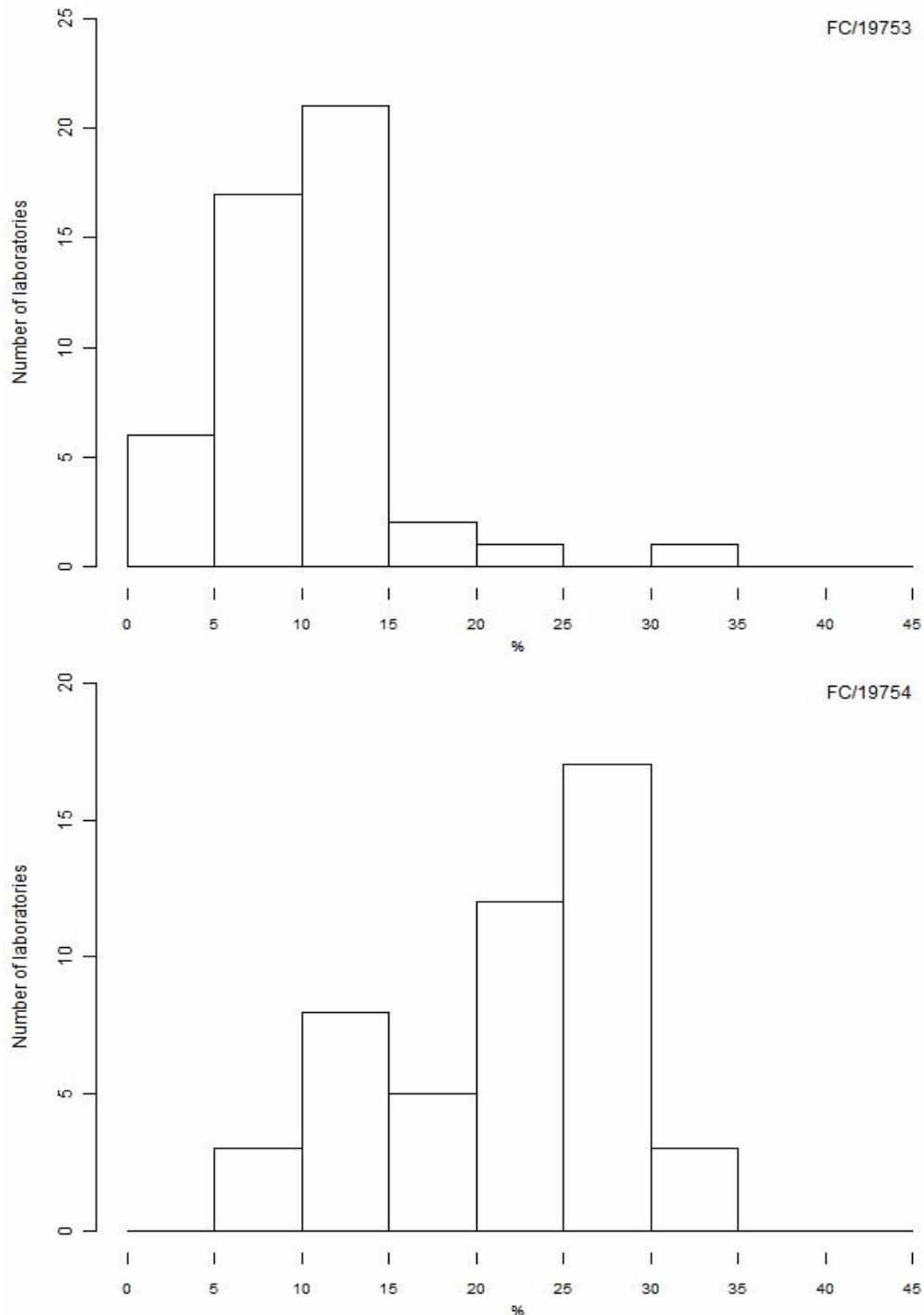
## CD19 %



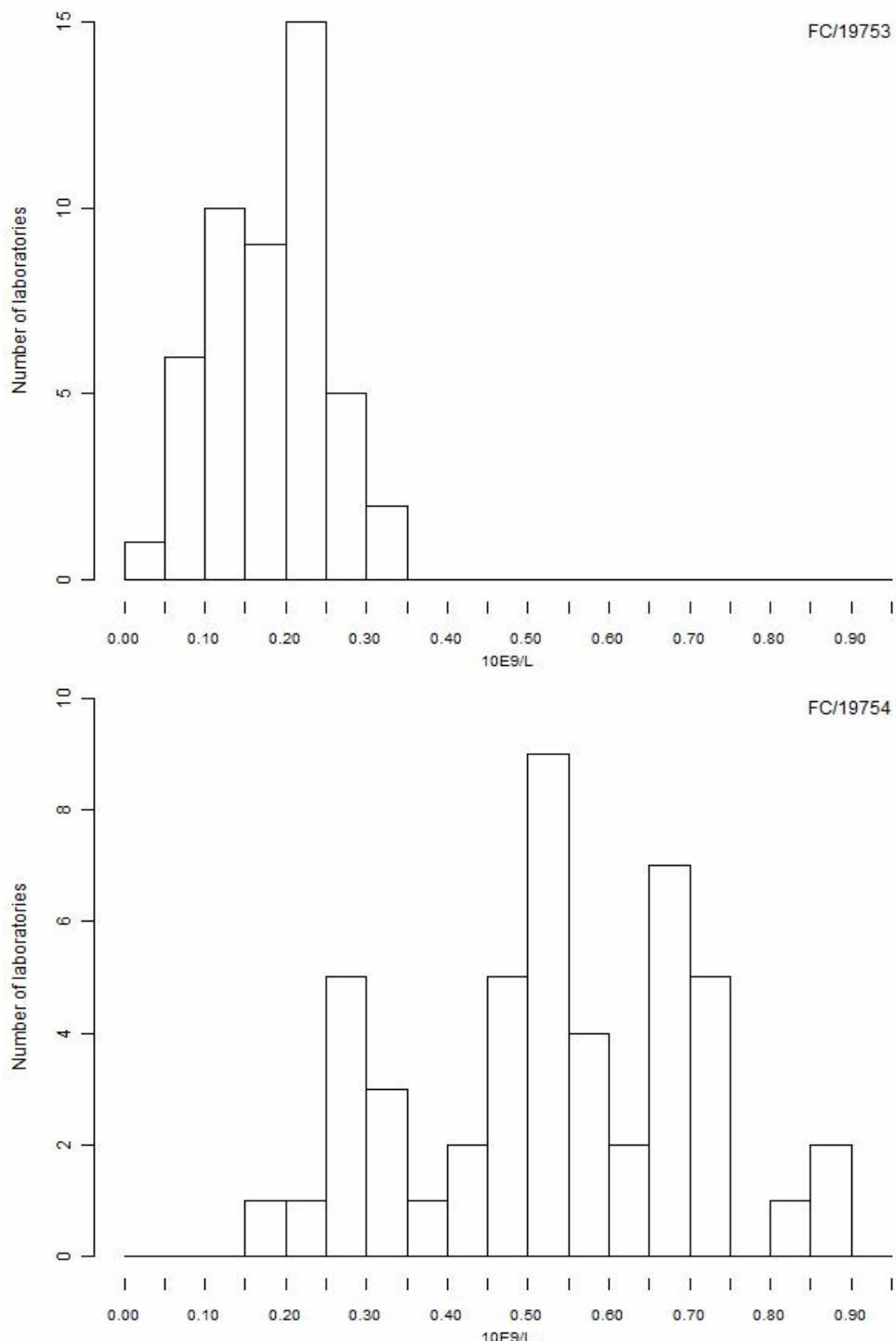
## CD19 10E9/L



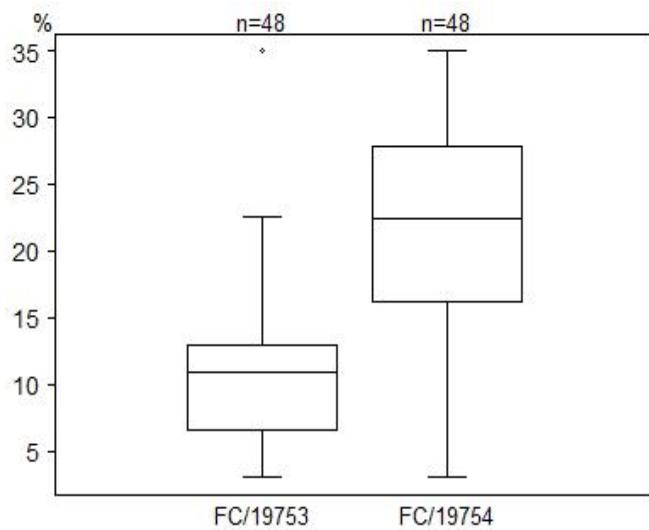
## NKcells %



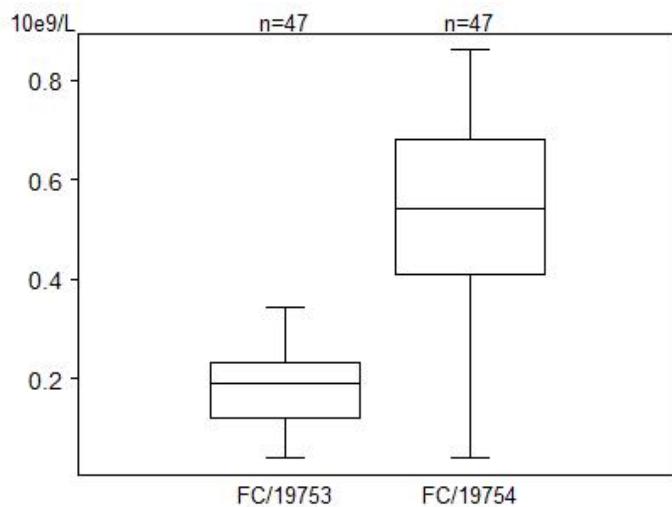
## NKcells 10E9/L



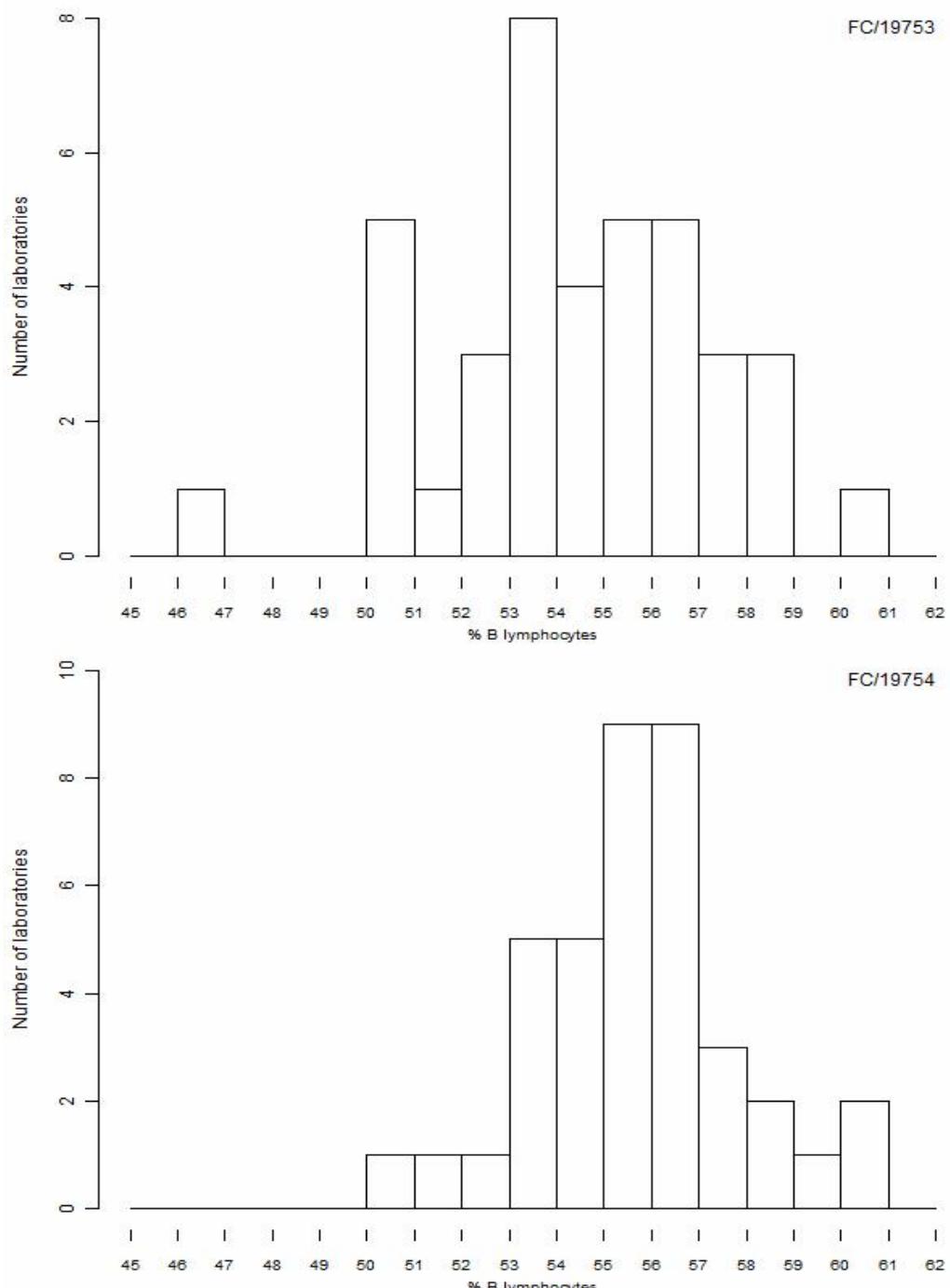
### NKcells %



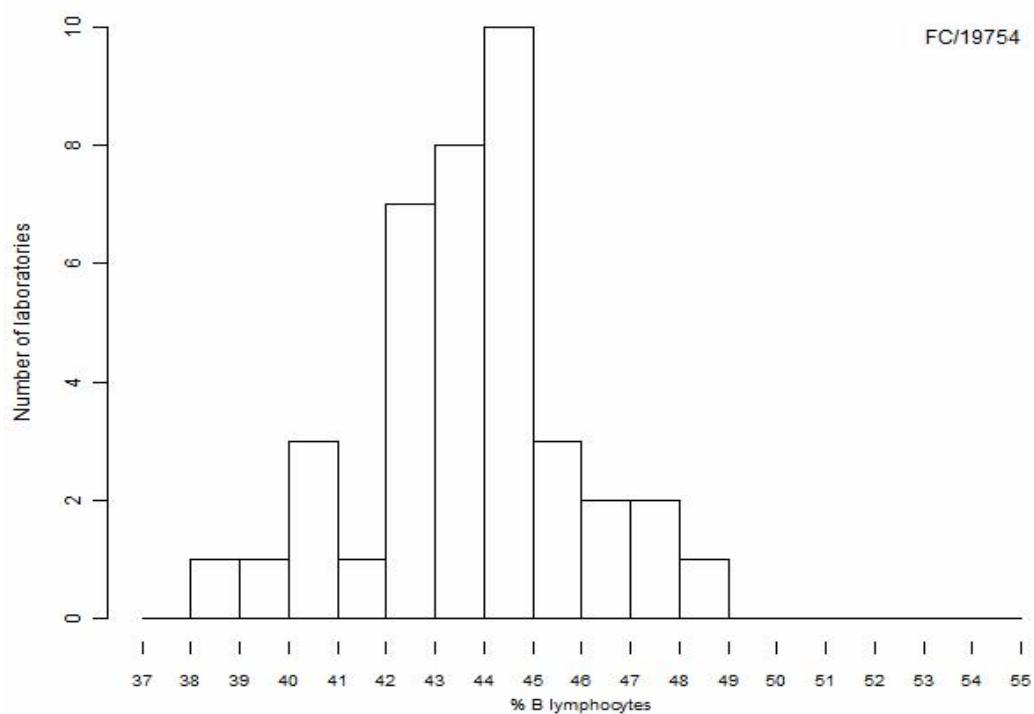
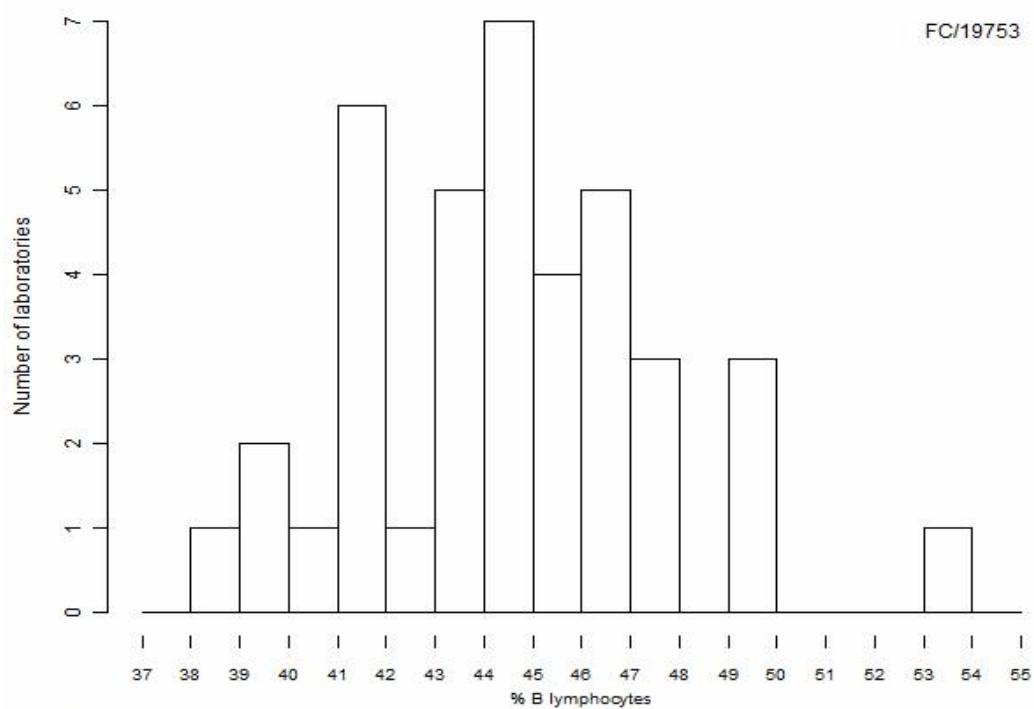
### NKcells 10E9/L



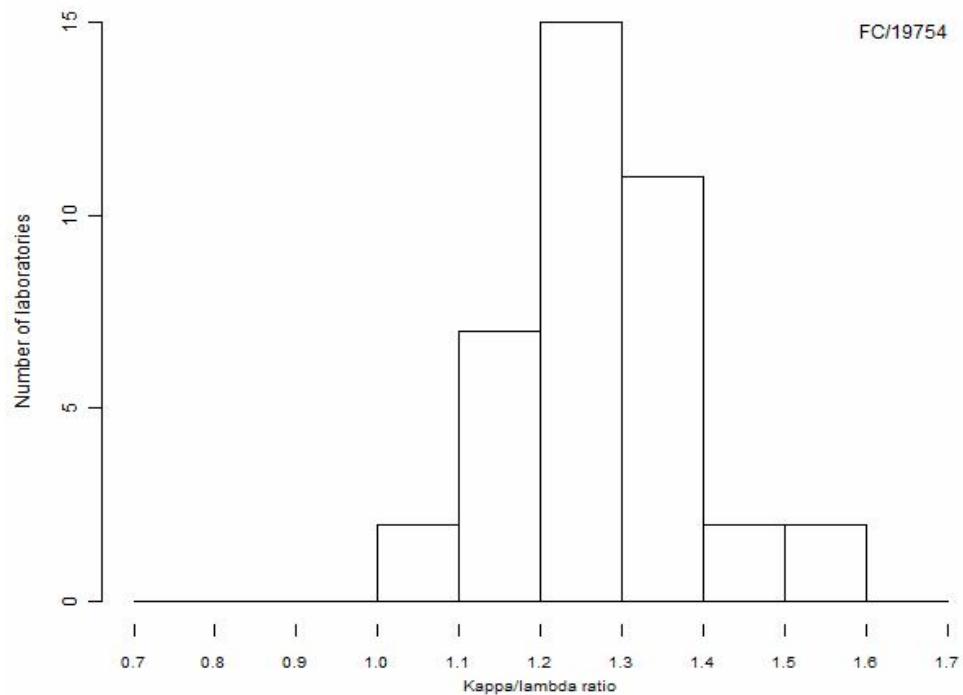
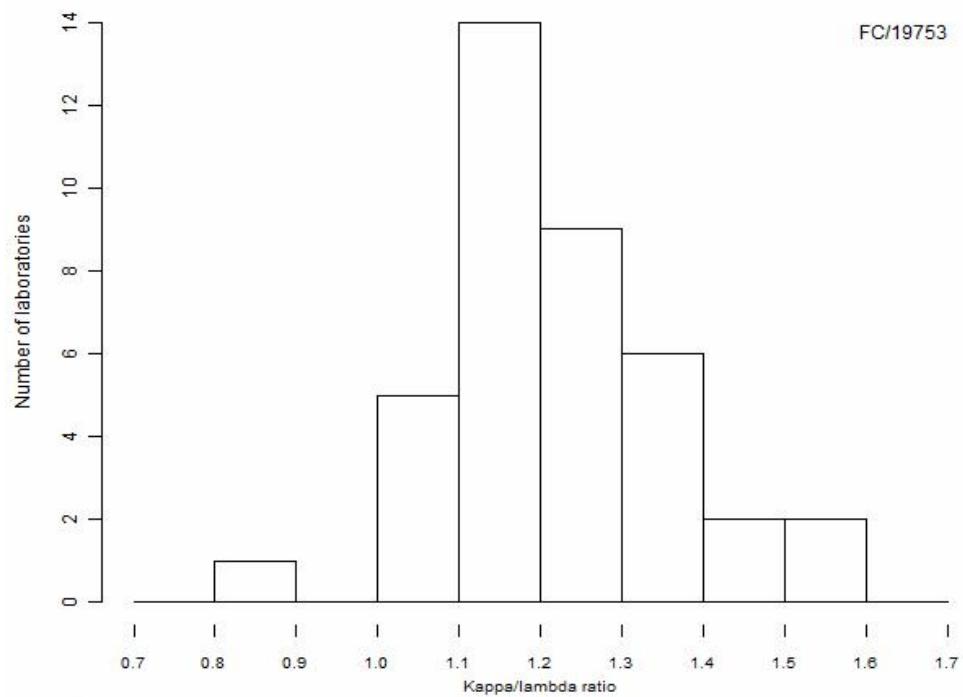
## Kappa % B lymphocytes



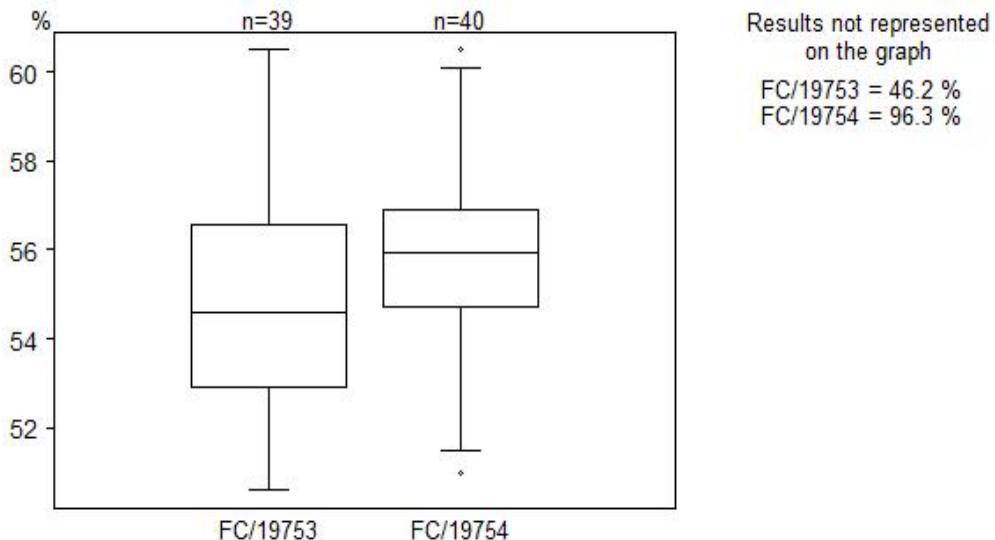
## Lambda % B lymphocytes



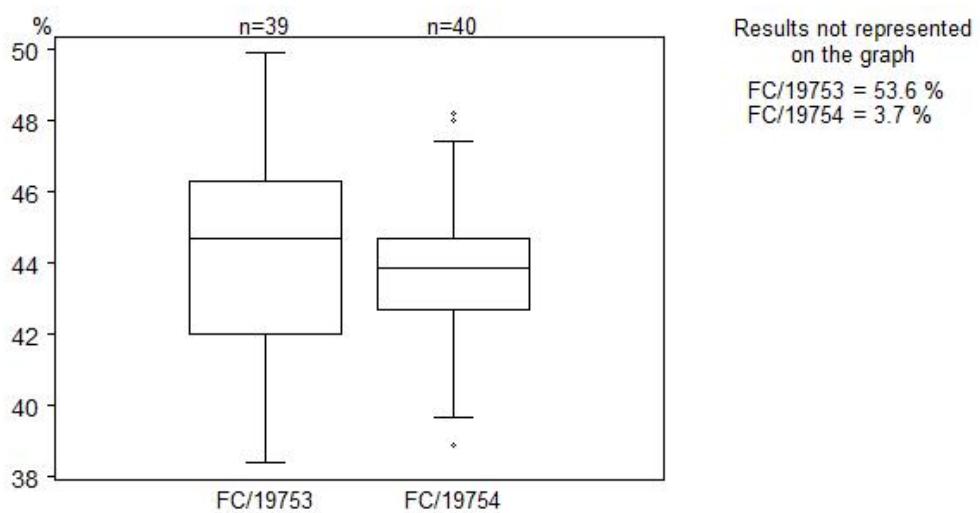
## Kappa/lambda



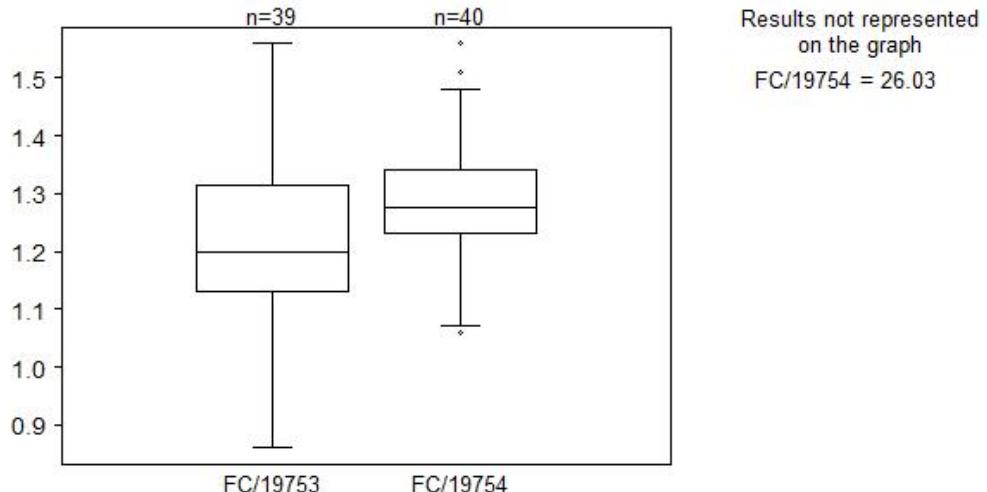
## Kappa % B lymphocytes



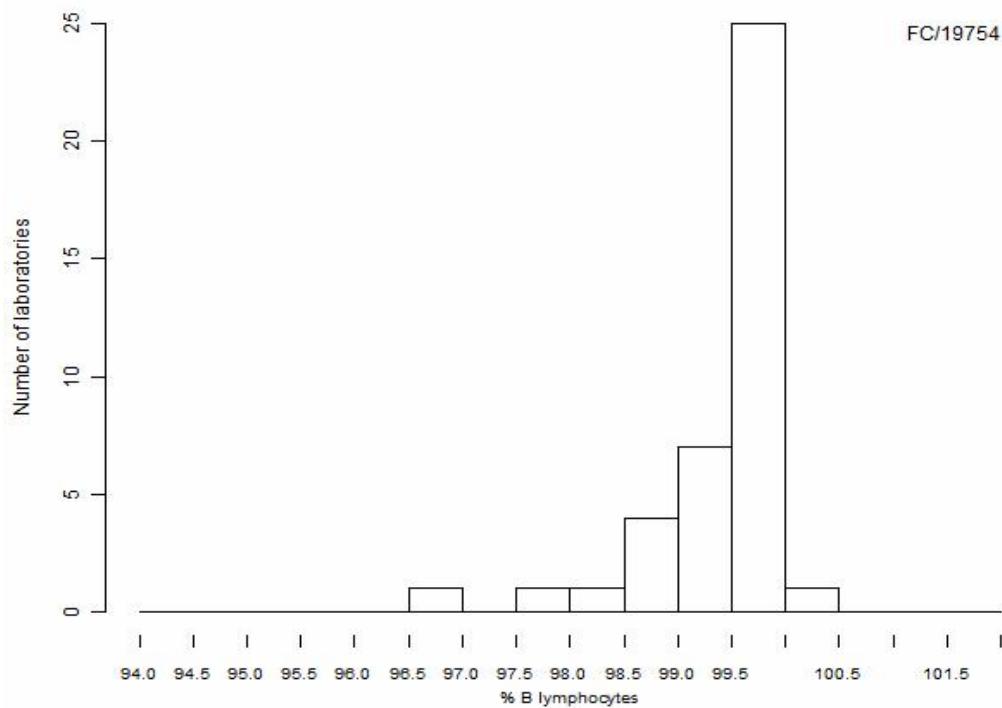
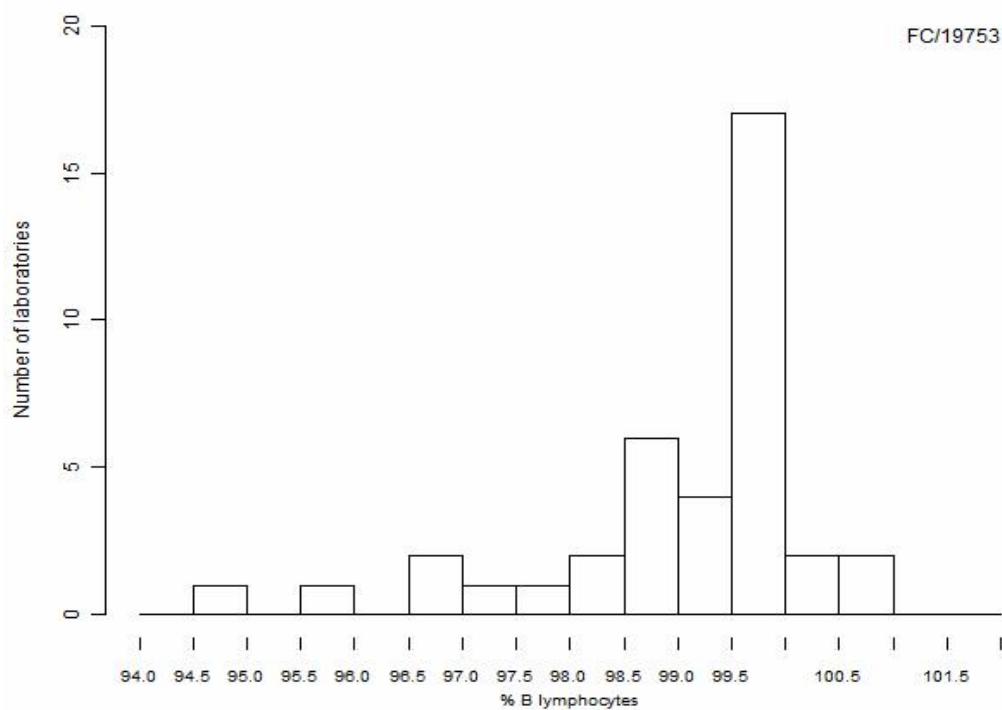
## Lambda % B lymphocytes



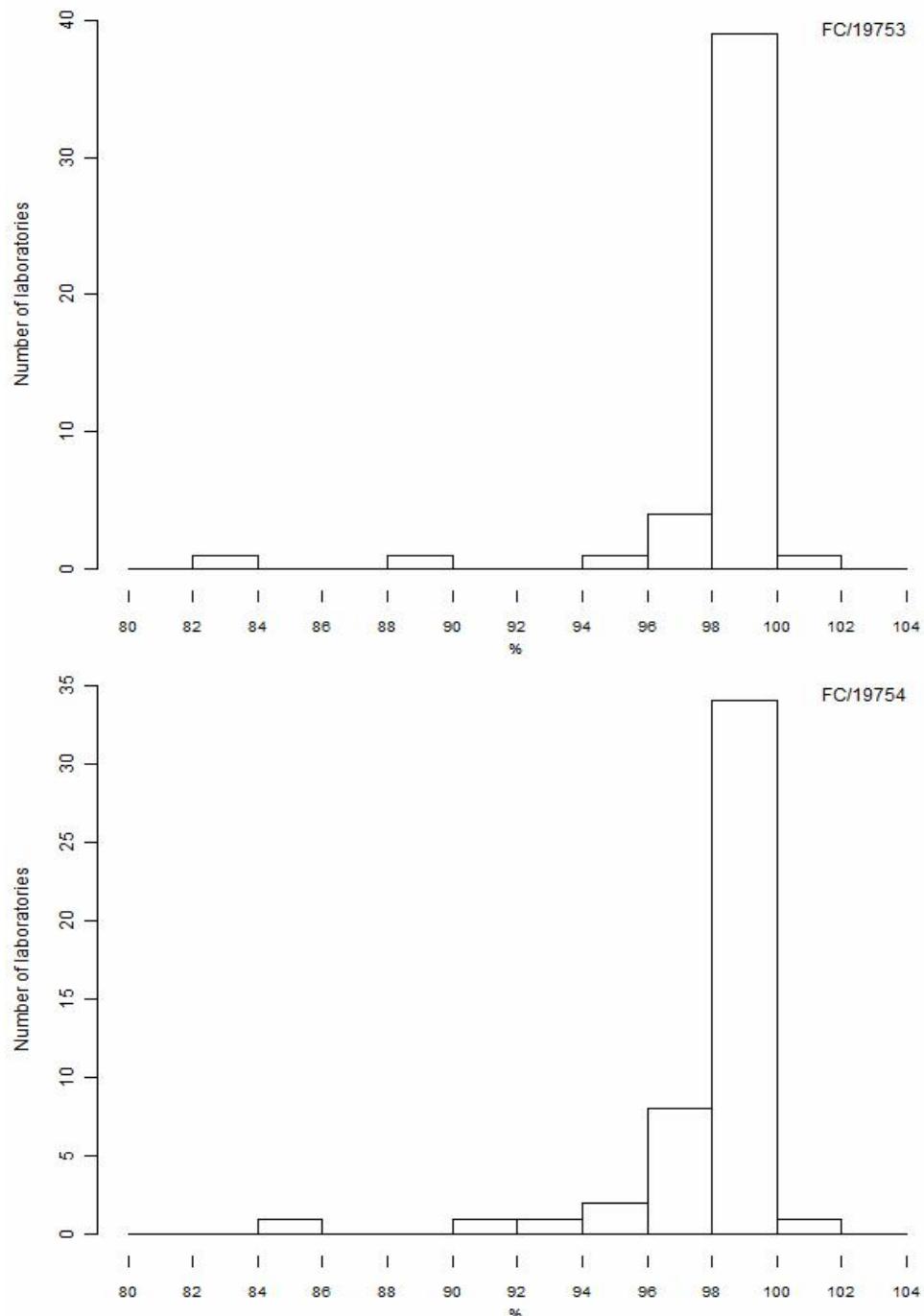
## Kappa/lambda



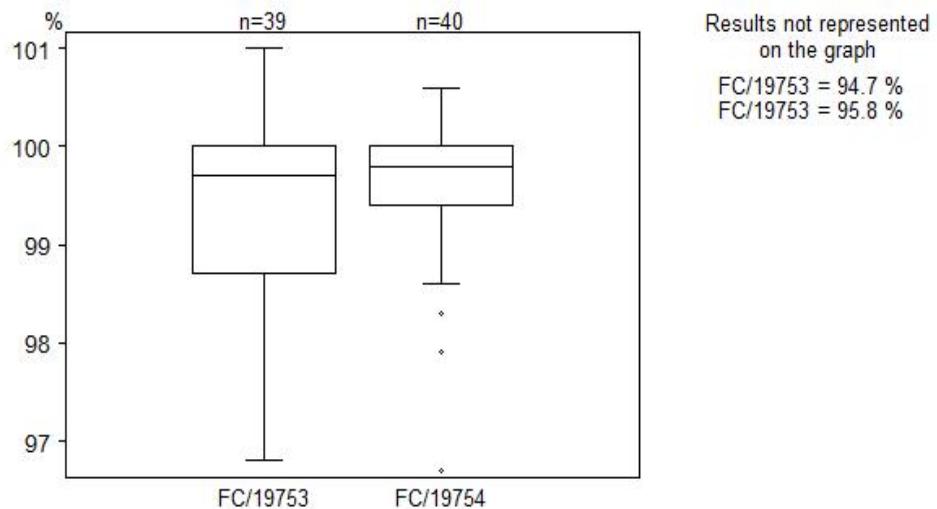
## Sum K+L % B lymphocytes



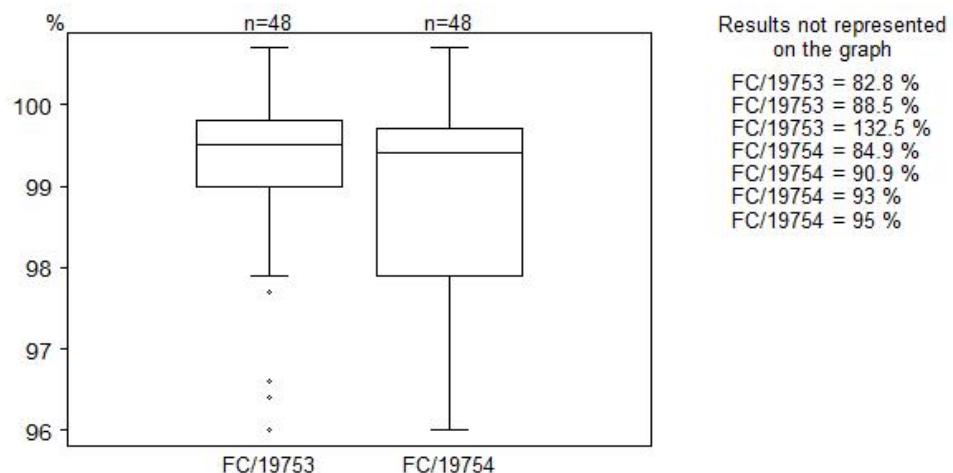
## Lymphosum %



## 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<sup>+</sup>% plus CD19<sup>+</sup>% 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%.

## SAMPLE STABILITY ASSESSMENT

Stability analysis was performed for each parameter.

In the first instance, the evolution of the reported results over time was examined. This is visualized through the boxplots of the medians and standard deviations per day.

Afterwards the measured variability was compared with the measured variability in the past.

This comparison is shown in the graphs “comparison of variability with the past”:

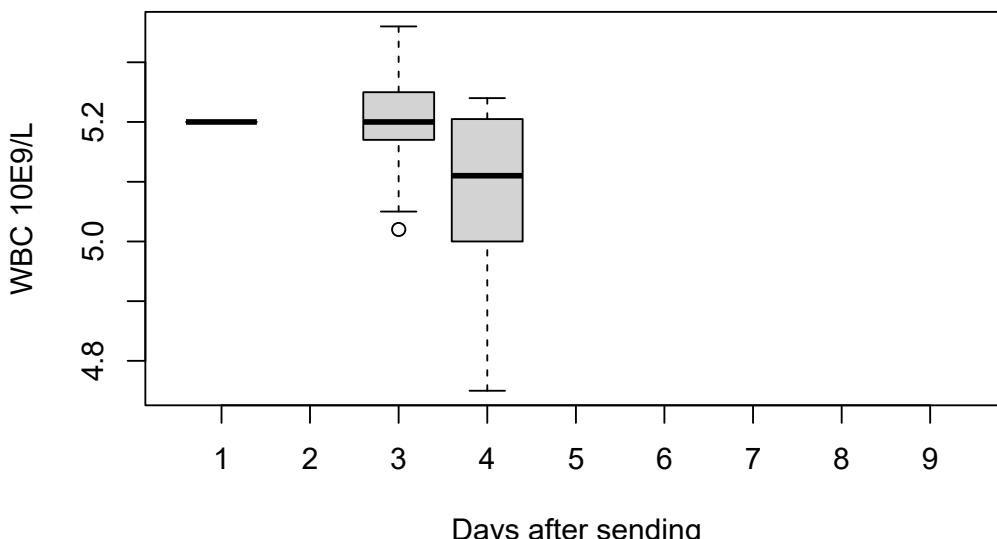
- the solid line represents the average variability for each concentration based on past data,
- the dotted lines show the 95% confidence region of the variabilities,
- the light gray circles show the variabilities of the past,
- the red dots the measured variability in 2023/1.

If the red dots fall outside the confidence region, there is a significant difference in the measured variability compared to the past, presumably caused by the lack of stability.

### WBC 10E9/L

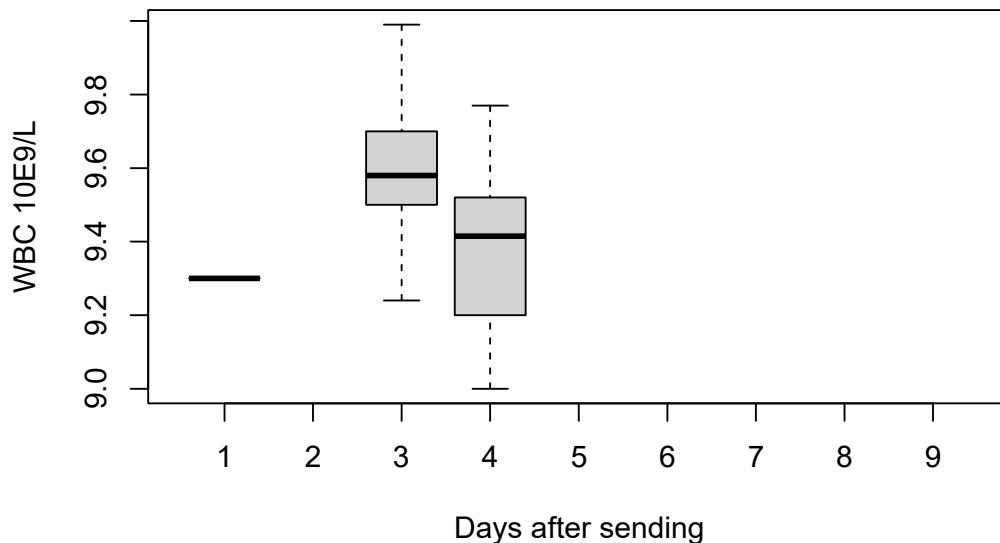
#### Evolution of parameter through the days

*Sample FC/19753*



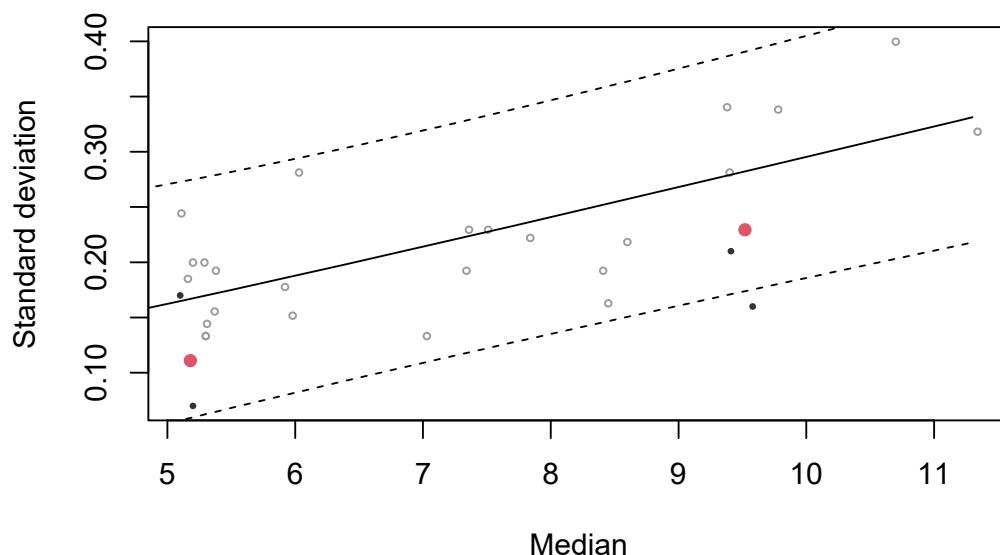
Days after sending	Number of data	Median	Standard deviation
1	1	5.2	0
3	31	5.2	0.07
4	15	5.1	0.17

Sample FC/19754



Days after sending	Number of data	Median	Standard deviation
1	1	9.3	0
3	31	9.58	0.16
4	15	9.41	0.21

Comparison of variability with the past

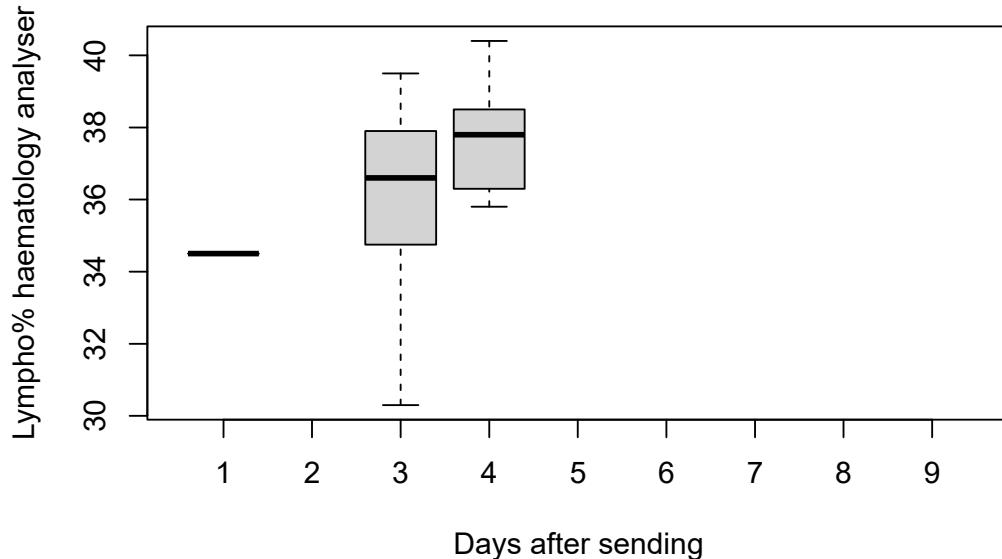


➔ Parameter may decrease with time, no significant effect on variability.

## Lympho% haematology analyser

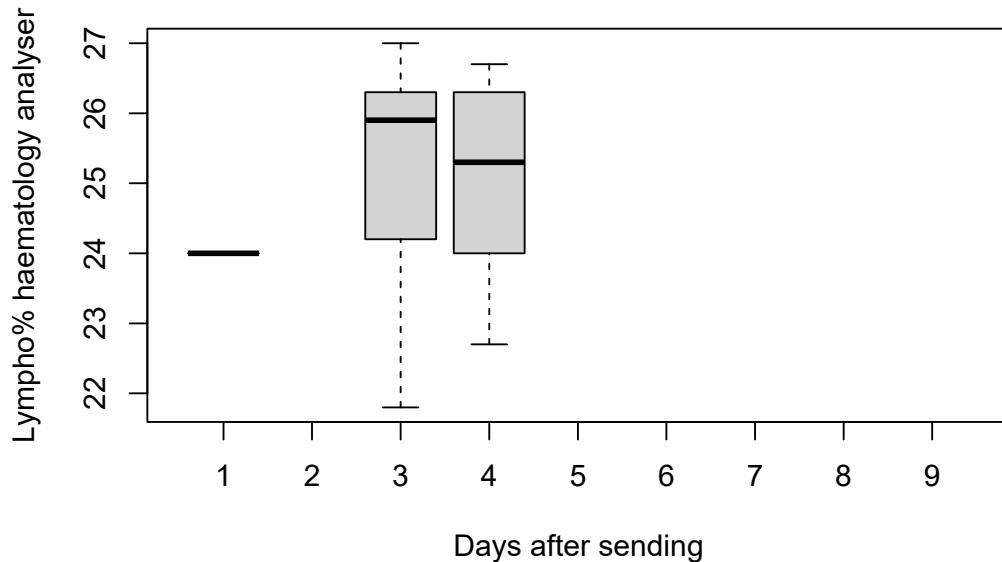
### Evolution of parameter through the days

Sample FC/19753



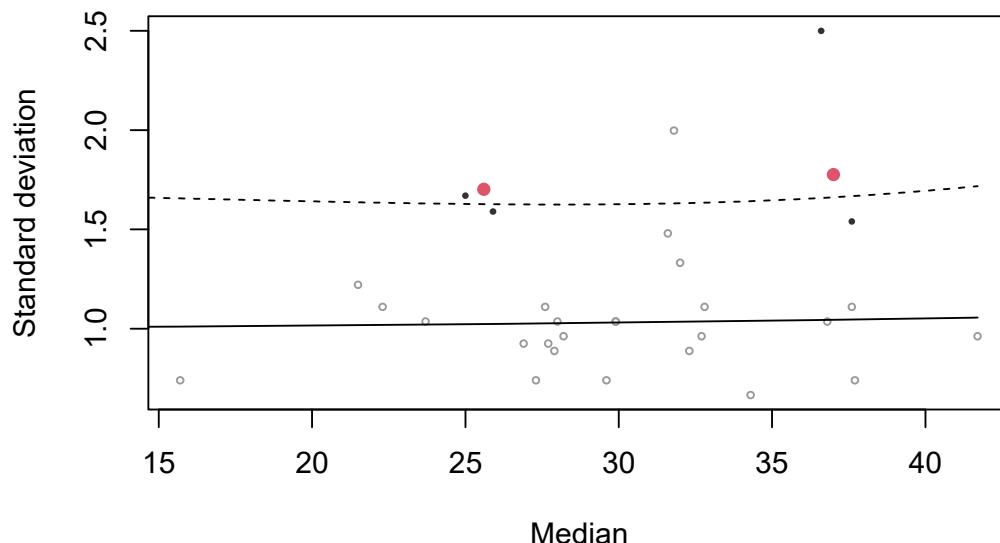
Days after sending	Number of data	Median	Standard deviation
1	1	34.5	0
3	30	36.6	2.5
4	14	37.6	1.54

Sample FC/19754



Days after sending	Number of data	Median	Standard deviation
1	1	24	0
3	30	25.9	1.59
4	14	25	1.67

## Comparison of variability with the past

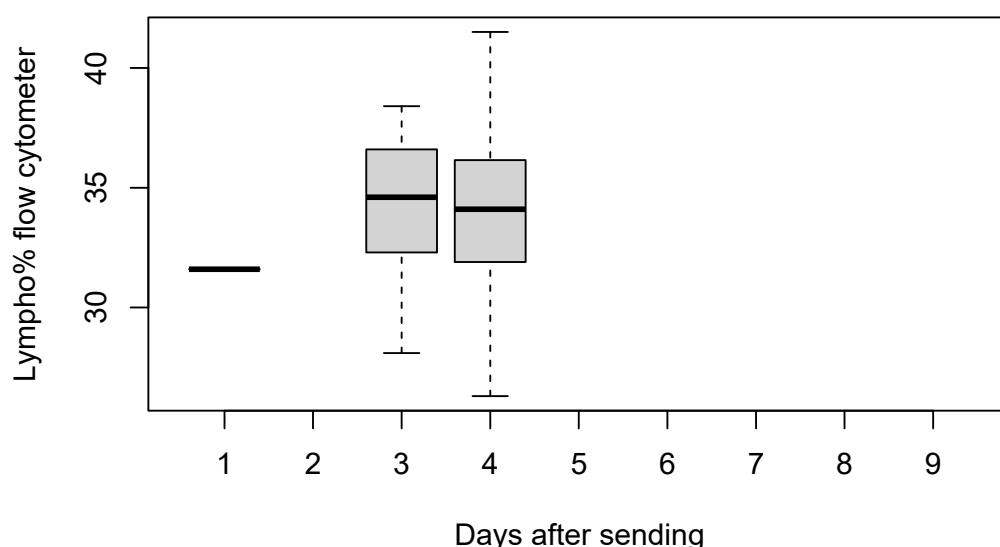


→ Parameter may increase with time, significant effect on variability.

## Lympho% flow cytometer

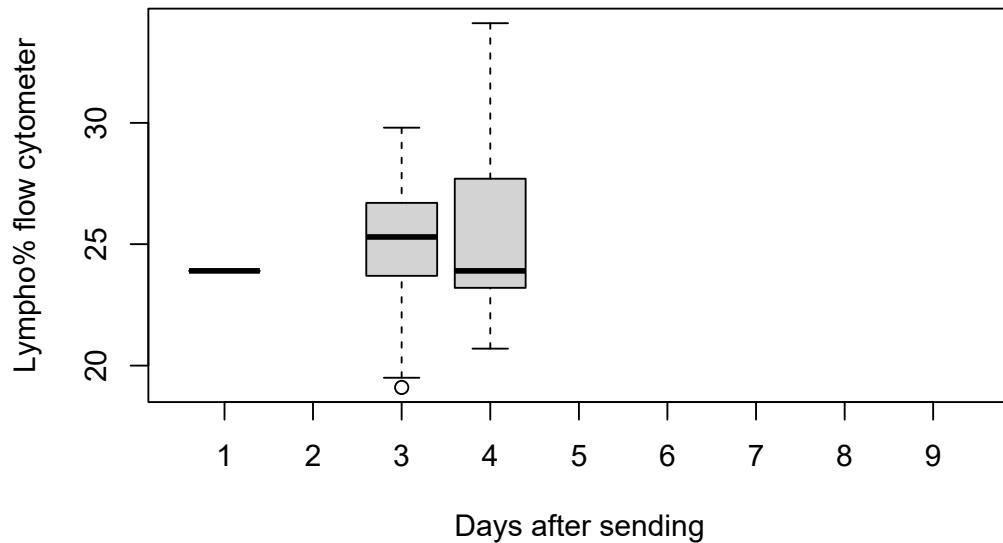
### Evolution of parameter through the days

*Sample FC/19753*

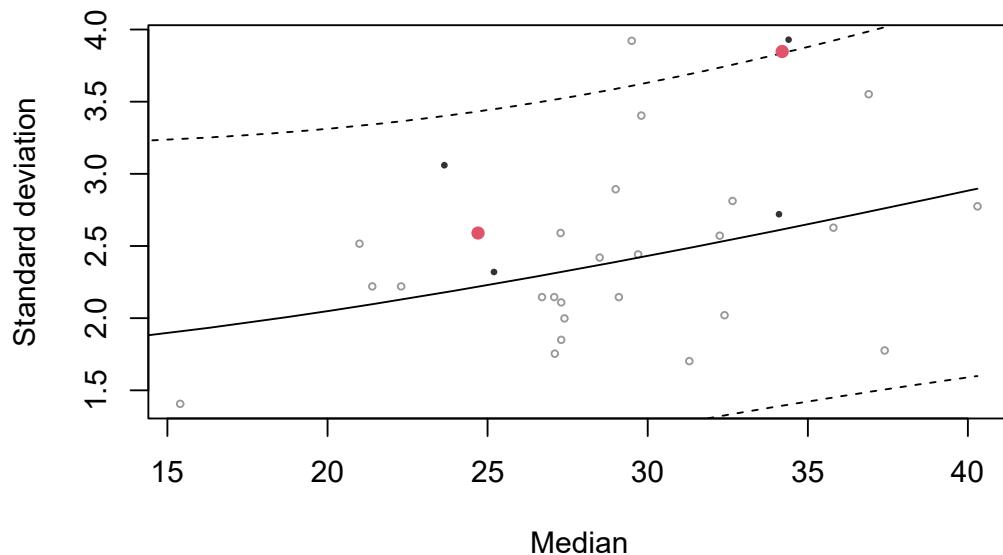


Days after sending	Number of data	Median	Standard deviation
1	1	31.6	0
3	28	34.4	3.93
4	12	34.1	2.72

Sample FC/19754



#### Comparison of variability with the past

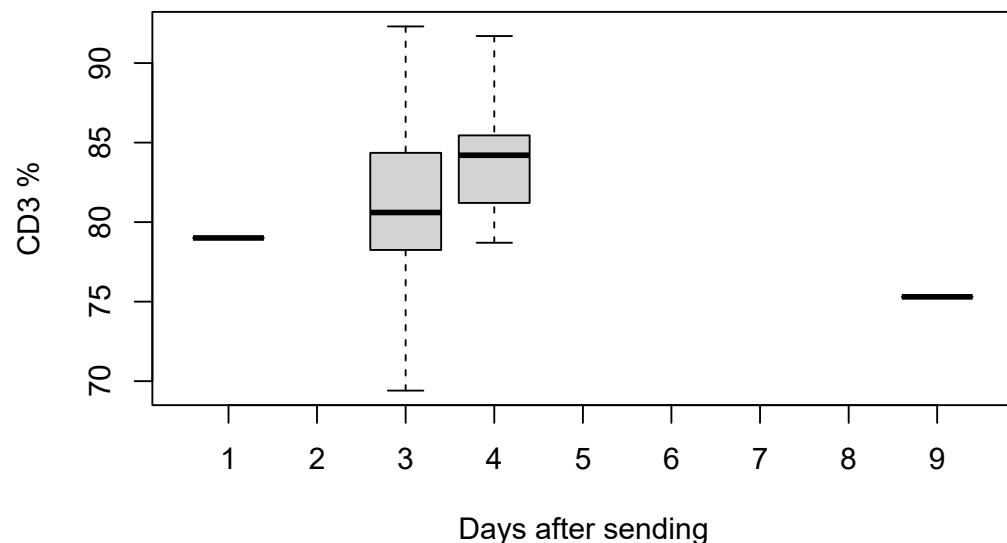


→ Parameter may increase with time, slight effect on variability.

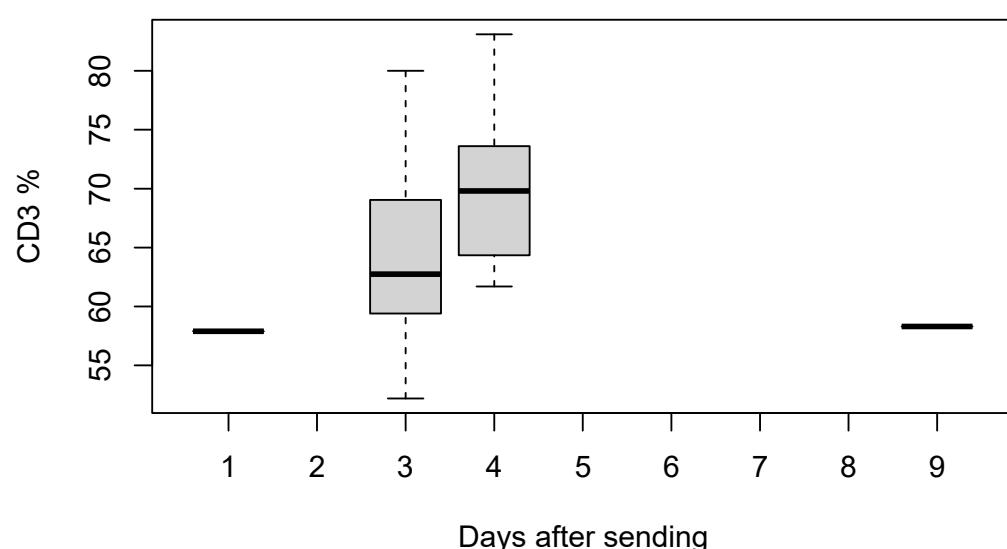
## CD3 %

### Evolution of parameter through the days

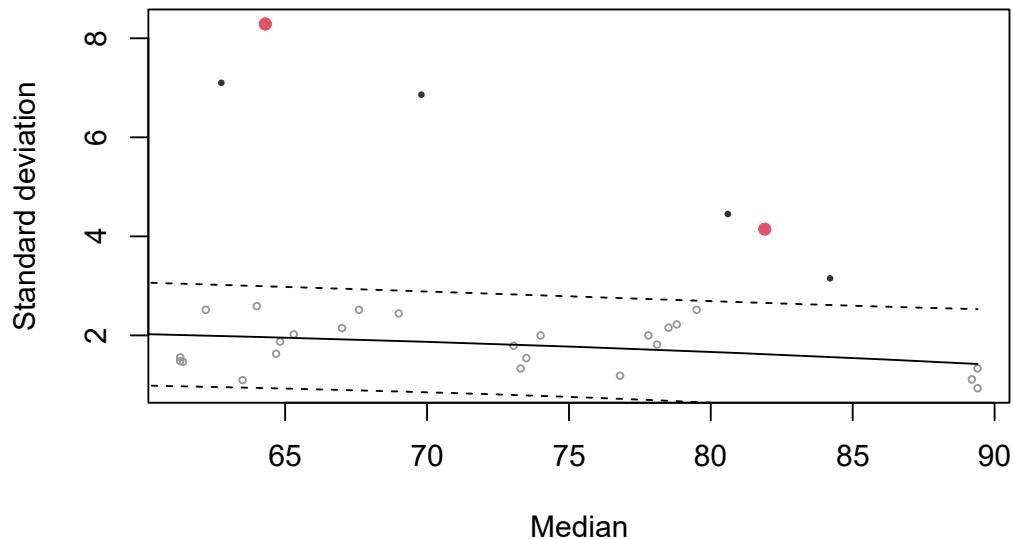
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past

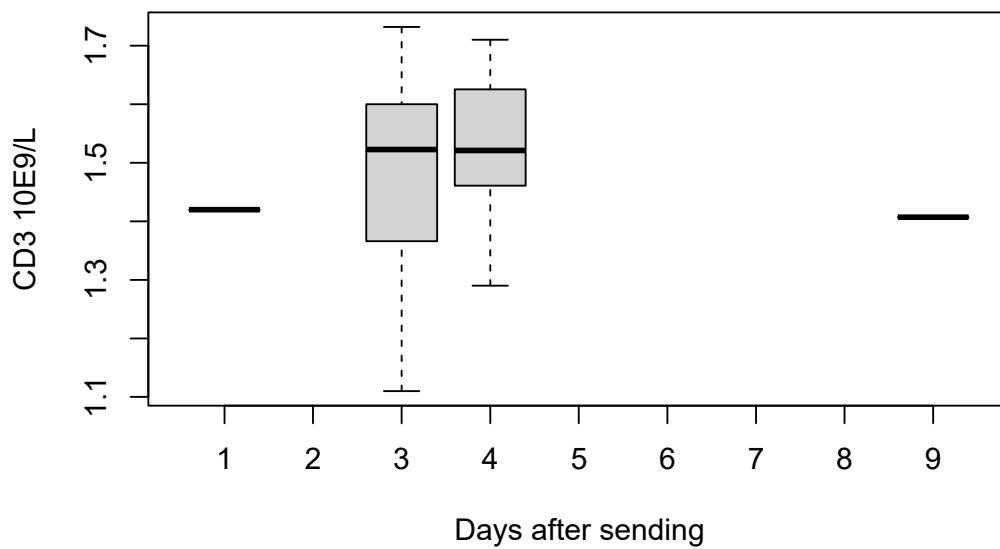


→ Parameter increases with time, significant effect on variability.

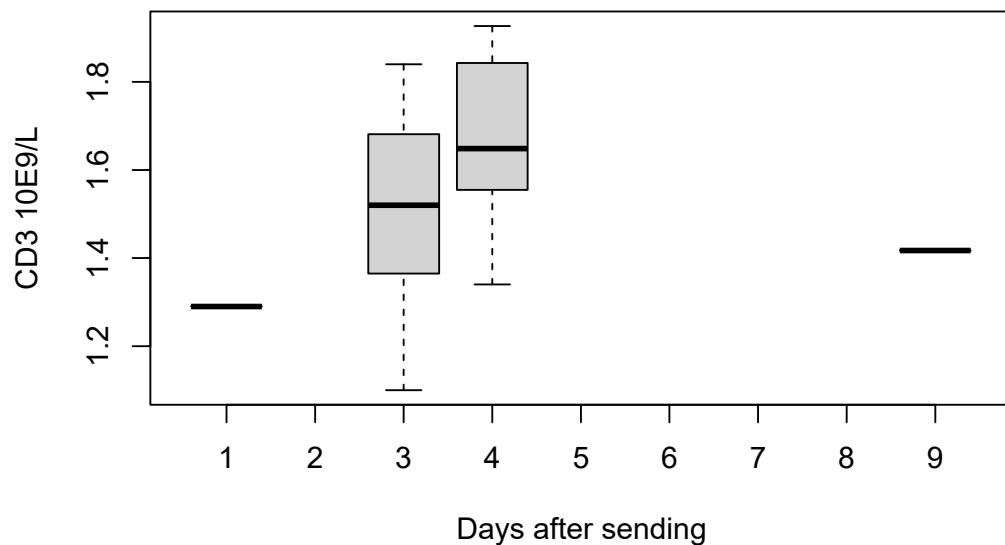
## CD3 10E9/L

### Evolution of parameter through the days

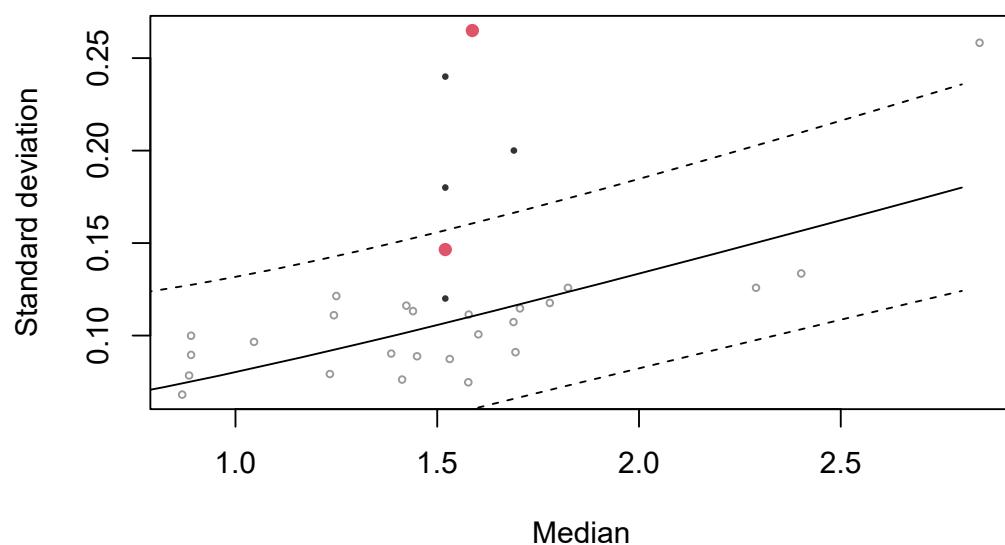
*Sample FC/19753*



Days after sending	Number of data	Median	Standard deviation
1	1	1.42	0
3	32	1.52	0.18
4	15	1.52	0.12
9	1	1.41	0



#### Comparison of variability with the past

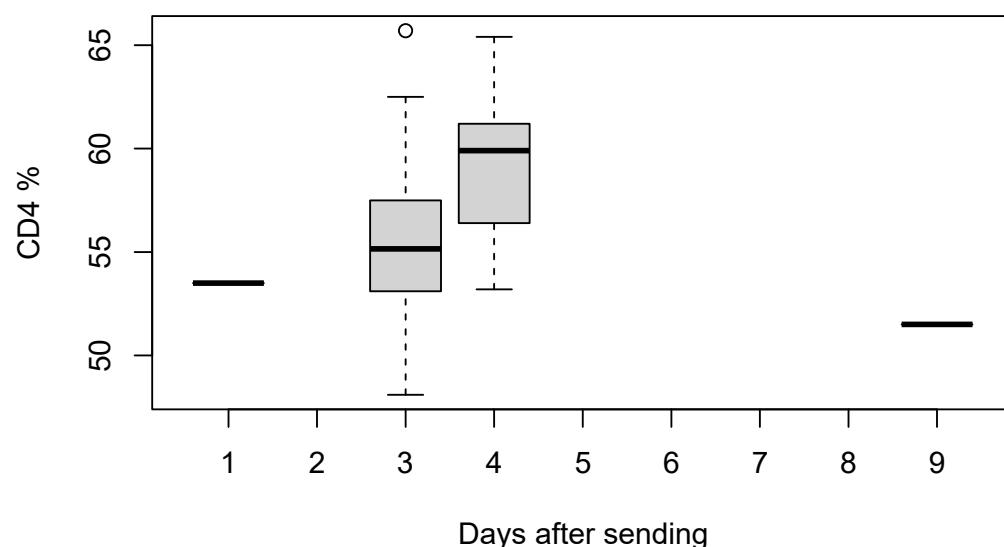


➔ Parameter increases with time, significant effect on variability at least for one sample.

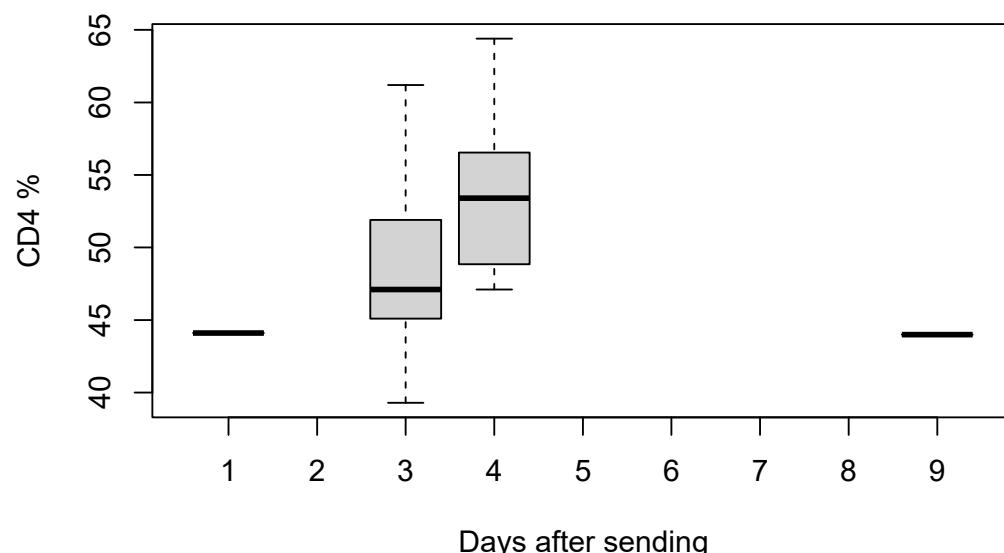
## CD4 %

### Evolution of parameter through the days

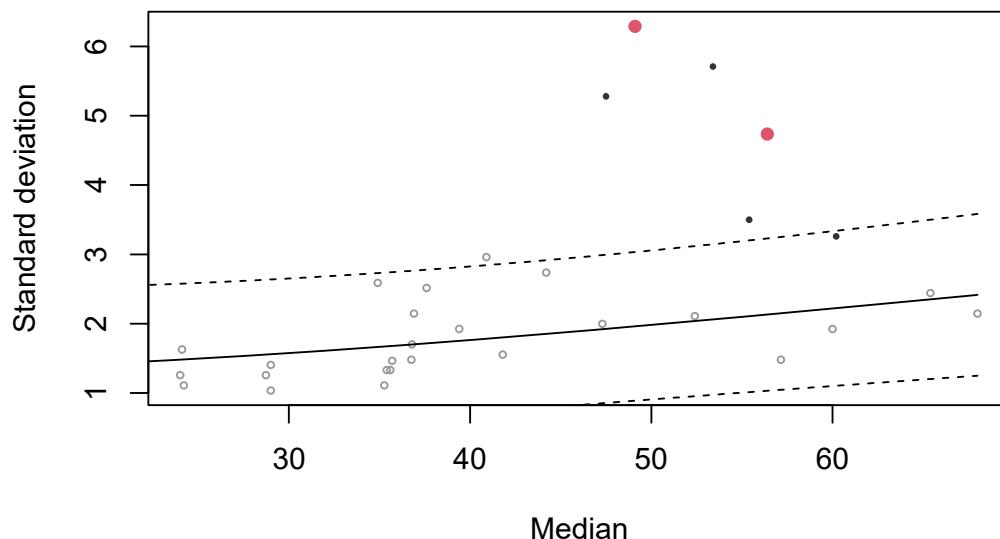
Sample FC/19753



Sample FC/19754



### Comparison of variability with the past

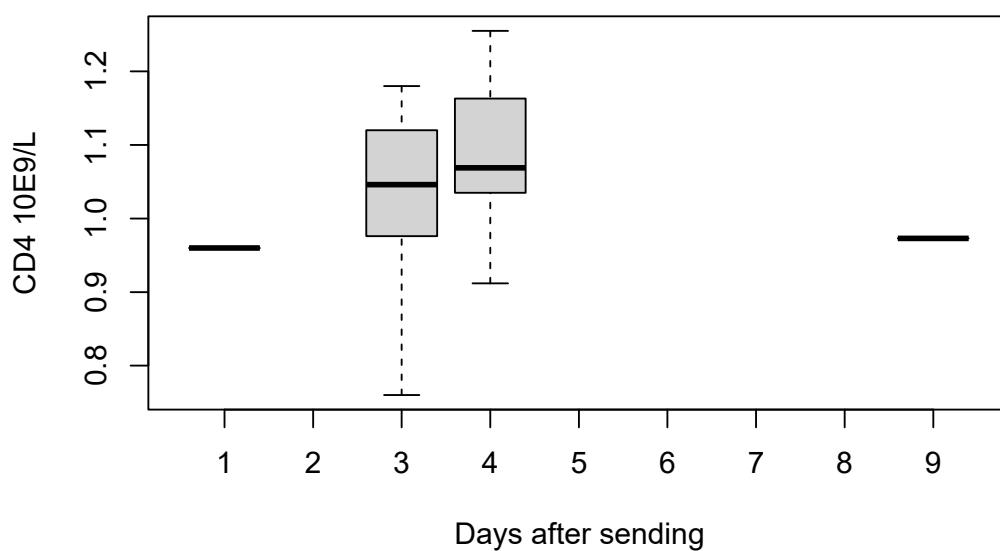


➔ Parameter increases with time, significant effect on variability.

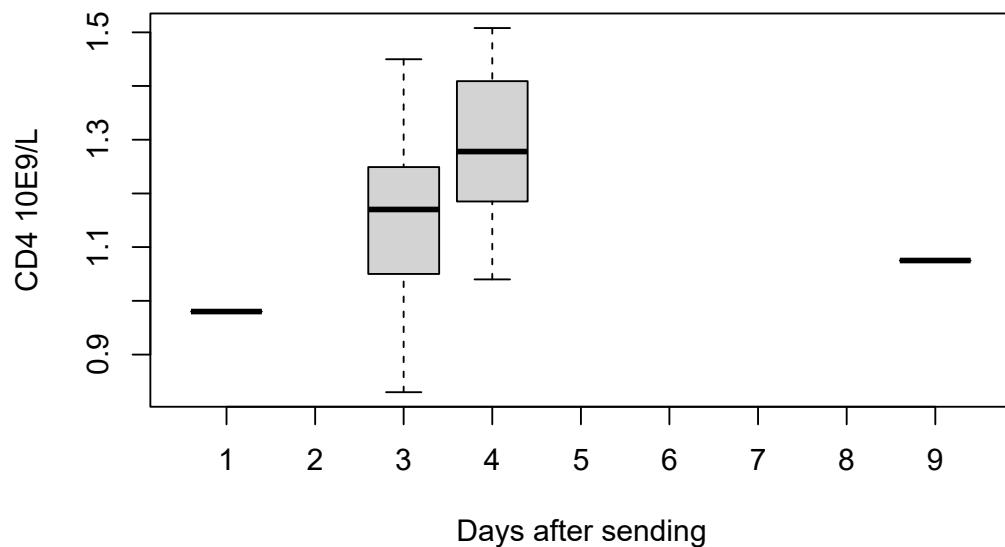
### CD4 10E9/L

#### Evolution of parameter through the days

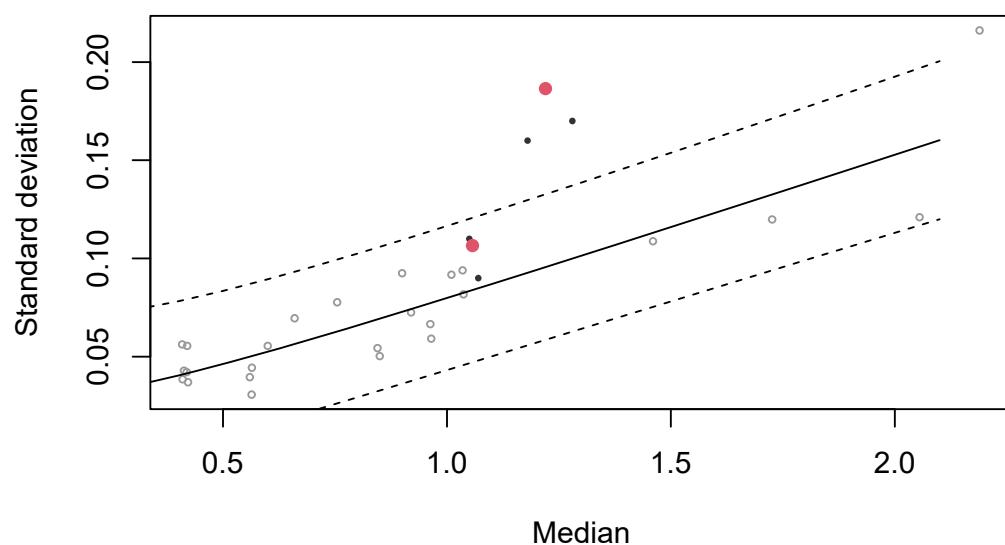
*Sample FC/19753*



Days after sending	Number of data	Median	Standard deviation
1	1	0.96	0
3	32	1.05	0.11
4	15	1.07	0.09
9	1	0.97	0



#### Comparison of variability with the past

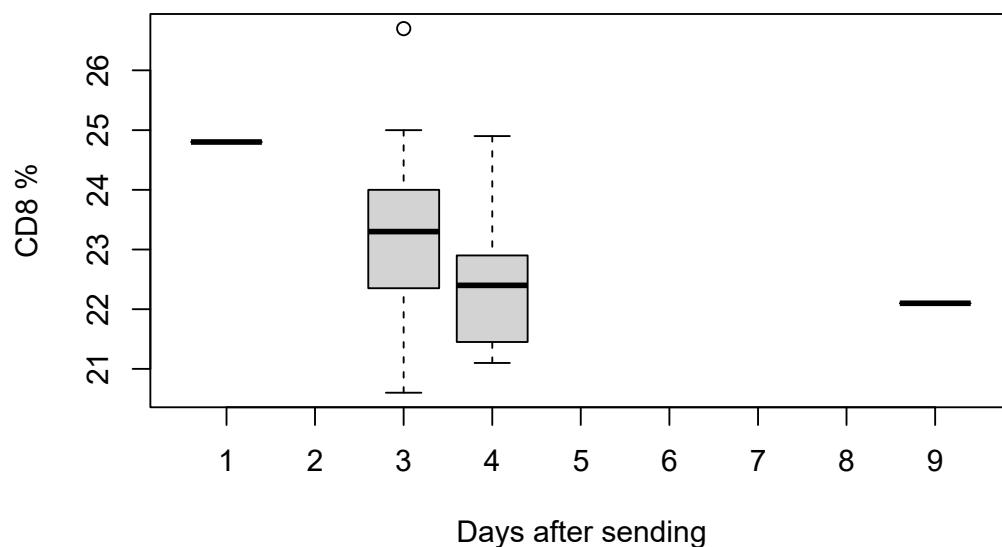


➔ Parameter increases with time, significant effect on variability at least for one sample.

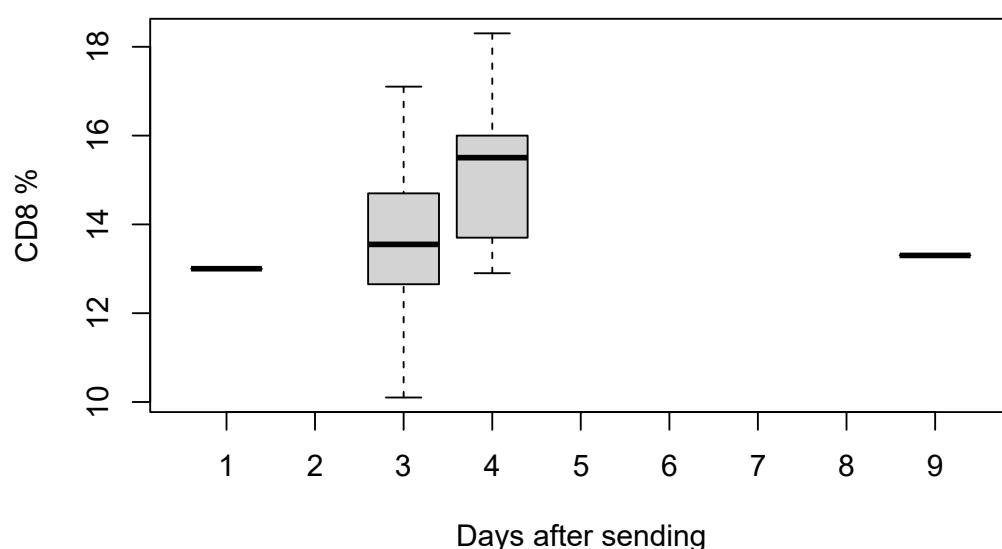
## CD8 %

Evolution of parameter through the days

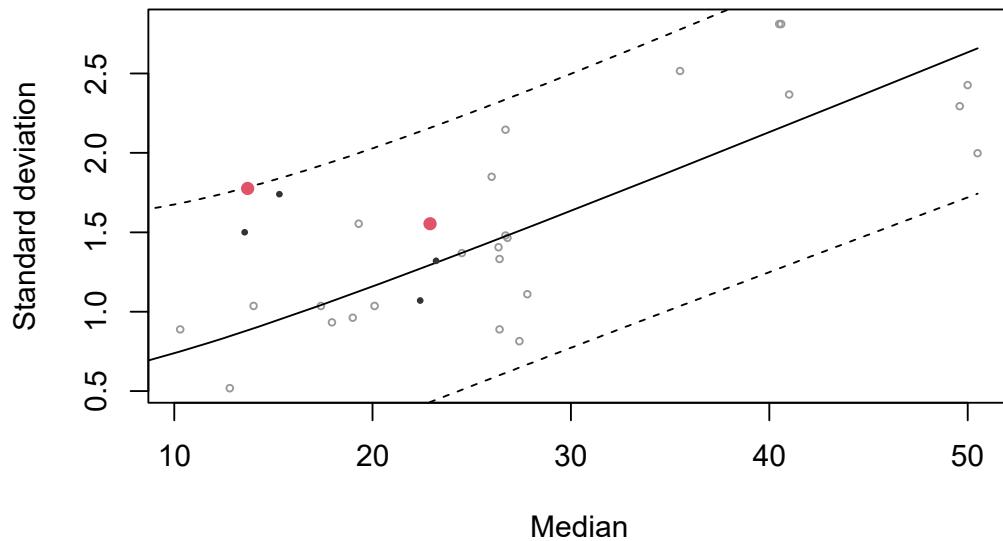
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past

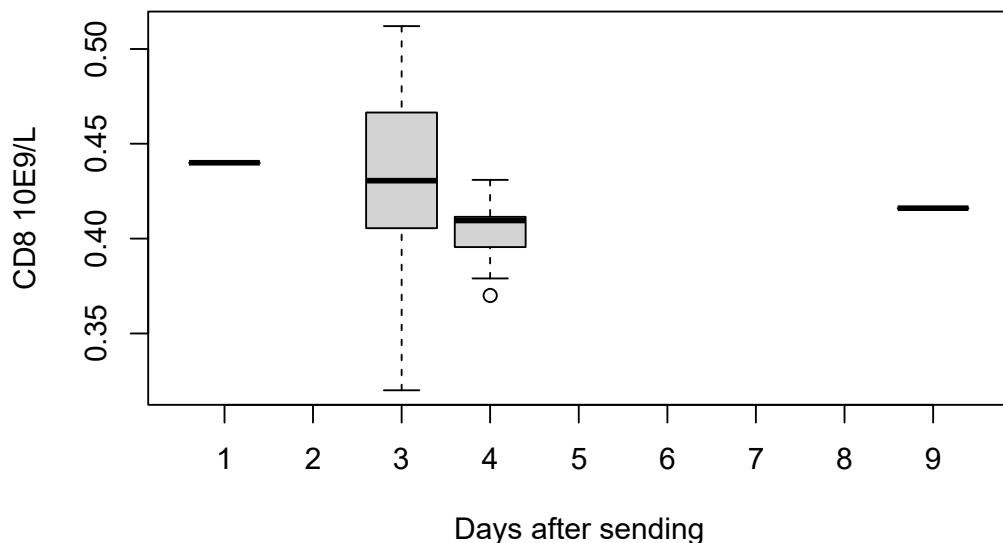


→ Unclear effect over time, no significant effect on variability.

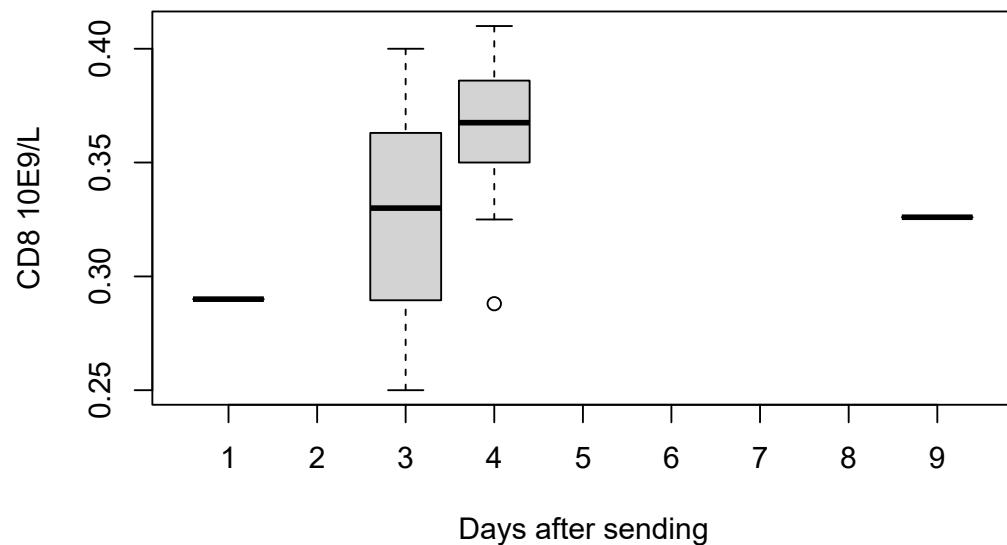
## CD8 10E9/L

### Evolution of parameter through the days

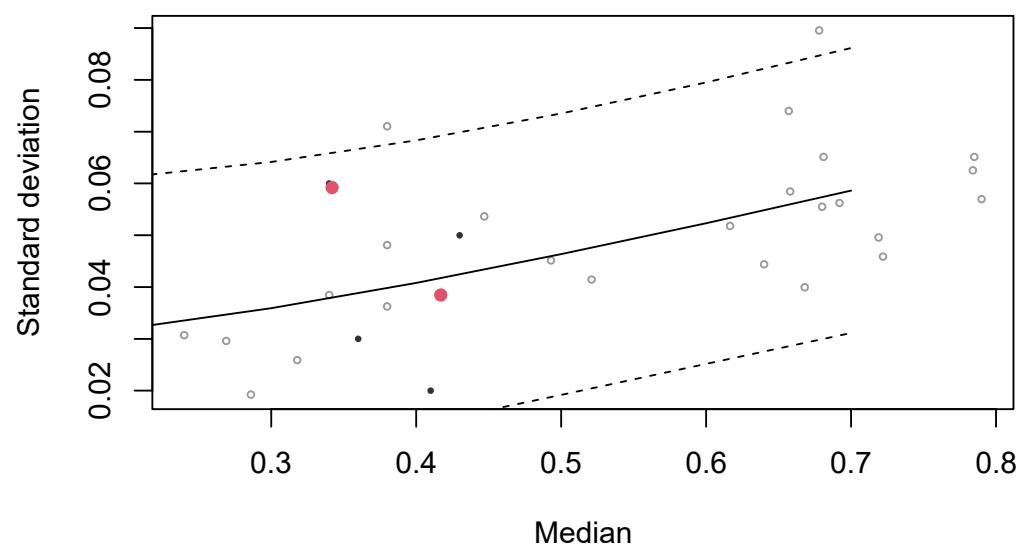
*Sample FC/19753*



Days after sending	Number of data	Median	Standard deviation
1	1	0.44	0
3	32	0.43	0.05
4	15	0.41	0.02
9	1	0.42	0



#### Comparison of variability with the past

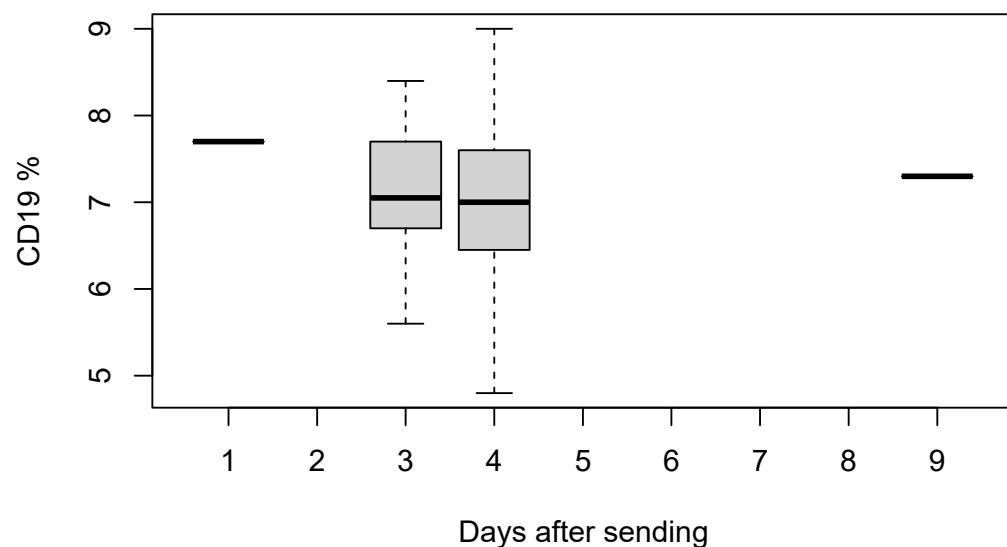


➔ Unclear effect over time, no significant effect on variability.

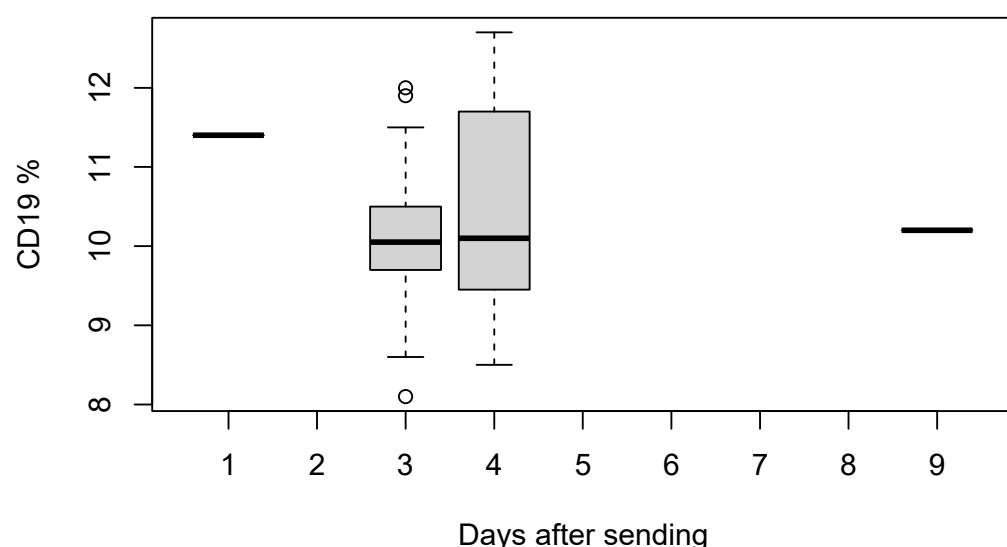
## CD19 %

### Evolution of parameter through the days

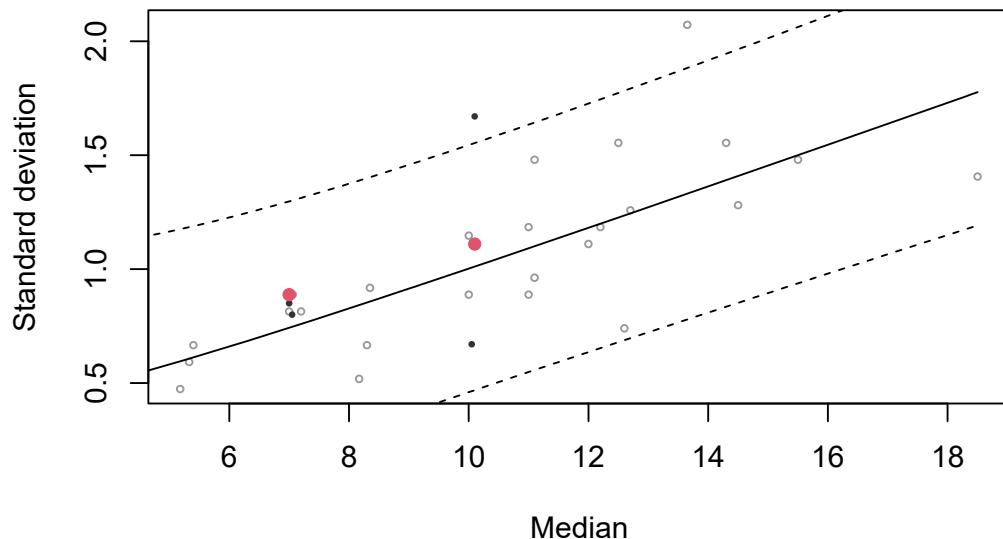
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past

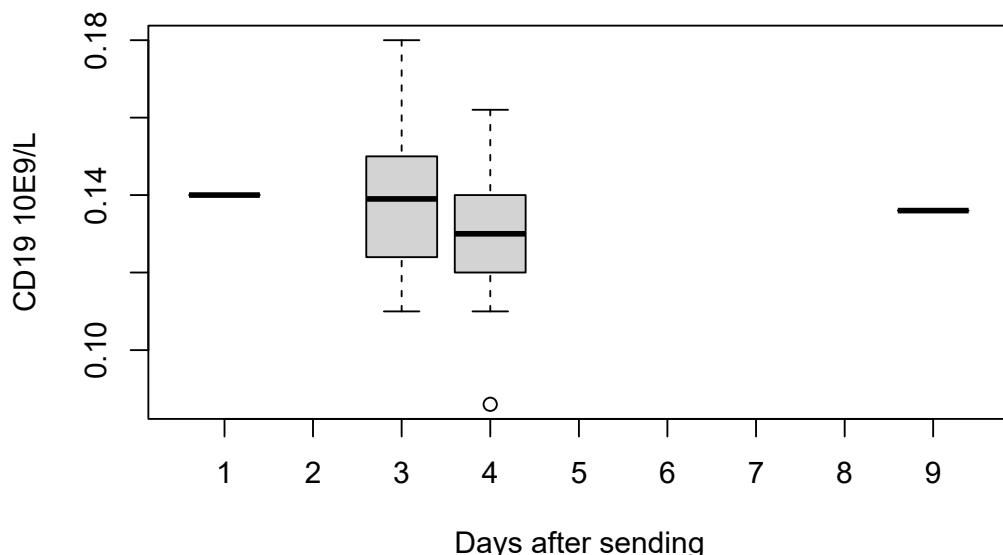


→ Parameter appears to be stable through time, no significant effect on variability.

## CD19 10E9/L

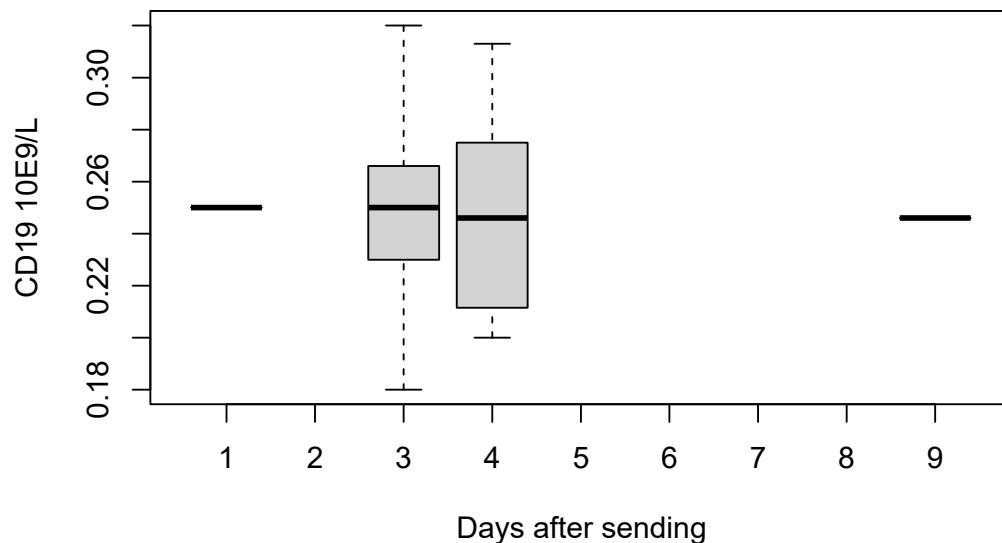
### Evolution of parameter through the days

*Sample FC/19753*



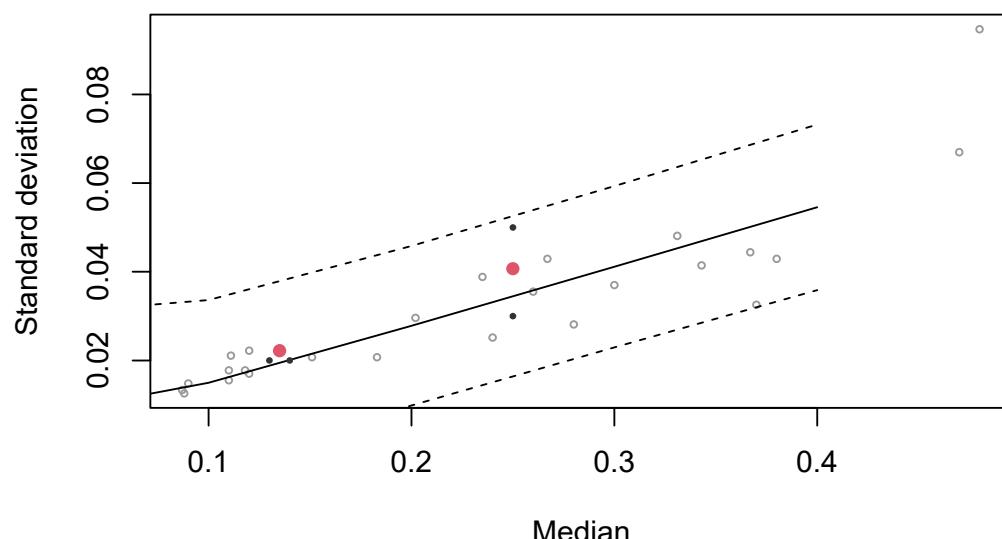
Days after sending	Number of data	Median	Standard deviation
1	1	0.14	0
3	32	0.14	0.02
4	15	0.13	0.02
9	1	0.14	0

Sample FC/19754



Days after sending	Number of data	Median	Standard deviation
1	1	0.25	0
3	32	0.25	0.03
4	15	0.25	0.05
9	1	0.25	0

**Comparison of variability with the past**

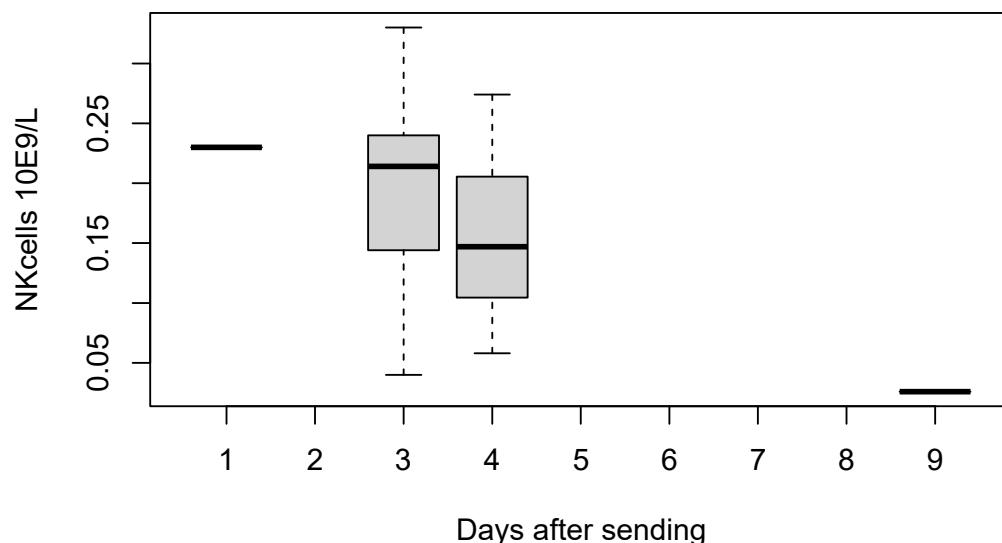


→ Parameter appears to be stable through time, no significant effect on variability.

## NKcells 10E9/L

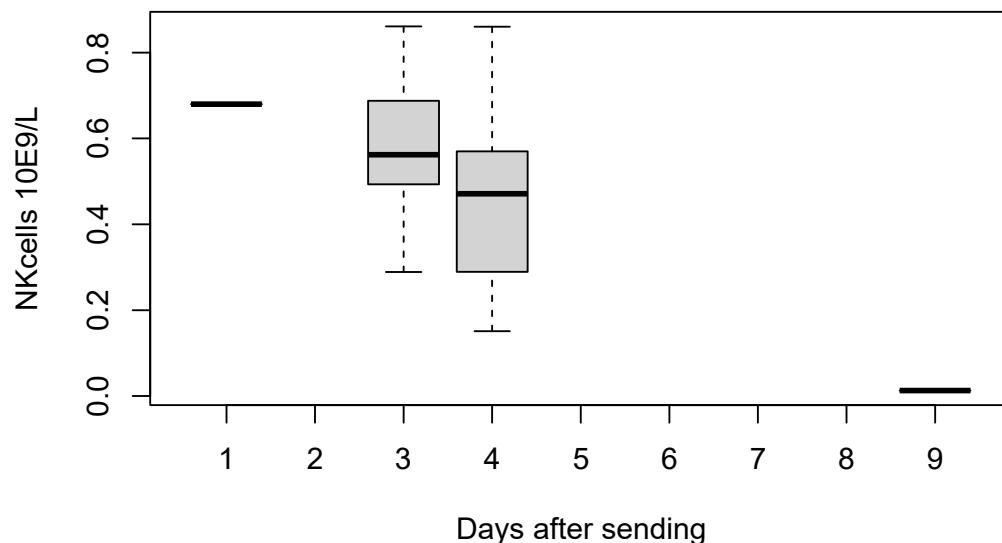
**Evolution of parameter through the days**

*Sample FC/19753*



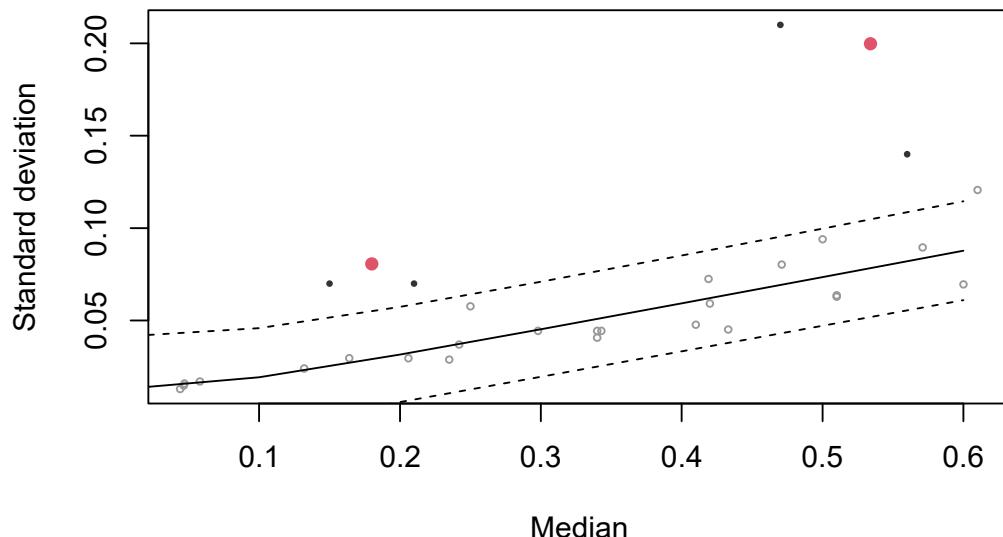
Days after sending	Number of data	Median	Standard deviation
1	1	0.23	0
3	32	0.21	0.07
4	15	0.15	0.07
9	1	0.03	0

*Sample FC/19754*



Days after sending	Number of data	Median	Standard deviation
1	1	0.68	0
3	32	0.56	0.14
4	15	0.47	0.21
9	1	0.01	0

## Comparison of variability with the past

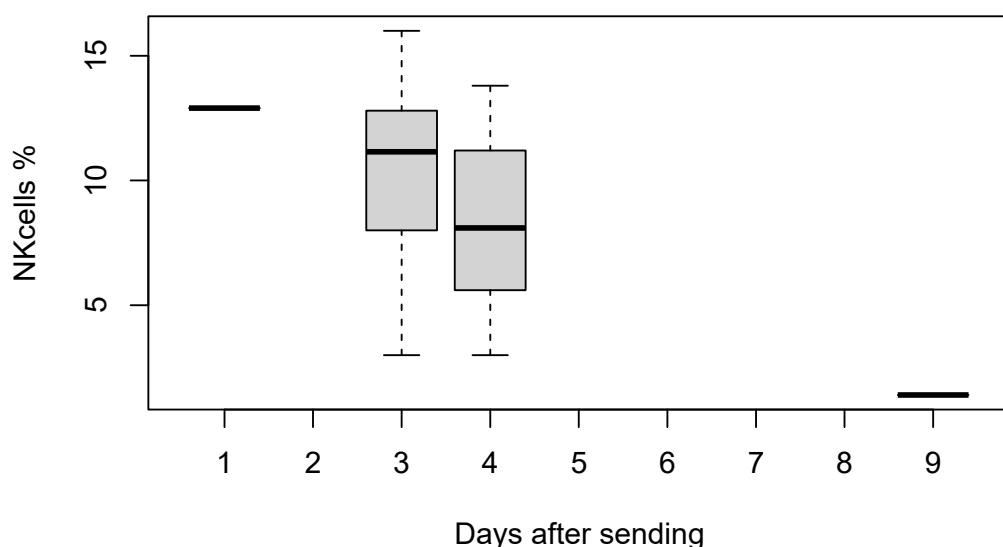


→ Parameter decreases with time, significant effect on variability.

## NKcells %

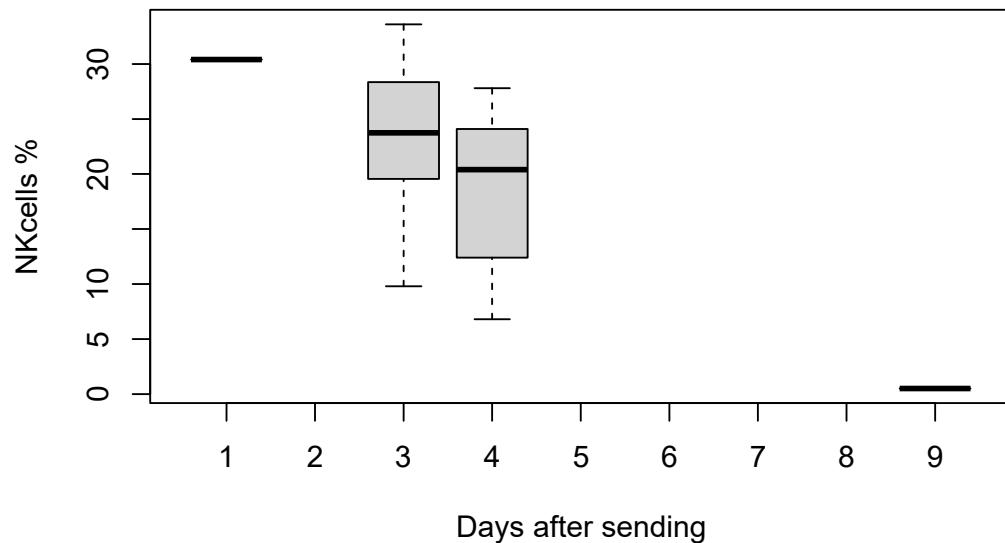
### Evolution of parameter through the days

*Sample FC/19753*

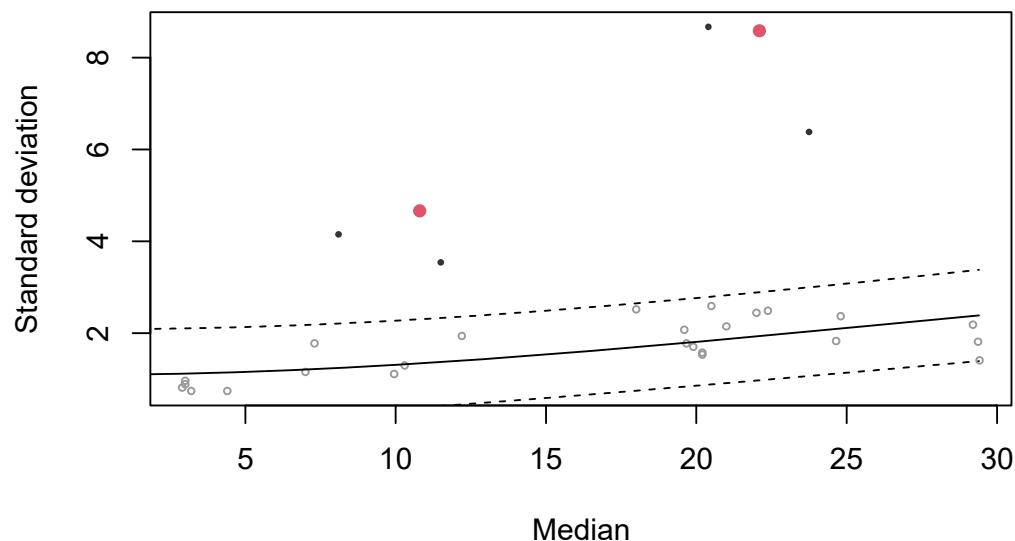


Days after sending	Number of data	Median	Standard deviation
1	1	12.9	0
3	32	11.5	3.54
4	15	8.1	4.15
9	1	1.4	0

Sample FC/19754



#### Comparison of variability with the past

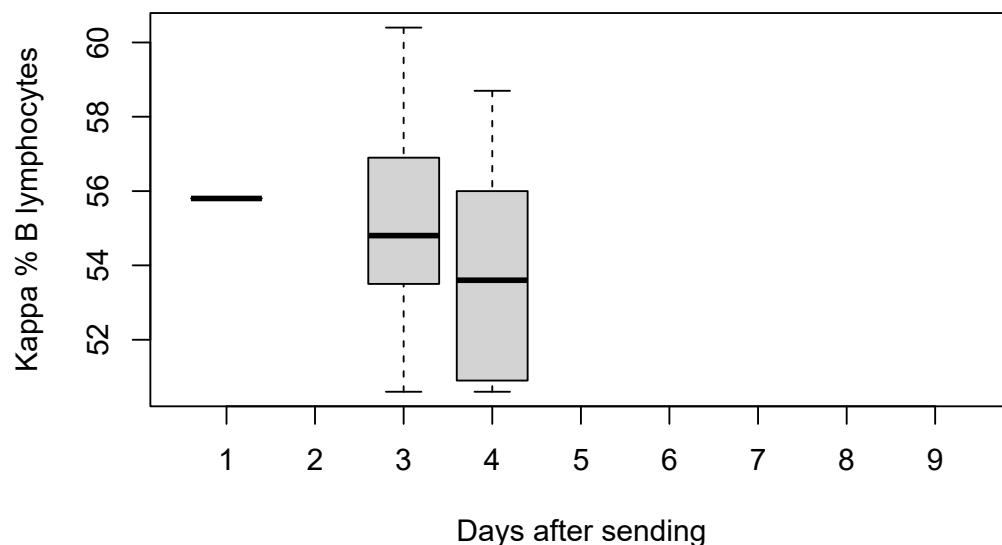


→ Parameter decreases with time, significant effect on variability.

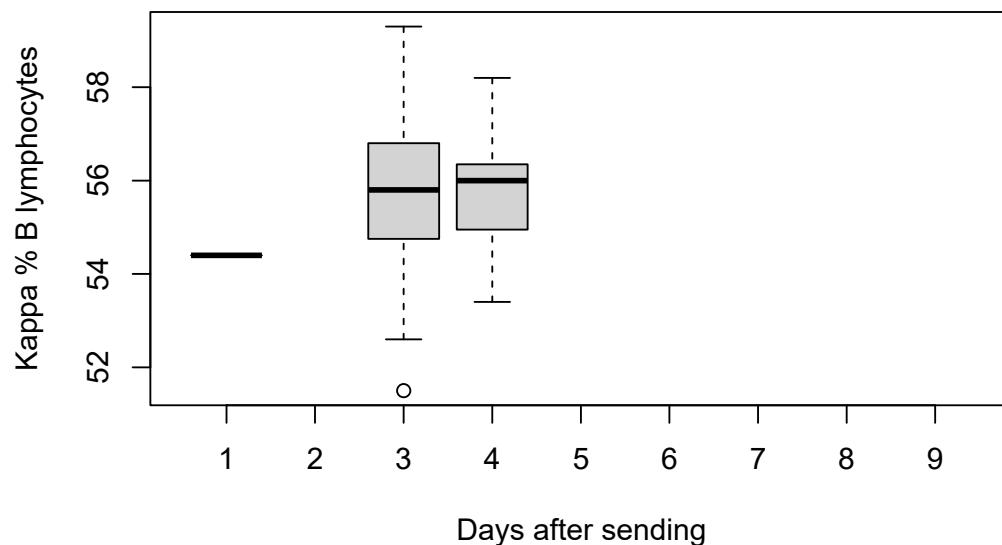
## Kappa % B lymphocytes

Evolution of parameter through the days

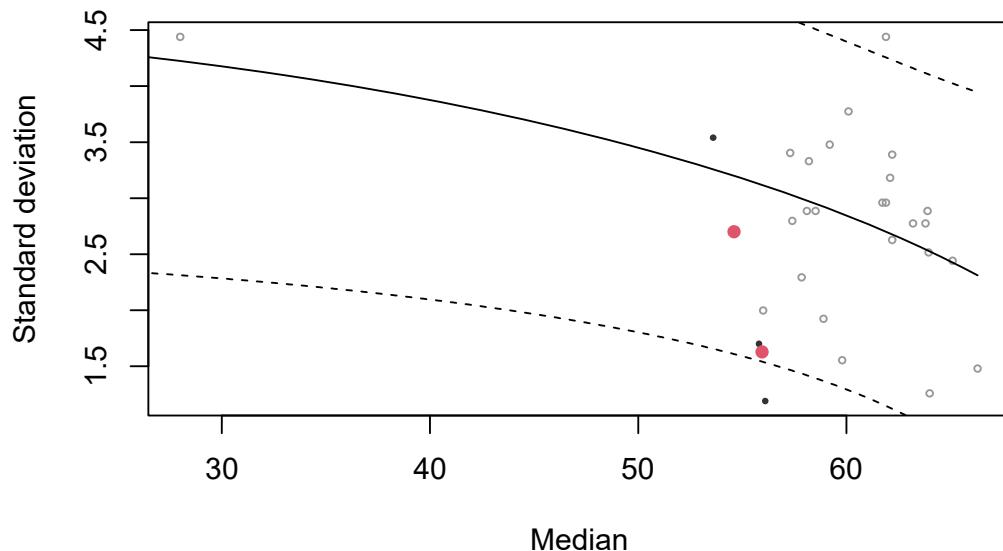
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past

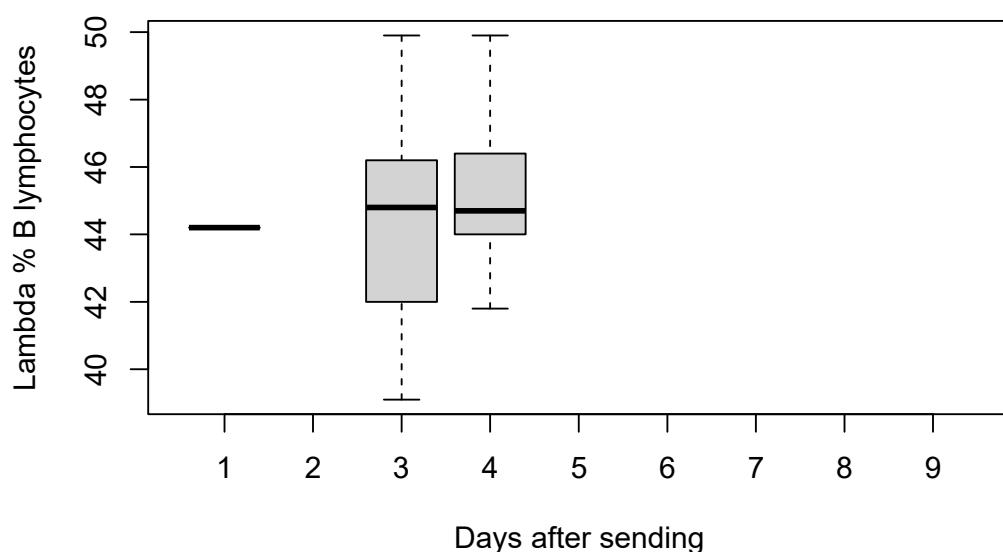


→ Parameter may decrease with time, no significant effect on variability.

## Lambda % B lymphocytes

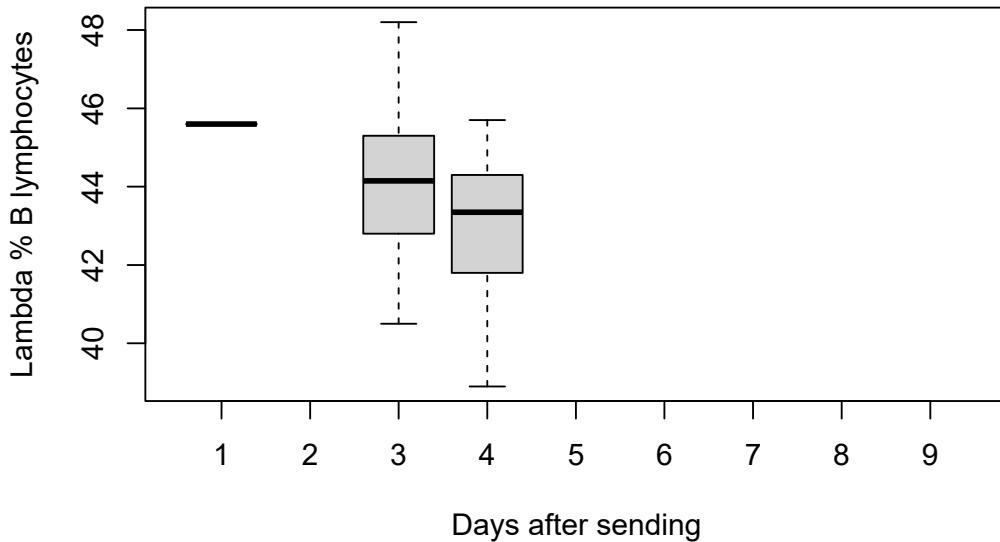
### Evolution of parameter through the days

*Sample FC/19753*

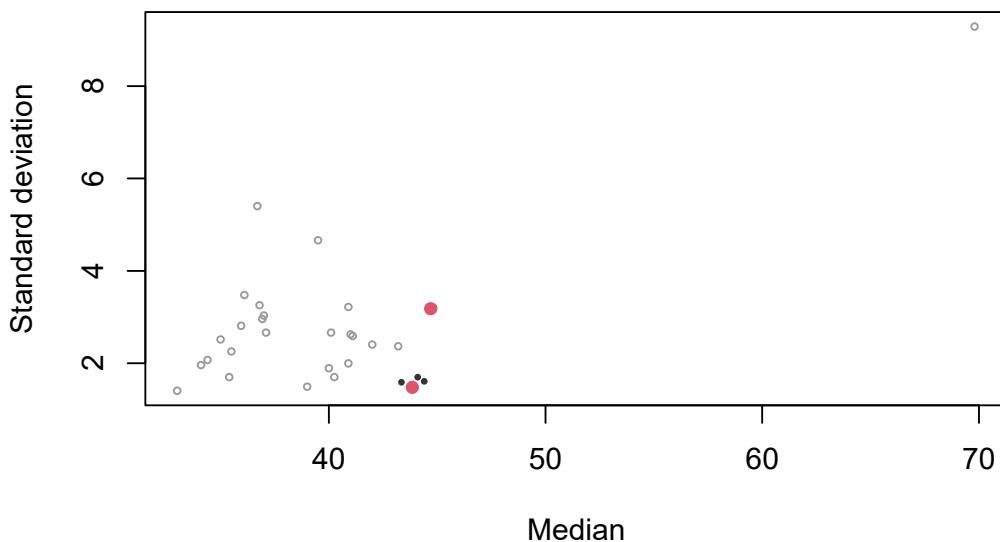


Days after sending	Number of data	Median	Standard deviation
1	1	44.2	0
3	24	44.85	3.21
4	14	44.4	1.61

*Sample FC/19754*



**Comparison of variability with the past**

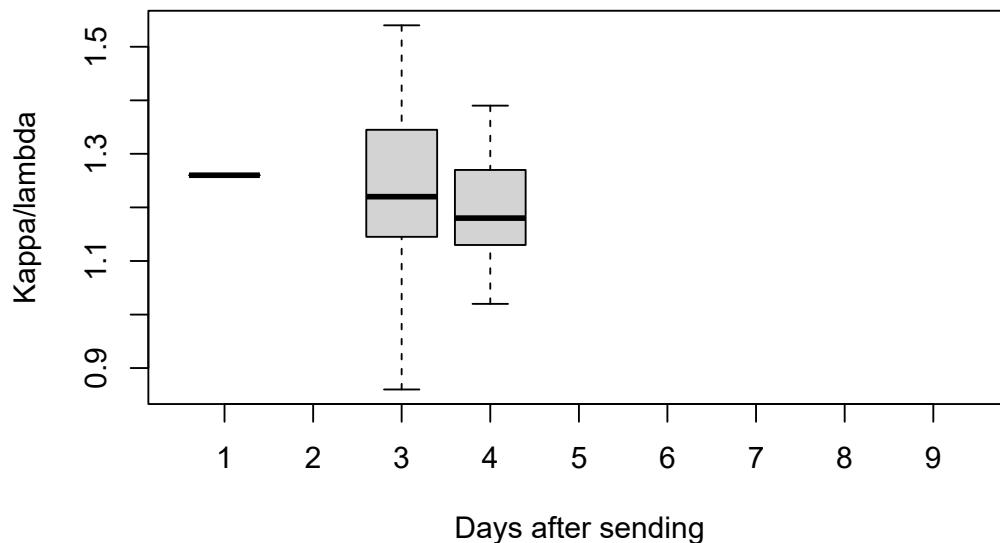


➔ Parameter may decrease with time, no significant effect on variability.

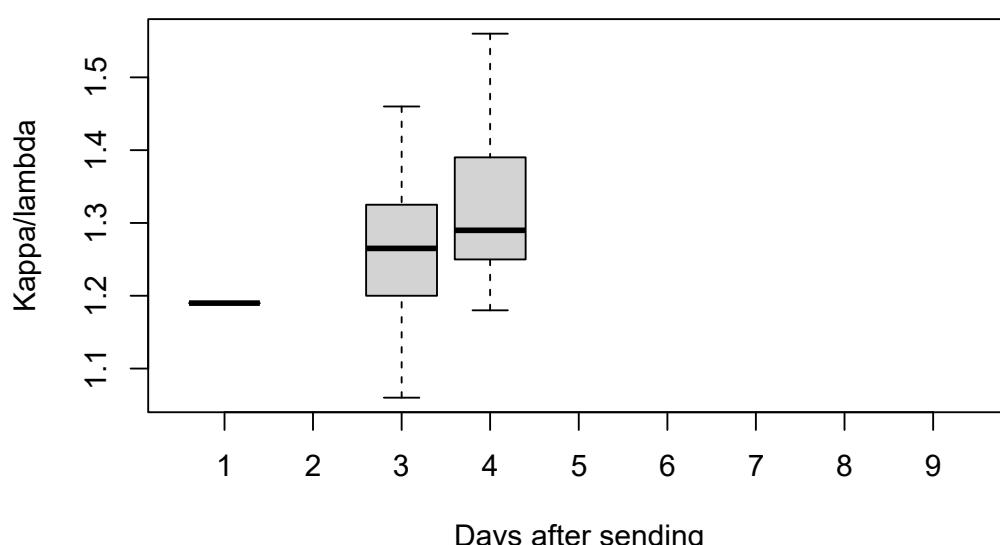
## Kappa/lambda

Evolution of parameter through the days

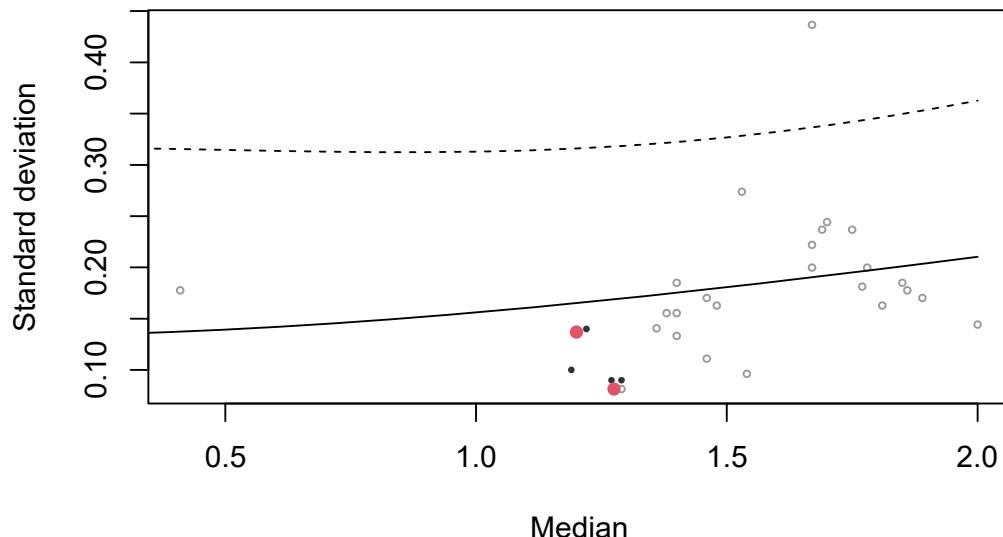
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past

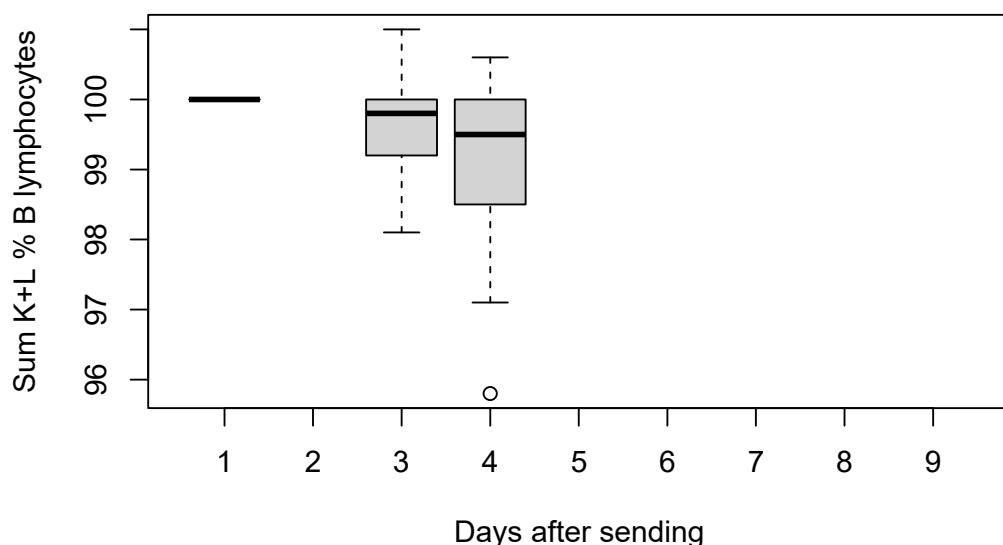


→ Parameter appears to be stable through time, no significant effect on variability.

## Sum K+L % B lymphocytes

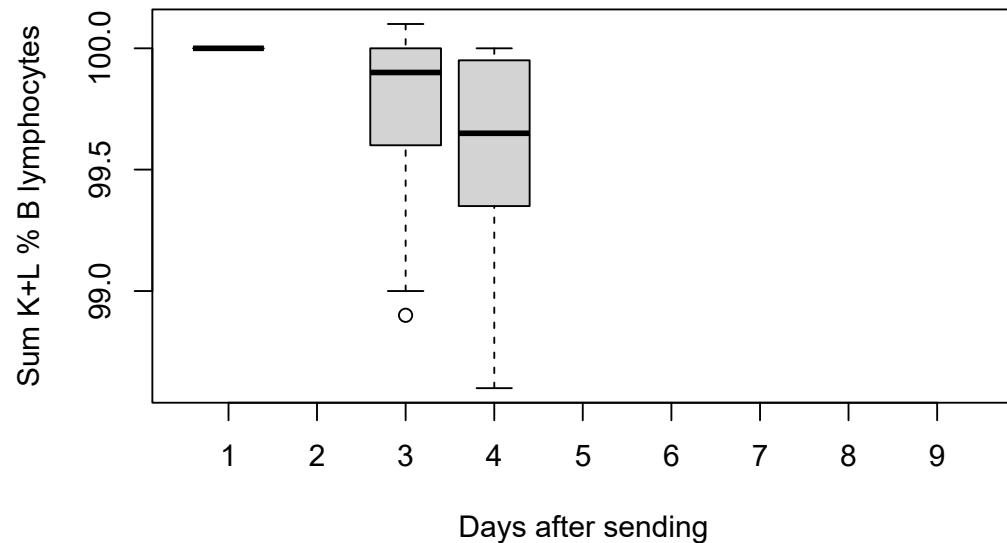
### Evolution of parameter through the days

*Sample FC/19753*

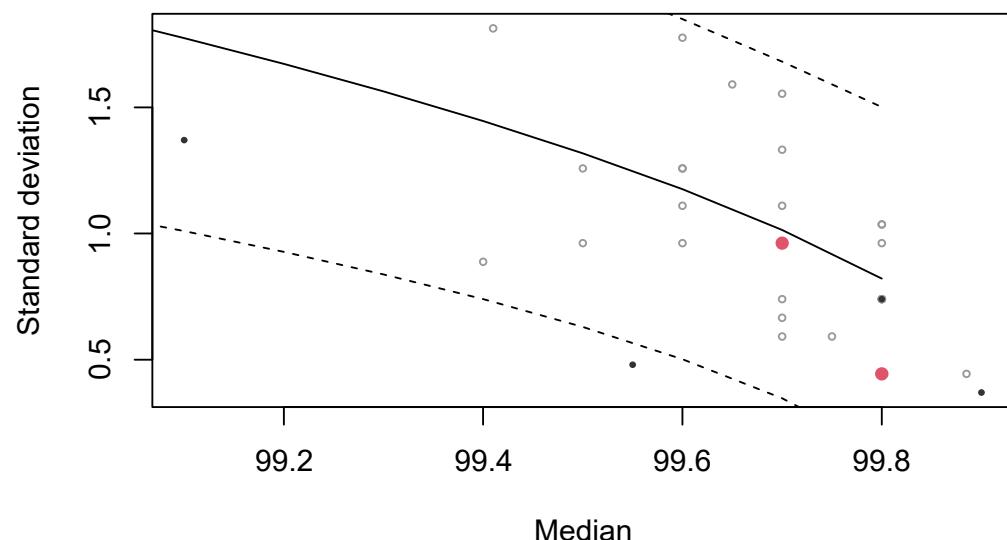


Days after sending	Number of data	Median	Standard deviation
1	1	100	0
3	24	99.8	0.74
4	14	99.1	1.37

Sample FC/19754



#### Comparison of variability with the past

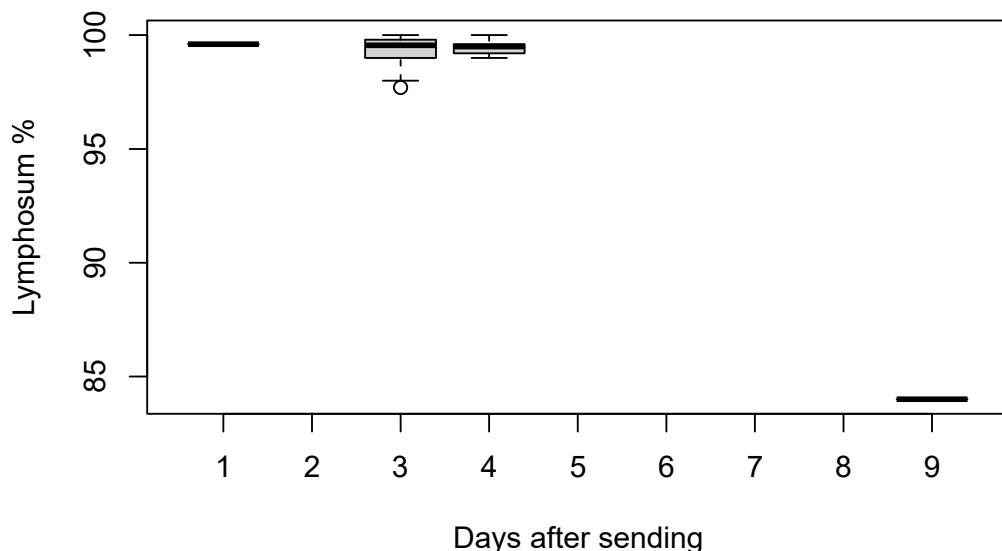


➔ Parameter decreases slightly with time, no significant effect on variability.

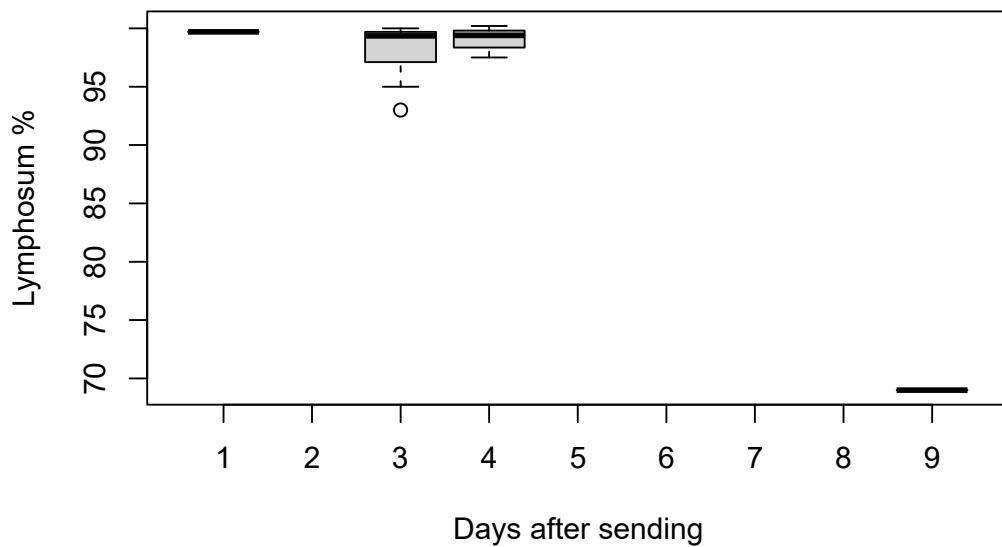
## Lymphosum %

Evolution of parameter through the days

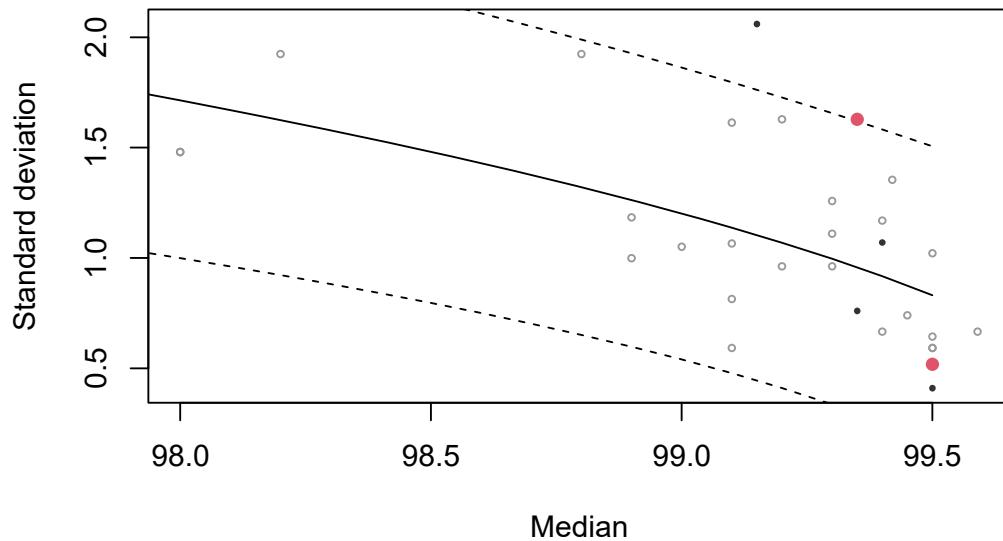
Sample FC/19753



Sample FC/19754



## Comparison of variability with the past



➔ Unclear effect over time, no significant effect on variability.

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END

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