BCP-ALL MRD EQA certificate

Participation and performance certificate of the EuroFlow BCP-ALL MRD EQA scheme

Round [YYYY X]

[Laboratory name]

[Organization name]

[City, Country]

[Additional info on certificate]

Code of the laboratory: A000

The laboratory at the Charles University, Prague, Czech Republic, operates as the leading expert laboratory of the BCP-ALL MRD scheme, with Michaela Reiterová, MD, PhD in the role of lead subject-matter expert. In addition, other EuroFlow subject-matter experts provide support with case selection, expert analysis, determination of consensus reference results, data analysis, performance evaluation, and reporting (see p. 15-16 for a complete list).

This BCP-ALL MRD EQA certificate, including the round summary report, was authorized by Prof. Dr. Jacques J.M. van Dongen, ESLHO EQA Program Coordinator, and was issued on behalf of ESLHO.

A guide through the certificate

This EQA certificate consists of five parts in which performance results are provided:

1) A wet part individual report, which provides:
   - Your results in p-score metrics
   - Your no. of nucleated cells measured compared to all

2) A wet part group report, which provides graphical representations of:
   - MedFI values for the 11 parameters reported per participant
   - No. of nucleated cells reported per participant

3) A dry part individual report, which provides:
   - A comparison of your MRD evaluation to the reference and all participants
   - A comparison of your immunophenotypic characterization to the reference

4) A dry part group report, which provides graphical representations of:
   - No. of MRD events, LoD, and LoQ reported per participant
   - No. of events corresponding to the reported LoD/LoQ and number of nucleated cells measured per participant

5) The Round summary report, which contains the round's summary statistics and the key issues.

Table of contents

Wet part individual report

The wet part of the EuroFlow BCP-ALL MRD EQA scheme is designed to mimic routine sample preparation, data acquisition, and data analysis as used for local diagnostic samples. The BCP-ALL MRD scheme is intended for laboratories that use the EuroFlow BCP-ALL MRD antibody panel in accordance with the relevant EuroFlow standard operating procedures (SOPs) in their routine diagnostics.

Laboratory A000	p-score		
	Control_1	**Control_2**	**Control_3**
CD81 (Mature B cells)			
CD123 (Dendritic cells)			
CD34 (Precursors)			
CD19 (Mature B cells)			
CD10 (Neutrophils)			
CD38 (Dendritic cells)			
CD38 (Monocytes)			
CD20 (Mature B cells)			
CD45 (Mature B cells)			
FSC (Mature B cells)			
SSC (Mature B cells)			

Figure 1: P-scores
For each evaluated population, white bars indicate the distance (calculated as p-score) of the individually reported MedFI values from the expected MedFI values in the EuroFlow reference data set. Grey boxes indicate that the distance is within the acceptable range and black boxes indicate that the distance is outside of the acceptable range. NR = not reported value
An explanation on how performance is scored can be found on p.14.

Summary: 30 out of 33 values in 3 samples are within the allowed range.

Performance rating: Successful (perfect score)

Laboratory A000	**Control_1**	**Control_2**	**Control_3**
Nucleated cells measured	6,522,998	8,105,324	13,965,244
Median of Nucleated cells measured across all samples in this round	5,223,853		

Figure 2: Nucleated cells measured
Numbers of nucleated cells measured that are lower than 4 million are indicated in black, and higher than 4 million are indicated in grey. The median of nucleated cells measured across all samples in the round is also provided. The minimum number of cells allowing MRD quantification of at least 0.001% is 4 million, based on the paper of Theunissen et al. (see the reference below).

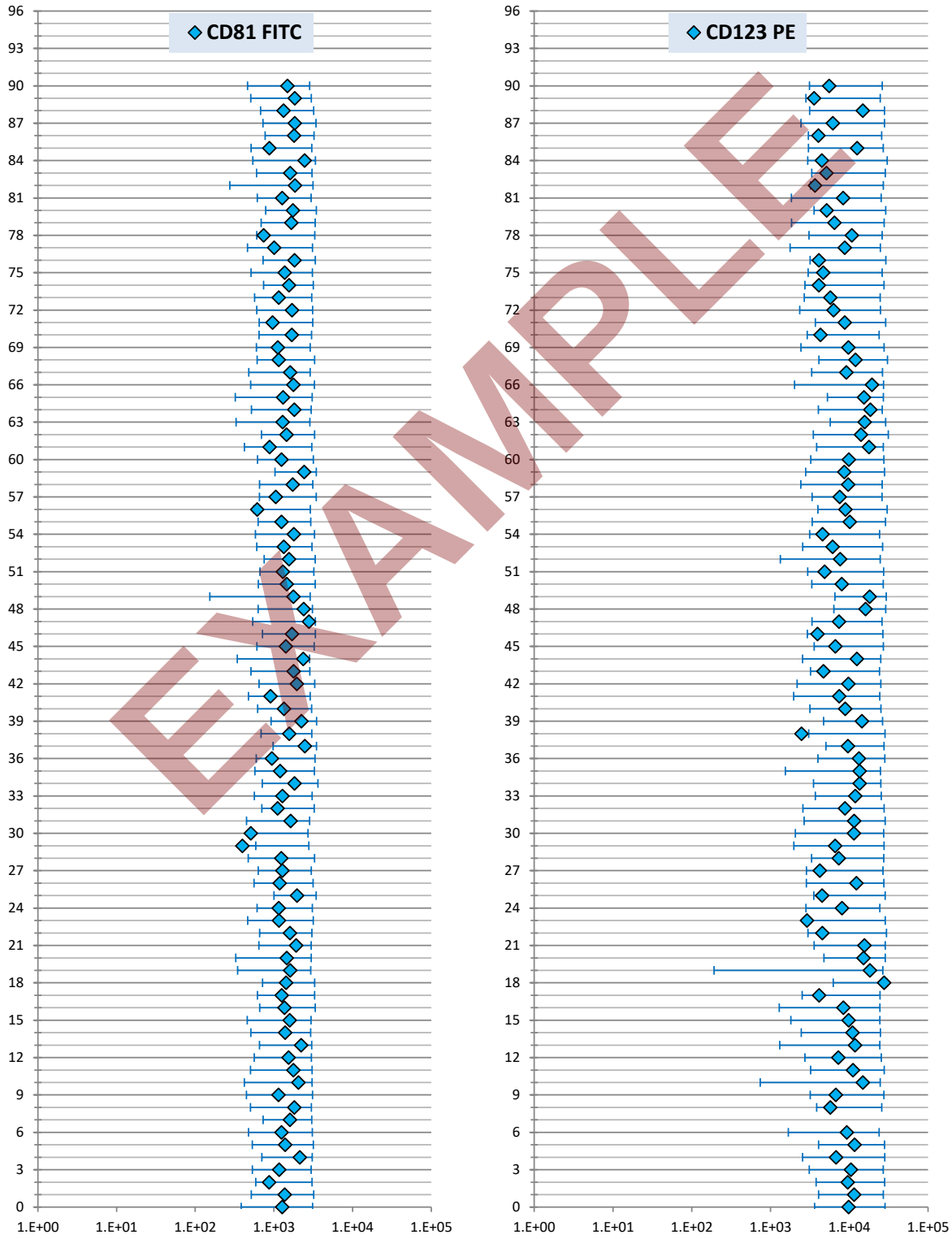
Summary: 3 out of 3 samples allow MRD quantification at a sensitivity of at least 0.001%

Reference: Theunissen P. et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017

Wet part group report

The MedFI values of the reported markers in the wet part (x-axis) are plotted against the file numbers in the current EQA round (y-axis).
File number 0 is the median of the EuroFlow reference dataset. The error bars show the allowed distance from this median.

Your file numbers are 1,2,3



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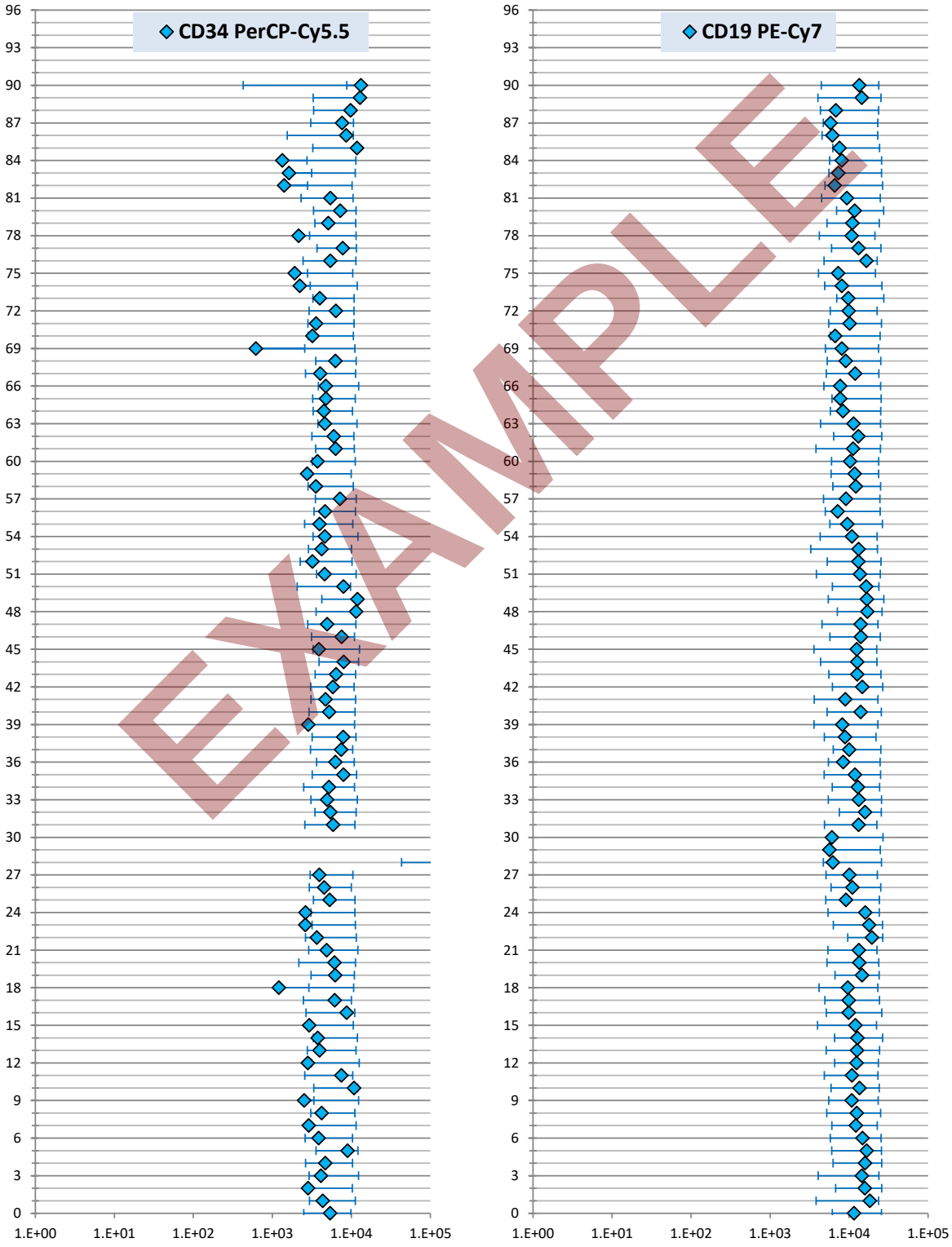
Your file numbers are 1,2,3



The MedFI values of the reported markers in the wet part (x-axis) are plotted against the file numbers in the current EQA round (y-axis).
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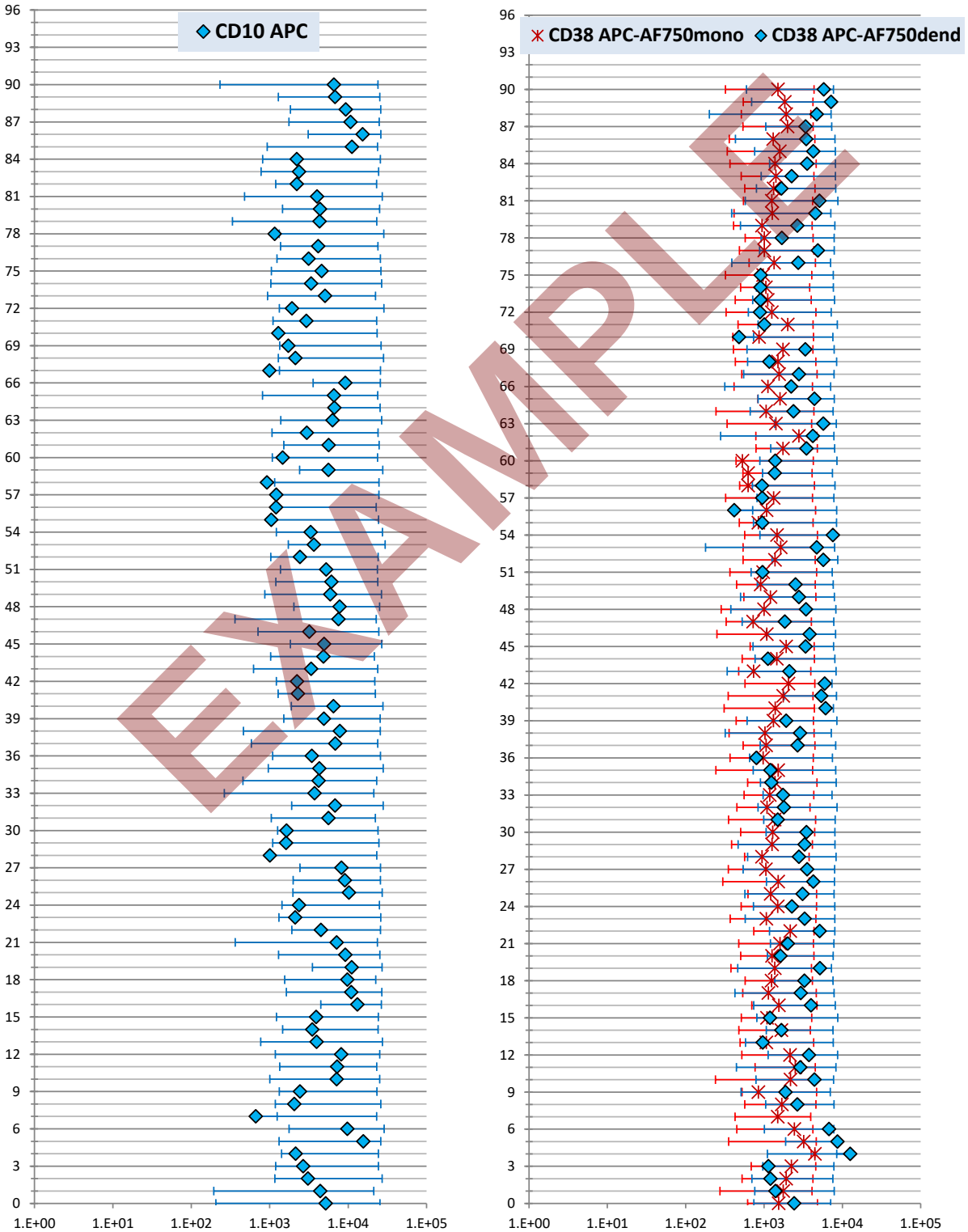
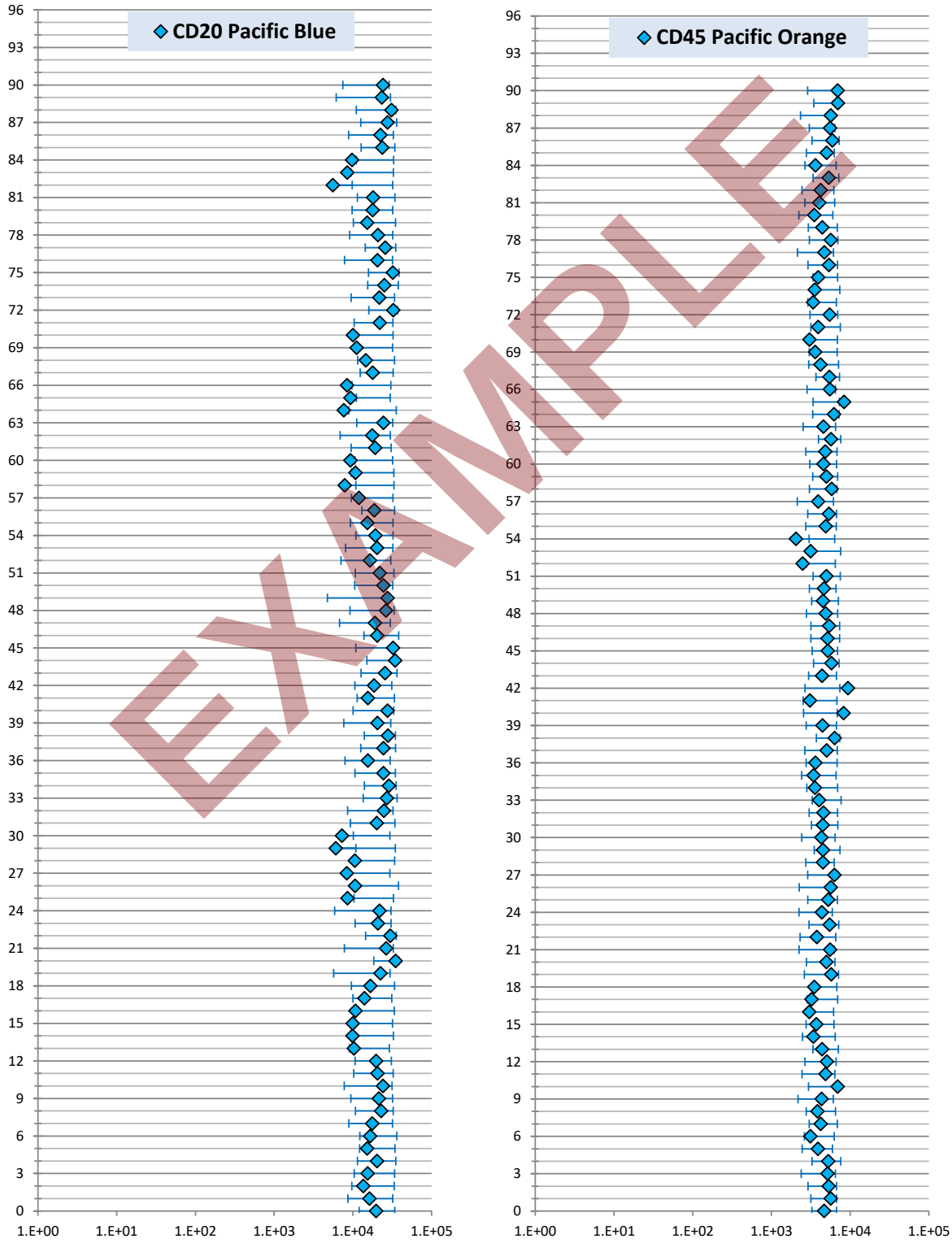
Your file numbers are 1,2,3



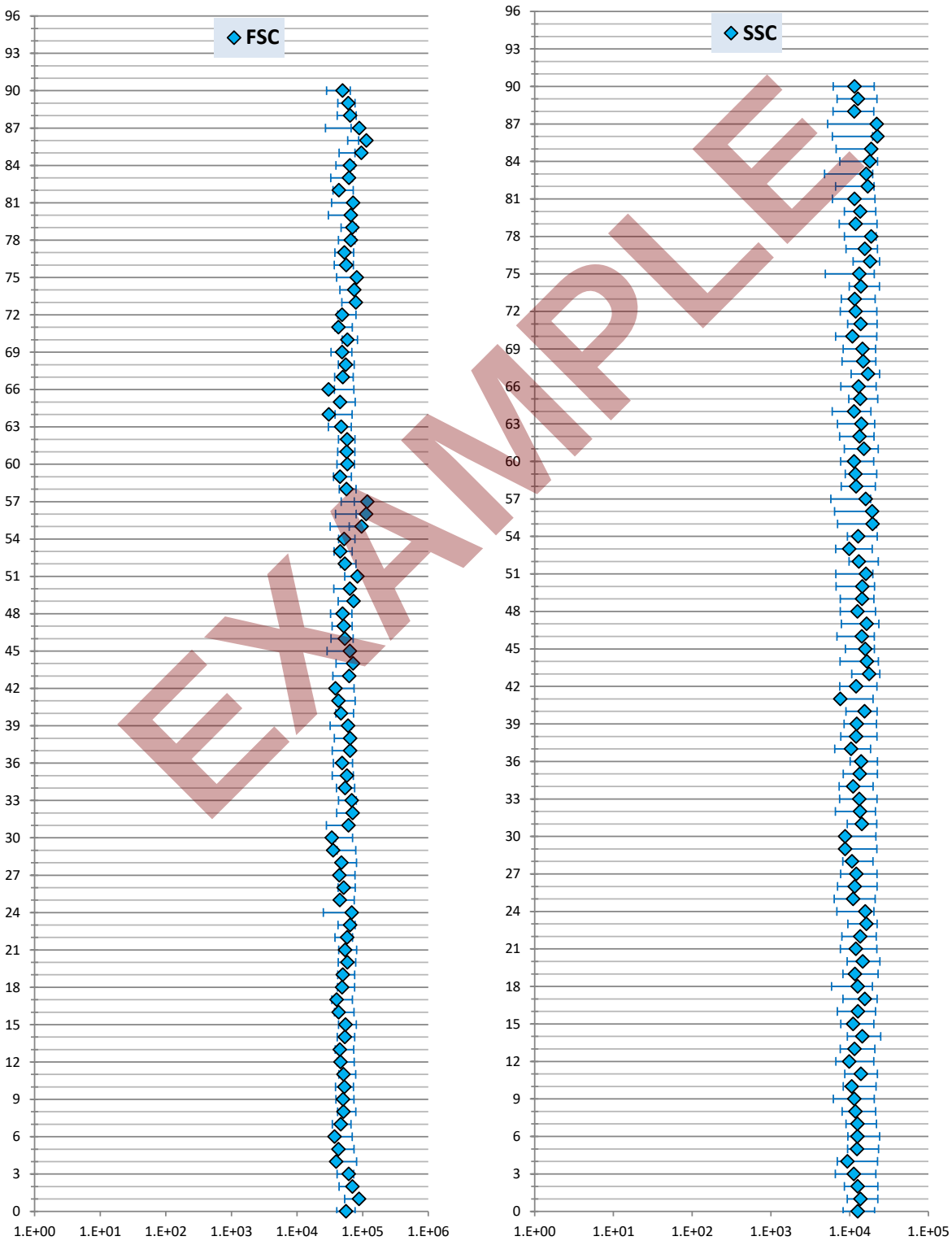
The MedFI values of the reported markers in the wet part (x-axis) are plotted against the file numbers in the current EQA round (y-axis).
File number 0 is the median of the EuroFlow reference dataset. The error bars show the allowed distance from this median.

Your file numbers are 1,2,3

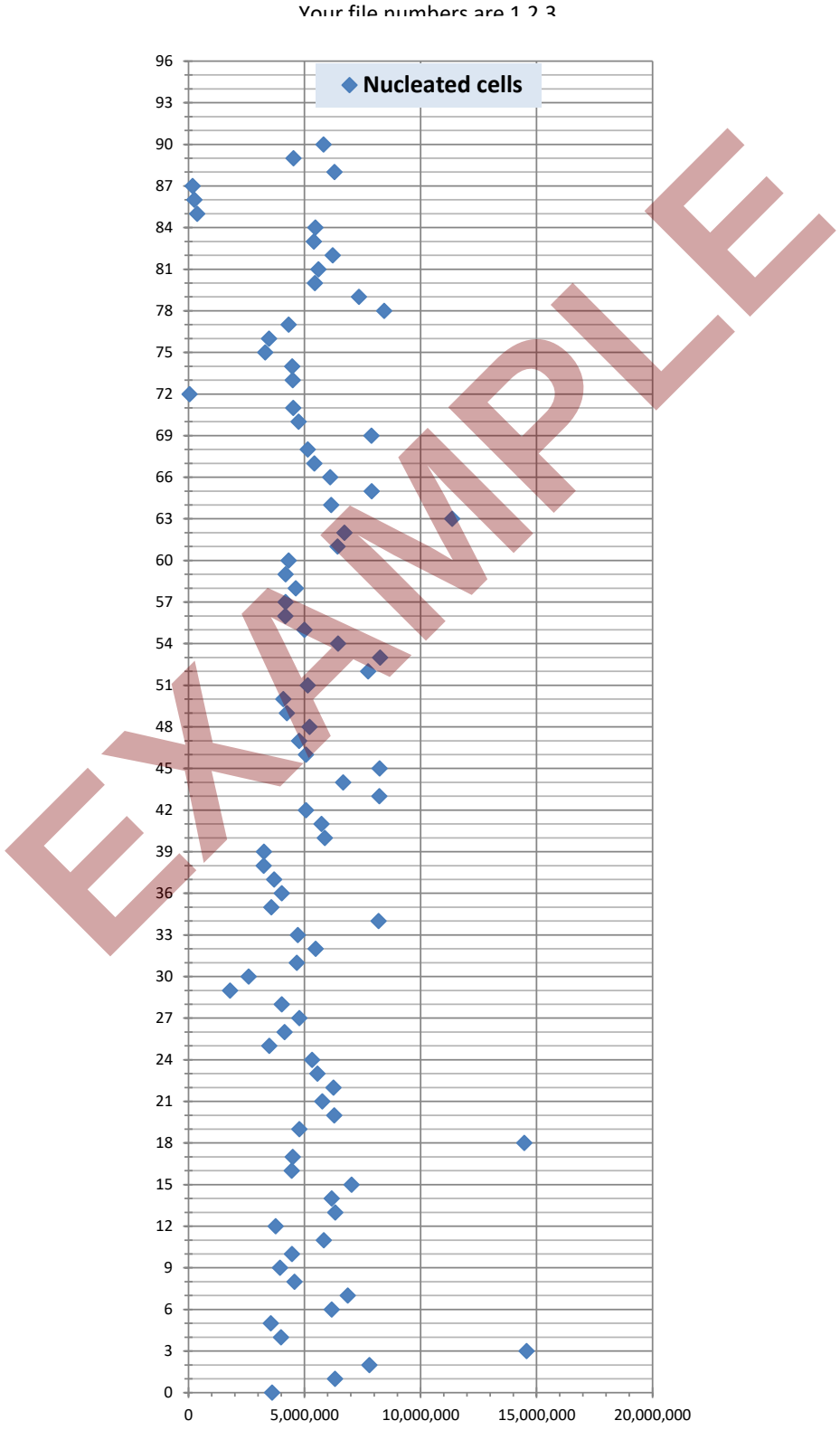


The MedFI values of the reported markers in the wet part (x-axis) are plotted against the file numbers in the current EQA round (y-axis).
File number 0 is the median of the EuroFlow reference dataset. The error bars show the allowed distance from this median.

Your file numbers are 1,2,3



The number of nucleated cells measured in the wet part (x-axis) is plotted against the file numbers in the current EQA round (y-axis). According to Theunissen et al. (2017), a minimum of 4 million cells is required to enable MRD quantification down to 0.001%. Therefore, values below 4 million are considered too low for reliable MRD quantification at that sensitivity.



Dry part individual report

MRD analysis in the merged files

Reference = median value of 3 experts´ results

All participants = the median (minimum-maximum) of all participants´ values reported in this round

Case 1			
	A000	*Reference*	*All participants*
Reported MRD (events)	67852	74190	69411 (0 - 74134)
MRD % calculation check:			
Reported MRD (% of nucleated cells)	*Reported number of nucleated cells in the merged file*		***Calculated MRD (%) (MRD events/Nucleated cells in the merged file*100)***
1.22	5485963		**1.2512**

Case 2			
	A000	*Reference*	*All participants*
Reported MRD (events)	238	262	235 (51 - 326)
MRD % calculation check:			
Reported MRD (% of nucleated cells)	*Reported number of nucleated cells in the merged file*		***Calculated MRD (%) (MRD events/Nucleated cells in the merged file*100)***
0.002	7895652		**0.003**

Case 3			
	A000	*Reference*	*All participants*
Reported MRD (events)	1499	1627	1569 (0 - 2092)
MRD % calculation check:			
Reported MRD (% of nucleated cells)	*Reported number of nucleated cells in the merged file*		***Calculated MRD (%) (MRD events/Nucleated cells in the merged file*100)***
0.012	13985472		**0.0113**

Immunophenotypic characterization of MRD cells in the merged files

Reference = mode value of 3 experts´ results

Each marker expression is indicated according to the following color code:

MARKER EXPRESSION	Not evaluated	Negative	Heterogenous	Positive

	Case 1		Case 2		Case 3	
	A000	*Reference*	*A000*	*Reference*	*A000*	*Reference*
CD20						
CD45						
CD81						
CD66c+CD123						
CD34						
CD19						
CD10						
CD38						

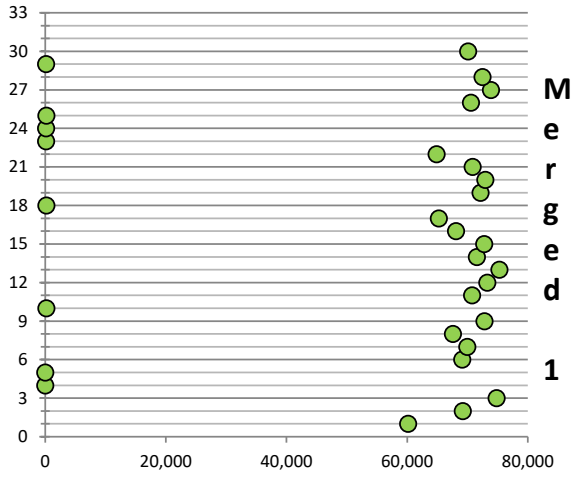
Dry part group report

Reported MRD events in the Merged files	Reported MRD events in the Control files (background)

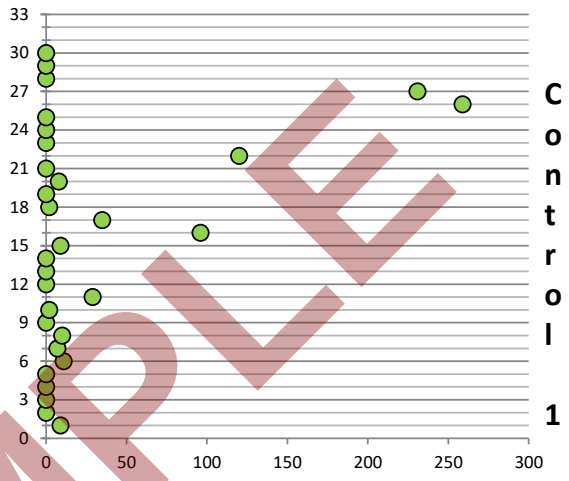
The reported number of MRD events (x-axis) measured in the three Merged files is plotted against the file numbers (y-axis) in the current EQA round.

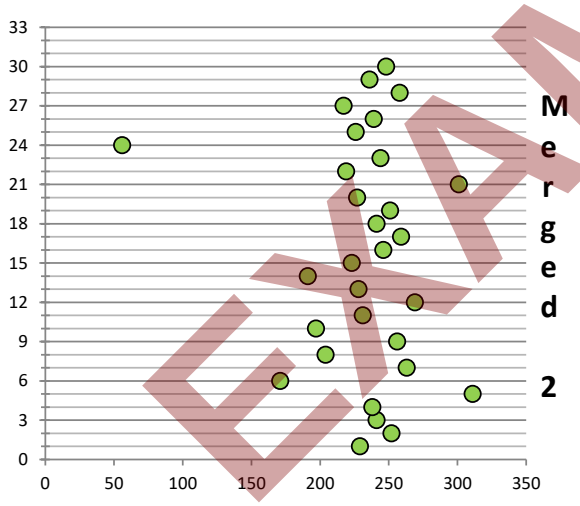
The reported number of background MRD events (x-axis) in the three Control files is plotted against the file numbers (y-axis) in the current EQA round

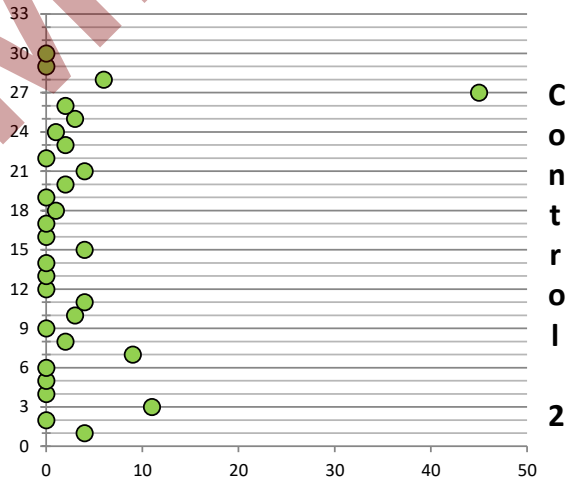
In these graphs, your files have the number 1

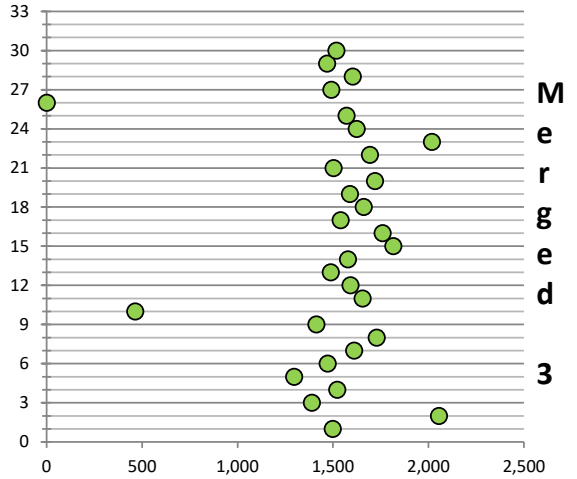


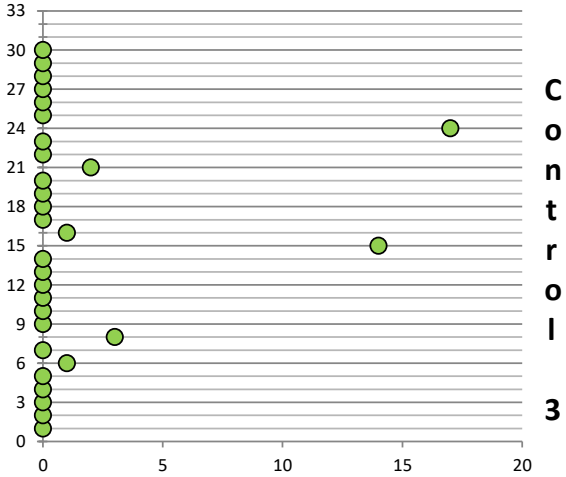
In these graphs, your files have the number 1







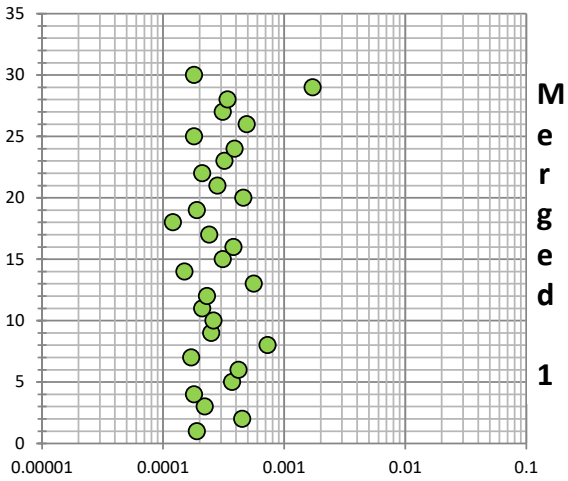


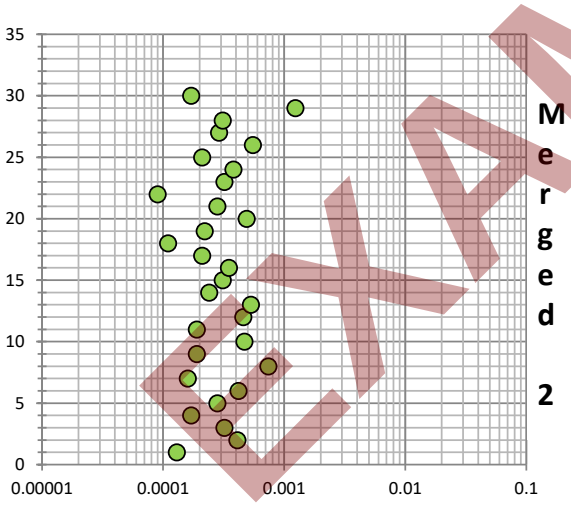


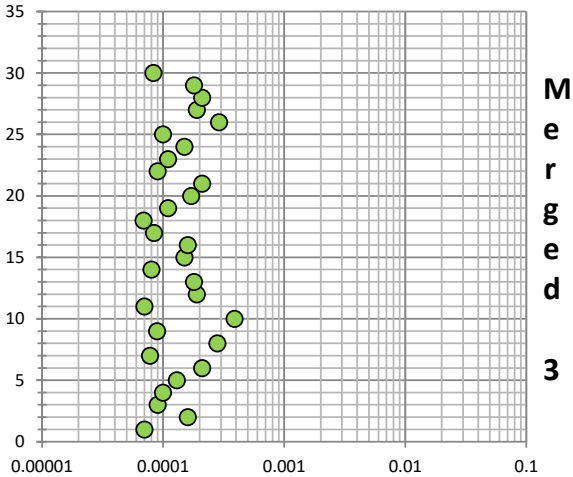
Reported LoD in the Merged files

The reported limit of detection (LoD) (%) (x-axis) in the three Merged files is plotted against the file numbers (y-axis) in the current EQA round.

In these graphs, your files have the number 1



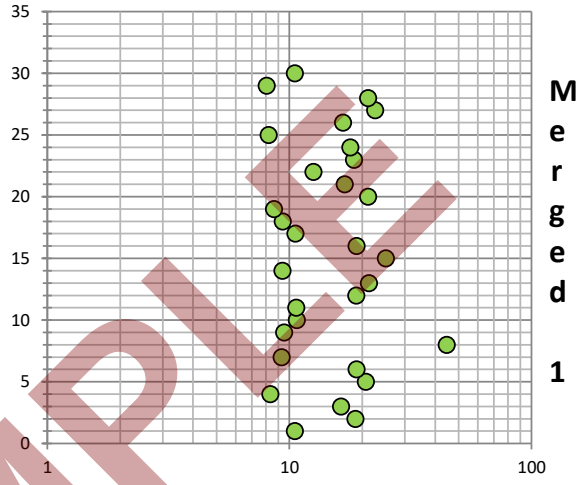


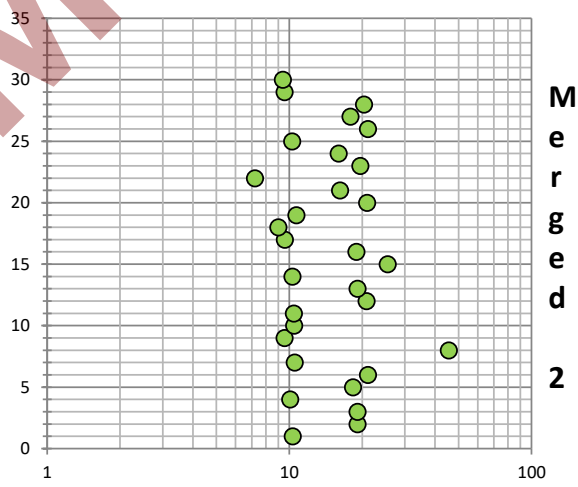


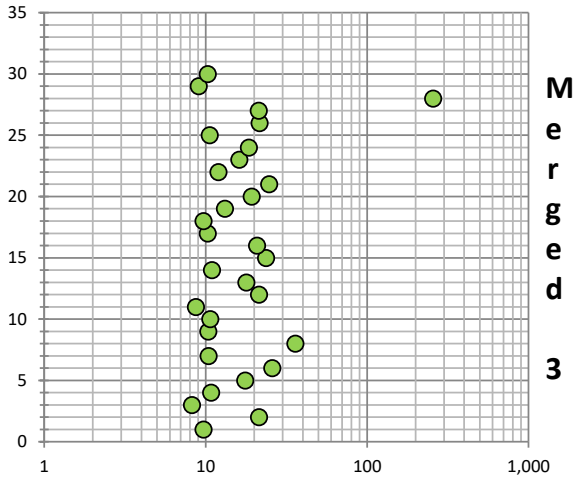
Calculated no.of LoD events in the Merged files

The calculated number of LoD events (i.e, **the number of cells in a cluster considered sufficiently large to conclude MRD positivity**, calculated as (reported LoD*reported nucleated cells in the merged file)/100)) (x-axis) in the three Merged files is plotted against the file numbers (y-axis) in the current EQA round.

In these graphs, your files have the number 1



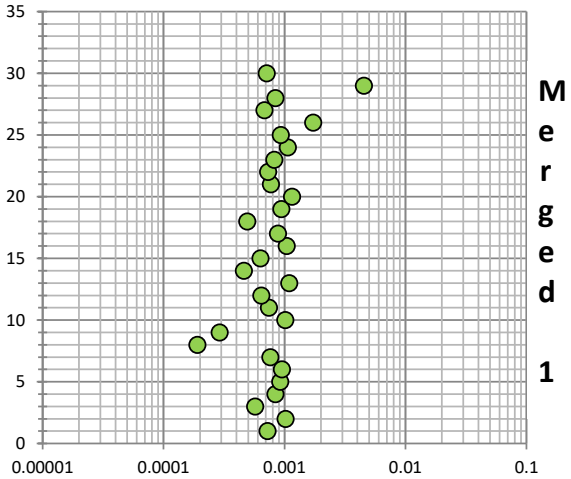


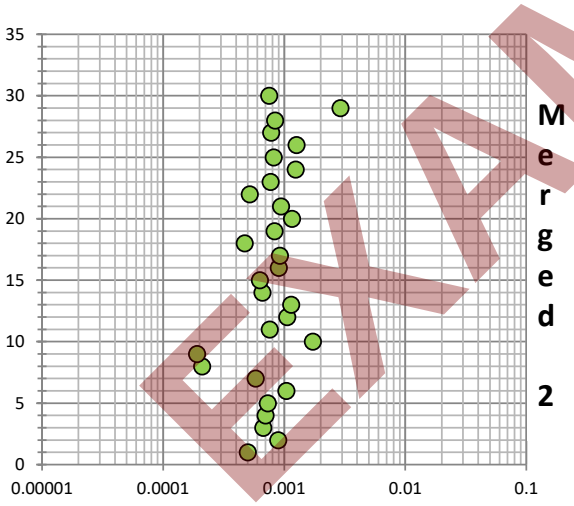


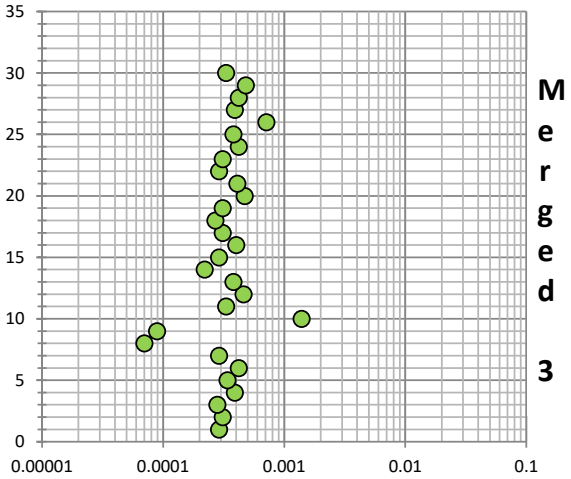
Reported LoQ in the Merged files

The reported limit of detection (LoQ) (%) (x-axis) in the three Merged files is plotted against the file numbers (y-axis) in the current EQA round.

In these graphs, your files have the number 1



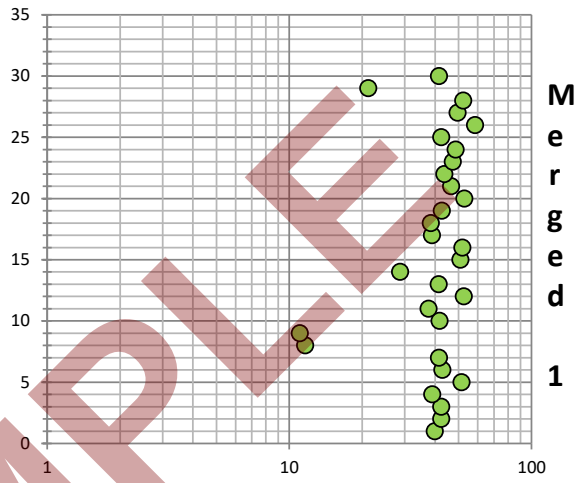


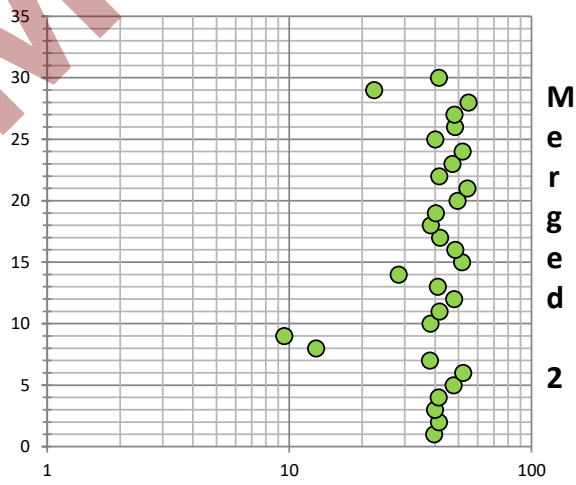


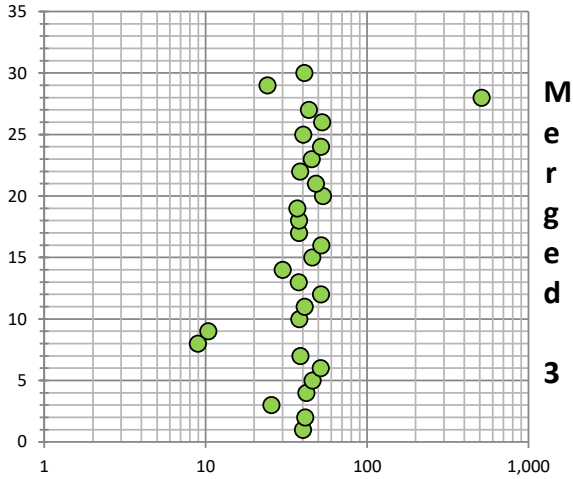
Calculated no.of LoQ events in the Merged files

The calculated number of LoQ events (i.e, **the number of cells in a cluster considered sufficiently large to conclude MRD positivity and to report a certain MRD value**, calculated as (reported LoQ*reported nucleated cells in the merged file)/100)) (x-axis) in the three Merged files is plotted against the file numbers (y-axis) in the current EQA round.

In these graphs, your files have the number 1







General

The objective of the EuroFlow BCP-ALL MRD EQA scheme is to evaluate the technical quality of the sample preparation and measurement on the flow cytometer by applying the EuroFlow BCP-ALL MRD Tube 1 antibody panel (wet part) and to evaluate the ability to analyze and interpret provided fcs files of patients with BCP-ALL (dry part). Participant-specific results are only known to you as EQA participant, ESLHO, and to the appropriate expert laboratories (e.g., for preparing the certificates).

Confidentialitv

Your raw data, performance results, and individual report are strictly confidential between your laboratory, ESLHO, and the Service Provider(s). Results may, however, be shared with other authorized parties for the purpose of quality improvement (e.g., accreditation bodies). Furthermore, under no circumstances may laboratories share this EQA certificate, including the Round summary report, with other laboratories not participating in the EQA scheme.

Data analysis and performance

Wet part

In the wet part you stained and measured three healthy donor samples. Your reported MedFI values were compared with the EuroFlow reference dataset (n=60 measurements across 11 laboratories) using performance score metrics. The p-score for each numerical value reported was calculated using the function below,

$$p-score = \frac{\log_{10} MedFI - \log_{10} qaMedFI}{D^{max}}$$

where qaMedFI is the median of the MedFIs in the reference data set and Dmax is the maximal allowed difference from qaMedFI. Dmax is determined by calculating the 5th and 95th percentiles of the differences between all MedFI values in the reference dataset and qaMedFI. These two percentiles are expressed as absolute values, and the larger value is used as Dmax.

The absolute value of the p-score equals or exceeds the value '1' when the maximum allowed difference from the reference dataset is exceeded. In such case, the reported value is considered out of range and therefore incorrect. Based on the calculation of Dmax, it is expected that 90 - 95% of the p-scores fall within the acceptable range. Thus, the performance of participants with a maximum of 3 incorrect values (out of 33 reported values) is scored as successful with a perfect score. Participants with 4 to 7 incorrect values are scored as successful with an acceptable score and those with more than 7 incorrect values as unsuccessful.

In summary, performance in the BCP-ALL MRD scheme is scored as follows:

- Successful (perfect score): 30, 31, 32, or 33 correct values
- Successful (acceptable score): 26, 27, 28, 29 correct values
- Unsuccessful: 25 or less correct values

In case all 3 reported values for a given marker are incorrect, this indicates a systematic error in that marker.

Dry part

In the dry part you merged the fcs files generated in the wet part with the fcs files provided by ESLHO. You analyzed the merged files and reported the number and the percentage of MRD events, the immunophenotype of the abnormal B cell population, the limit of detection and the limit of quantitation of the assay. The dry part does not include a performance scoring system. Instead, you can determine your performance by comparing your results to that of the reference result (defined as the median or mode value of three subject-matter experts from EuroFlow-affiliated Service Providers) and to the group's median (and min. - max. range).

Task division

The following persons are involved in the design and operation of the BCP-ALL MRD EQA scheme:

Name	Organization/Institute	Role	Tasks
ESLHO			
Prof. Dr. Jacques J. M. van Dongen	ESLHO, Zutphen, NL	EQA Program Coordinator	Final responsibility over EuroFlow EQA program. Review and authorization of the BCP-ALL MRD EQA certificate.
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer (lead)	Overall responsible for organization and operation of the BCP-ALL MRD scheme by ESLHO.
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer	Supports in the organization and operation of the BCP-ALL MRD scheme. Back-up for review and
Lead expert laboratory			
Dr. Michaela Reiterová	Charles University, Prague, CZ	Lead subject-matter expert	Pre-round: Case collection organization, evaluation of expert data, choosing files for box C Post-round: Round summary report
Dr. Naděžda Brdičková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Supports preparation of the rounds and case selection. Post-round: Cleaning and analysis of the submitted results, preparation of the EQA certificate, support in performance evaluation and reporting.
Dr. Ester Mejstříková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Prof. Dr. Tomáš Kalina	Charles University, Prague, CZ	Subject-matter expert	Post-round: Round summary report
Additional expert laboratories			
Dr. Paula Fernandez	Canton Hospital Aarau, Aarau, CH	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Mattias Hofmans	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Pauline Breughe	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Anna Laqua	University Hospital Schleswig-Holstein, Kiel, Germany	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Joana Desterro	Portuguese Institute of Oncology, Lisbon, Portugal	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results

Dr. Joana Rodríguez	Portuguese Institute of Oncology, Lisbon, Portugal	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Maria Soares	Portuguese Institute of Oncology, Lisbon, Portugal	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Chiara Buracchi	Tettamanti Foundation, Monza, Italy	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Anja de Jong	Princess Máxima Center, Utrecht, The Netherlands	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Bartosz Perkowski	Medical University of Silesia, Zabrze, Poland	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Robby Engelmann	University of Rostock, Rostock, Germany	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Juan Flores-Montero	Flow Cytometry Units, Hematology Department of the University Hospital of Salamanca and University of Salamanca, Salamanca, Spain	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Susana Delfa	Flow Cytometry Units, Hematology Department of the University Hospital of Salamanca and University of Salamanca, Salamanca, Spain	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Elen Oliveira	Federal University of Rio de Janeiro, Rio de Janeiro, BR	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results

For more information or in case you have questions about the EuroFlow BCP-ALL MRD EQA scheme, or other EuroFlow EQA schemes, please contact EuroFlow.EQA@eslho.org.

Round summary report

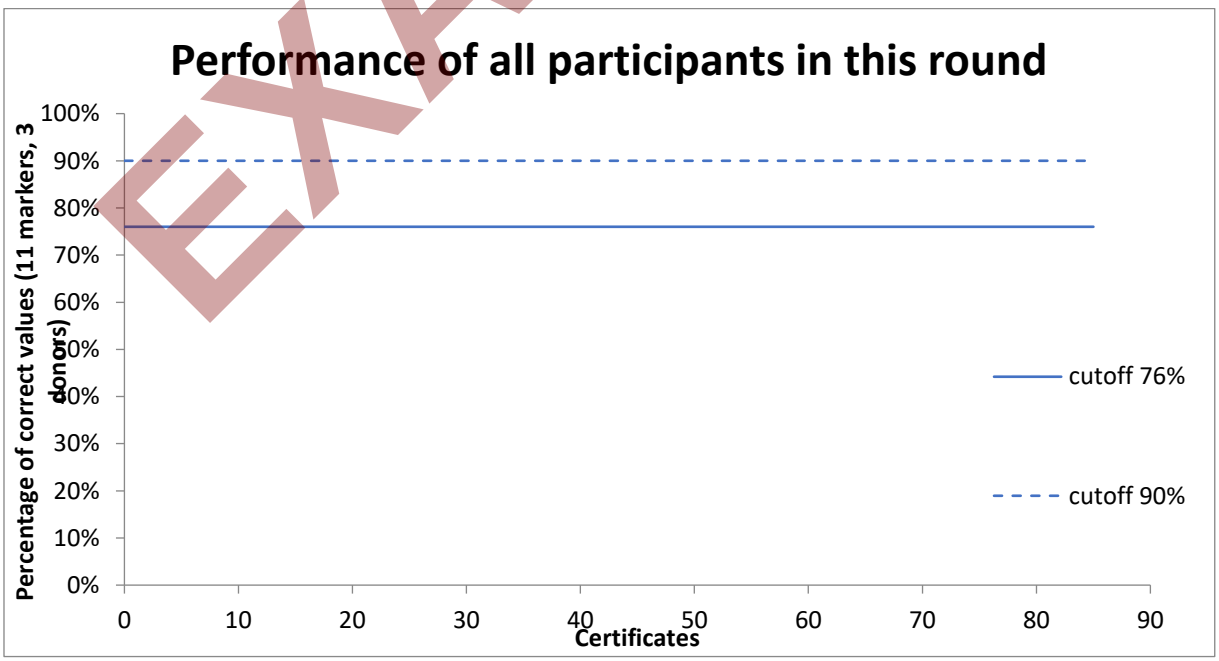
<The round summary report offers an expert analysis of the round's results, highlighting key findings, commonly observed issues, frequent mistakes, and important reminders. Designed as both a practical and educational resource, this report provides participants with valuable expert insights into areas that may require attention and improvement. By identifying what went wrong and outlining points to focus on in future rounds, the report supports continuous learning and helps strengthen and improve diagnostic practice within your laboratory.>

Thank you for participating in round …

In total…

Based on this assessment …

Wet part:



Note: The EQA target values (qaMedFI and D^{max}) of the BCP-ALL MRD EQA wet part are used for calculating the p-score (see references 3 and 8) and were determined based on 60 BCP-ALL MRD FCS files from 11 EuroFlow laboratories, acquired according to the EuroFlow protocols on BD FACS Canto (n=15) and BD FACS Lyric (n=45).

1) Reagents

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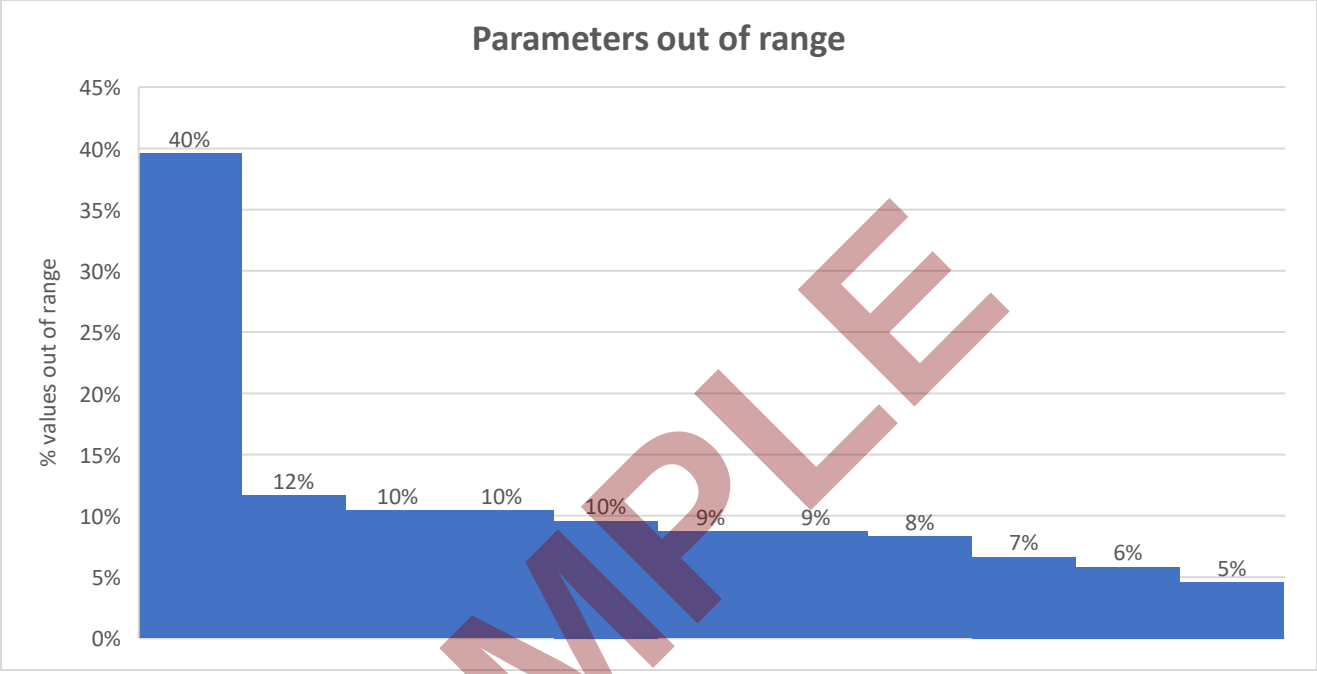

Dry part:

MRD analysis in the merged file

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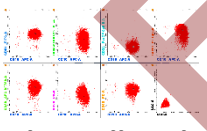
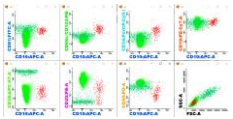
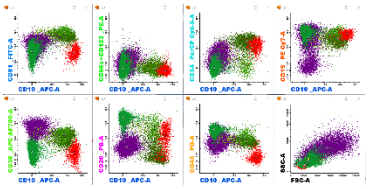
File 1: BCP-ALL blasts only



File 2: All nucleated cells without debris



File 3: All events



1. **MRD value**, calculated as *(MRD events/total number of cells)*100%*, …
2. **LoD**, calculated as *(LoD events/all nucleated cells)*100%*, …
3. **LoQ**, calculated as *(LOQ events/all nucleated cells)*100%*, …

Additionally, participants also reported the "*number of MRD-like background events*" …

Immunophenotypic characterization of MRD cells

…

Important reminders and key issues

Wet part:

…

Dry part:

…

Authorization

This EuroFlow BCP-ALL MRD EQA certificate is authorized by …

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Date Signature

Resources & Literature

www.euroflow.org (SOP protocols, recommended reagents, Lyric setup files)

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