LST EQA certificate

Participation and performance certificate of the EuroFlow LST EQA scheme

Round [YYYY X]

[Laboratory name]

[Organization name]

[City, Country]

[Additional info on certificate]

Code of the laboratory: A000_1

The laboratory at the Charles University, Prague, Czech Republic, operates as the leading expert laboratory of the LST scheme, with Prof. Dr. Tomáš Kalina in the role of lead subject-matter expert. In addition, other EuroFlow subject-matter experts provide support with data analysis, performance evaluation, reporting, and responding to technical questions (see p. 10 for a complete list).

This LST 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 three parts in which performance results are provided:

1) An individual report, in which your results in p-score metrics are provided.
2) A group report, in which a graphical representation of the MedFI values extracted from each participant's uploaded cyt files is provided anonymously.
3) The Round summary report, which contains the round's summary statistics and frequently found mistakes.

Table of contents

Individual report

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

Instrument	p-score		
BD Lyric	**Donor 1**	**Donor 2**	**Donor 3**
CD8 on CD8+ T cells			
Igλ on Lambda+ B cells			
Igκ on Kappa+ B cells			
CD56 on CD56++ NK cells			
CD5 on T cells			
CD19 on B cells			
CD3 on T cells			
CD38 on Monocytes			
CD4 on CD4+ T cells			
CD20 on B cells			
CD45 on T cells			

Figure 1: P-scores
For each evaluated population, white boxes 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.9.

Summary: 31 out of 33 values in 3 samples are within the allowed range.

Performance rating: Successful (perfect score)

Group report

If you uploaded a cyt file with the correct, compatible EQA profile (as indicated in the scheme instructions), you can view the graphical comparisons of your data with the data reported by the other participants in this EQA round on the following pages.

Your file numbers are 1,2,3. To see the graphical comparison of your data with others in the current round, look for these numbers on the y axis in the following graphs.

Figure 2A. MedFI values (circle) of the given markers on the gated peripheral blood lymphocyte subsets (x axis) plotted against the file numbers in the current EQA round (y axis). Color codes represent **IgL pos B-cells (orange)**, **IgK pos B-cells (red)**, **CD56 bright NK-cells (green)**, **CD4 pos T-cells (light blue)** **and** **CD8 pos T-cells (dark blue)**.

Your file numbers are 1,2,3

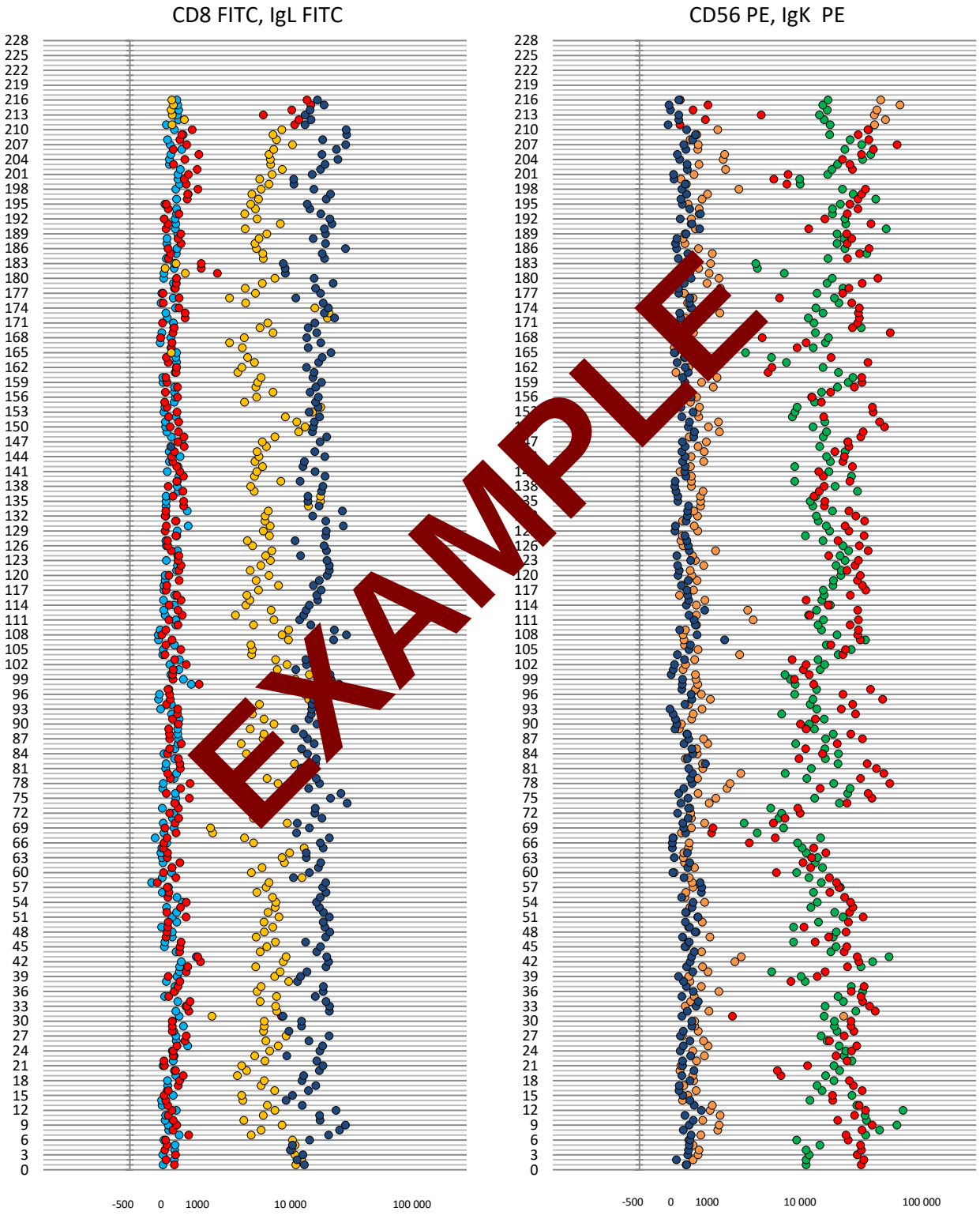


Figure 2B. MedFI values (circle) of the given markers on the gated peripheral blood lymphocyte subsets (x axis) plotted against the file numbers in the current EQA round (y axis). Color codes represent **IgL pos B-cells (orange)**, **IgK pos B-cells (red)**, **CD56 bright NK-cells (green)**, **CD4 pos T-cells (light blue)** **and** **CD8 pos T-cells (dark blue)**.

Your file numbers are 1,2,3

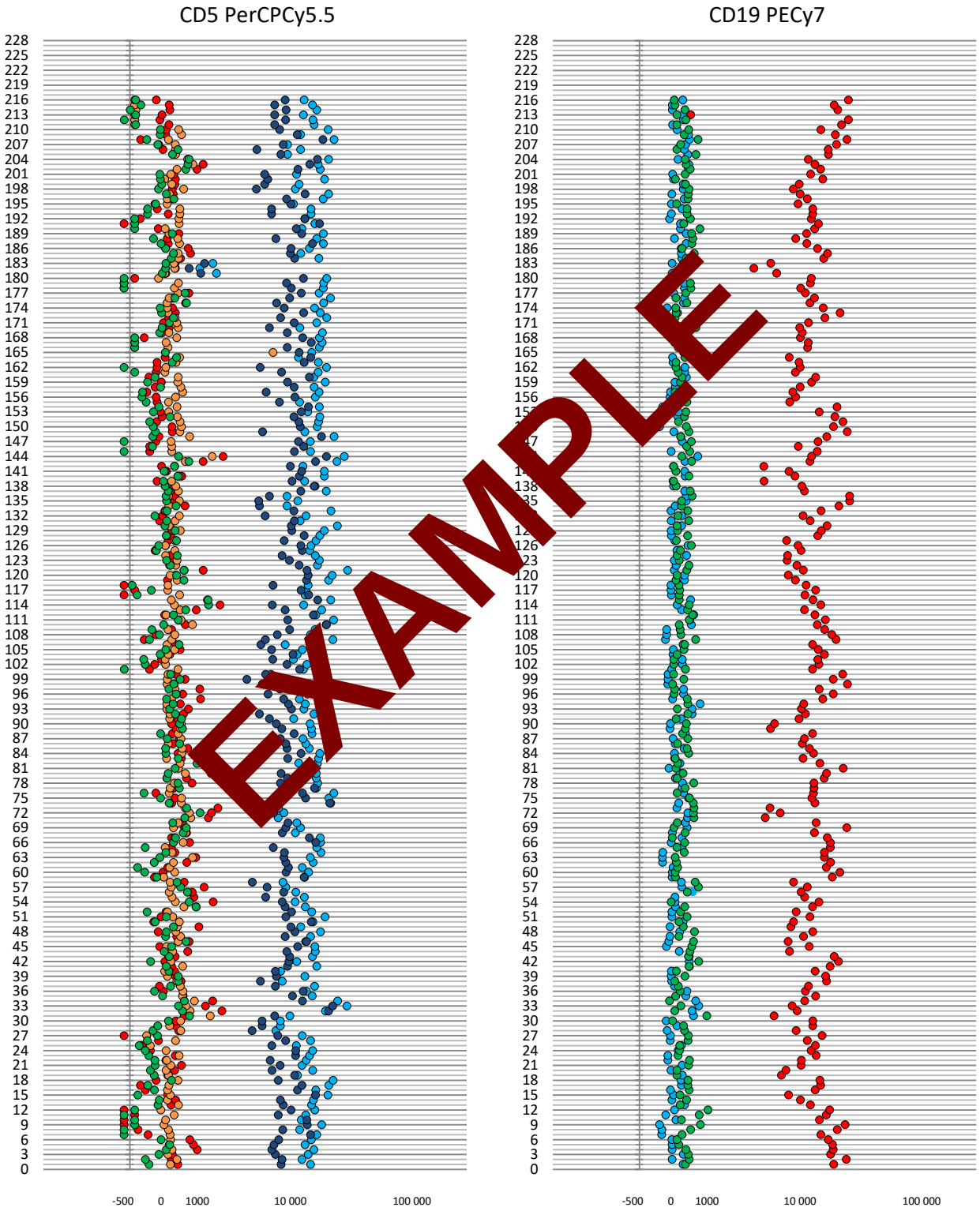


Figure 2C. MedFI values (circle) of the given markers on the gated peripheral blood lymphocyte subsets (x axis) plotted against the file numbers in the current EQA round (y axis). Color codes represent **IgK pos B-cells (red)**, **CD56 bright NK-cells (green)**, **CD8 pos T-cells (dark blue)** and **Monocytes (pink)**.

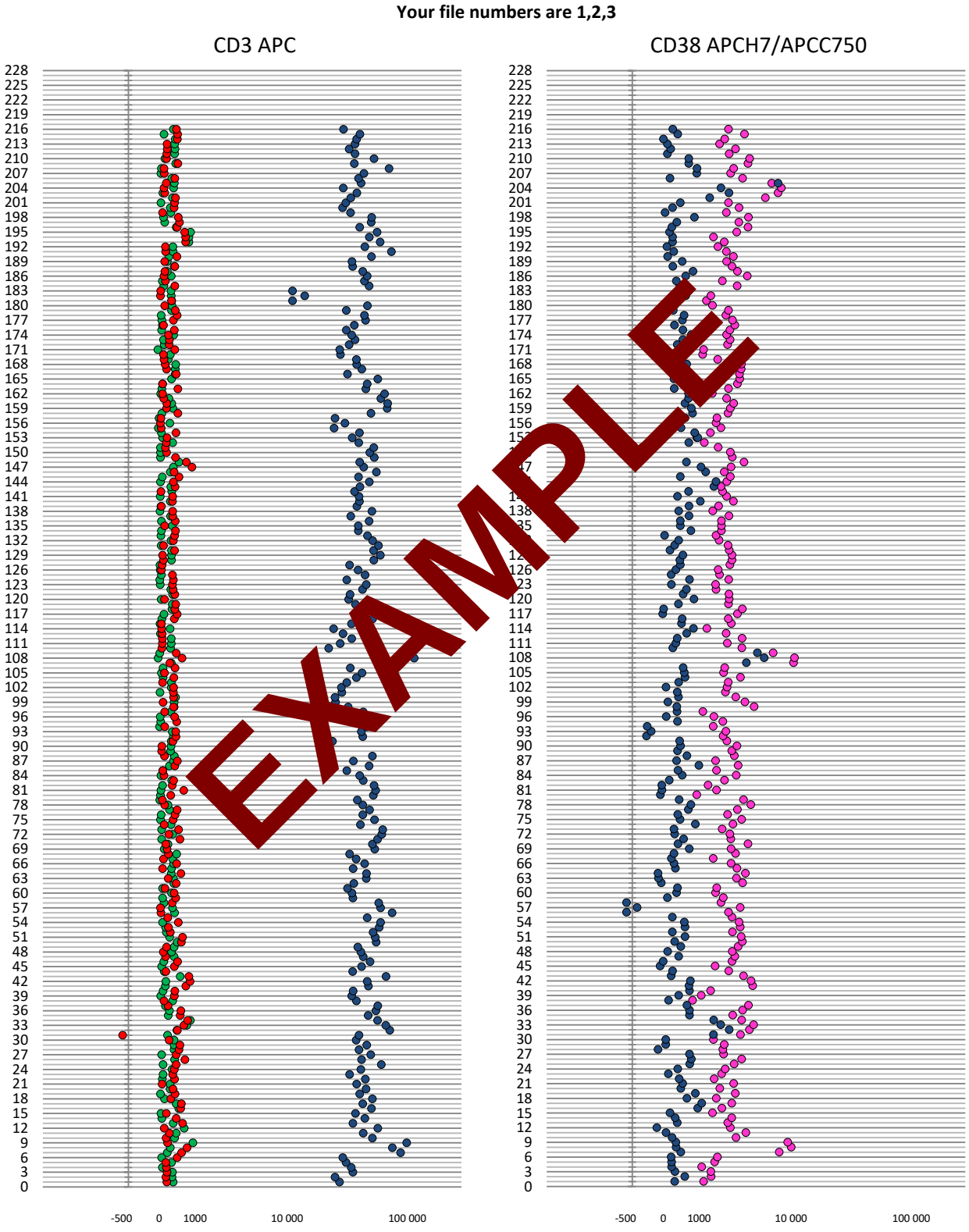
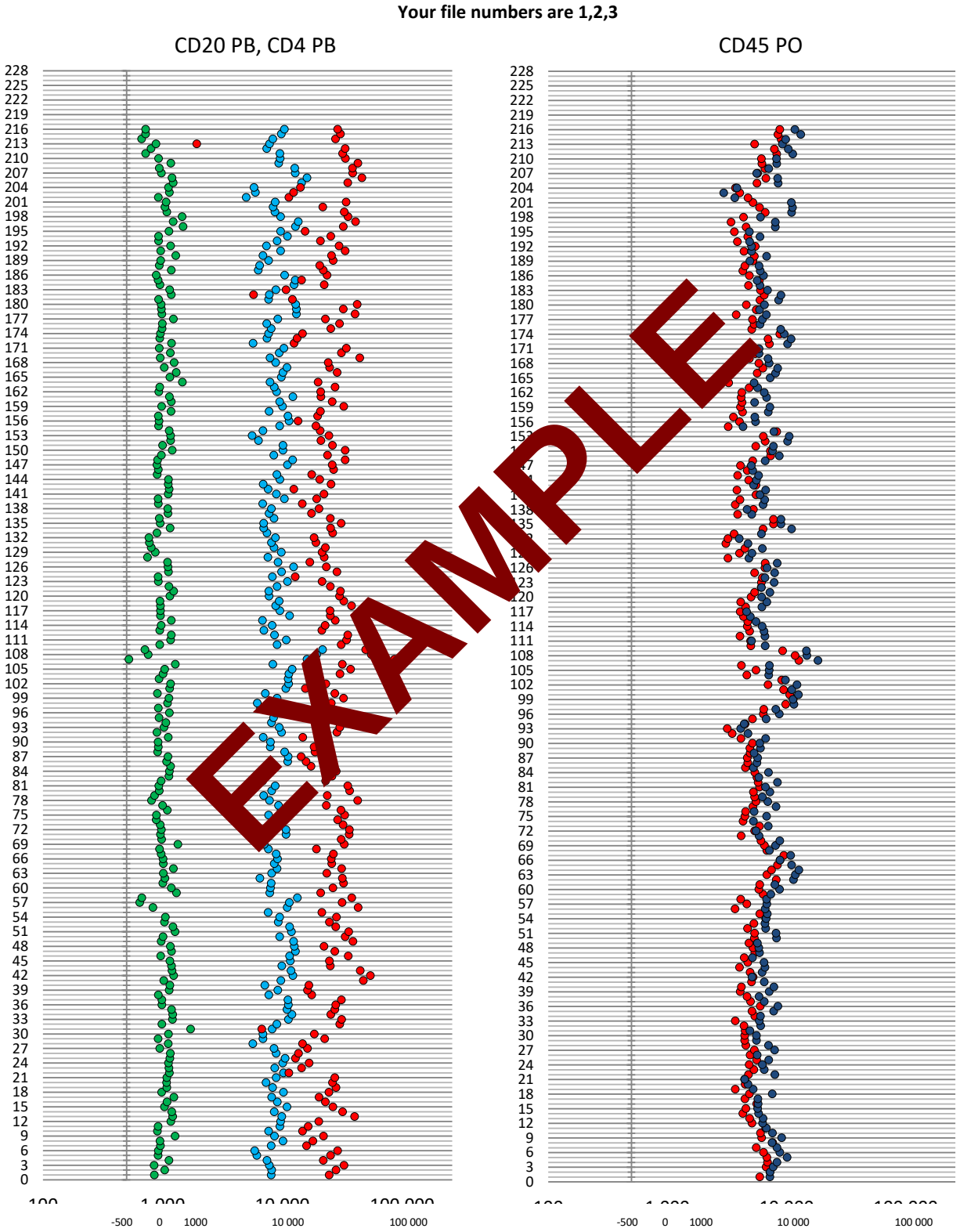


Figure 2D. MedFI values (circle) of the given markers on the gated peripheral blood lymphocyte subsets (x axis) plotted against the file numbers in the current EQA round (y axis). Color codes represent **IgK pos B-cells (red)**, **CD56 bright NK-cells (green)**, **CD4 pos T-cells (light blue)** **and** **CD8 pos T-cells (dark blue)**.



General

In the EuroFlow LST EQA scheme, three healthy-donor peripheral blood samples drawn locally at the participant's institution are used. No samples are provided by ESLHO. The scheme tests the technical performance of the participant's instrument setup, sample preparation, measurement, and analysis of the LST tube. The scheme does not test the diagnostic interpretation.

Confidentiality

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

Your reported MedFI values were compared with the reference data set (Kalina et al., 2015, 2019) 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 (or the 10th and 90th percentiles for less uniform markers) 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 (or 80 – 90% for less uniform markers). 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 LST 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.

Task division

The following persons are involved in the design and operation of the LST 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; authorizes the LST round report.
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer (lead)	Overall responsible for organization and operation of the LST scheme by ESLHO.
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer	Supports in the organization and operation of the LST scheme.
Lead expert laboratory			
Prof. Dr. Tomáš Kalina	Charles University, Prague, CZ	Lead Subject-matter expert	Designed the original LST scheme, expert input, review of the results and the summary, responds to participants´ questions about the technical aspects of the scheme and participant results, provides targeted help to participants.
Dr. Naděžda Brdičková	Charles University, Prague, CZ	Subject-matter expert	Assists in preparing the rounds, cleaning and analysis of the data submitted, preparing the certificates, and preparing the round summary with feedback to the participants.

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

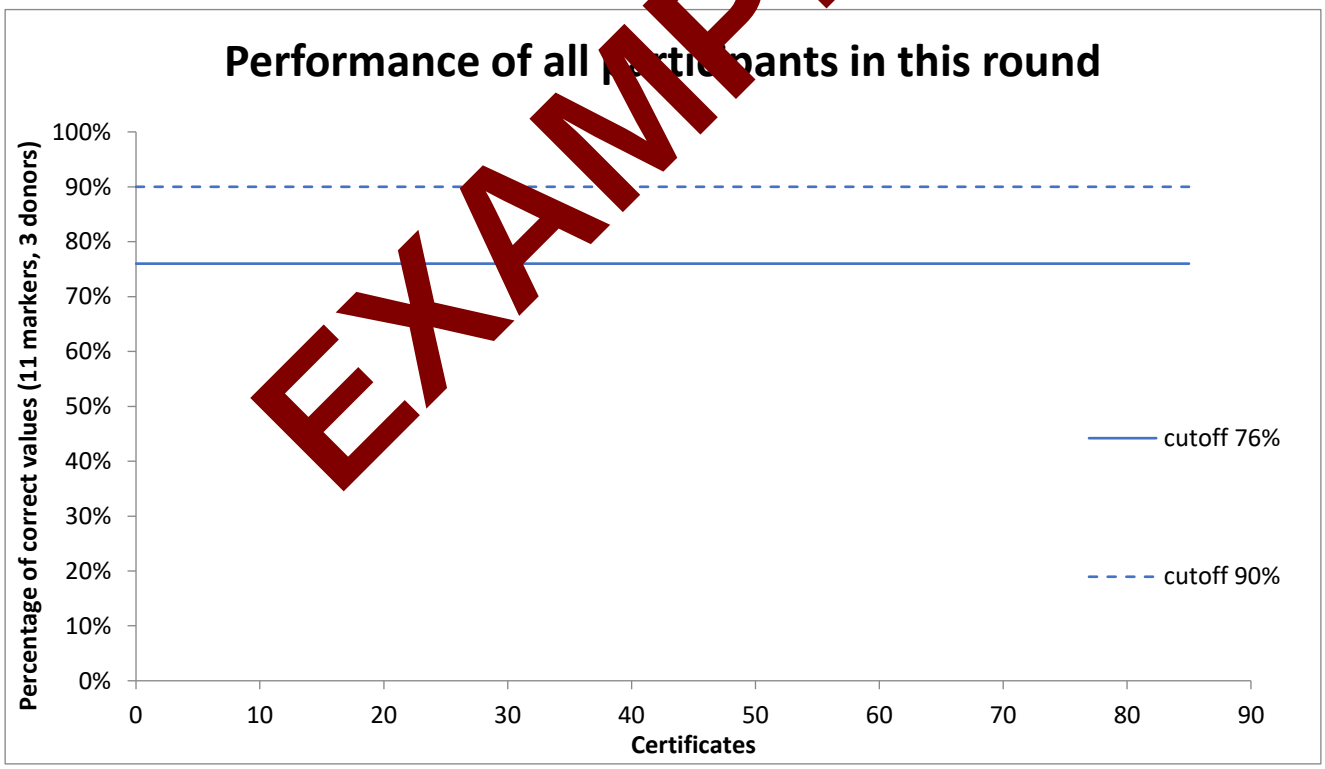
Round summary report

<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 …



Note: The EQA reference values (qaMedFI and Dmax – see references 3 and 5) were calculated based on 96 measurements in 15 laboratories, acquired according to the EuroFlow protocols.

Frequently found mistakes

1) Reagents

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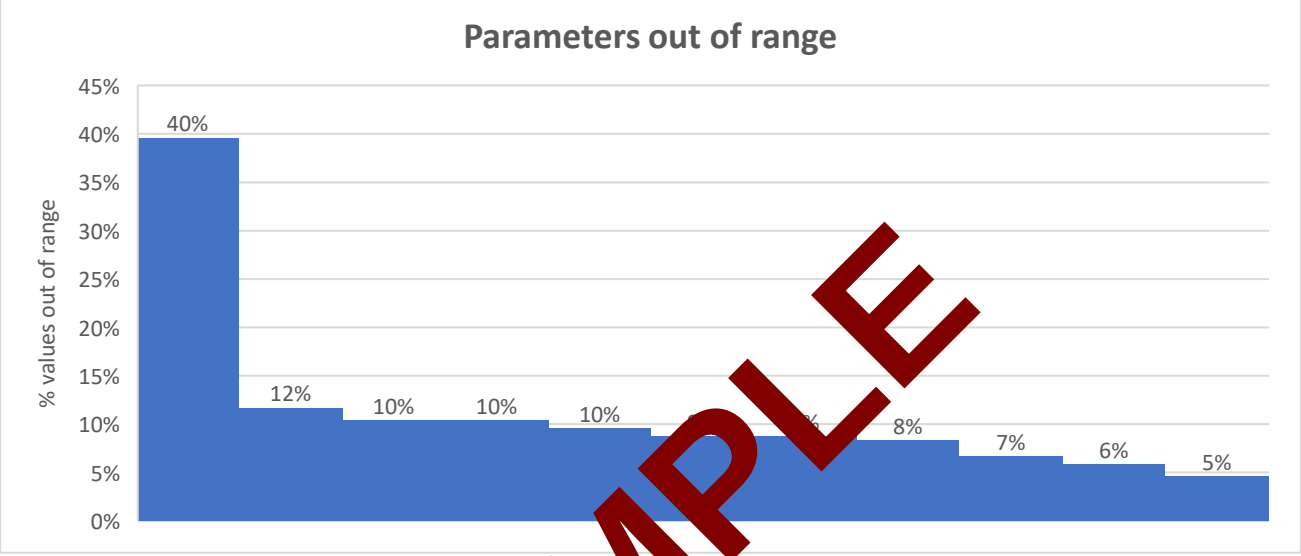

2) Use of alternative reagents

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3) Cyt files

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4) Transformation of the data to the 18-bit scale.

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5) The Rainbow beads 7th peak MedFI values

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6) Kappa and Lambda staining

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7) Acquisition of a sufficient number of cells

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8) NK CD56 bright cell gating

…

9) Overcompensation

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This EuroFlow LST EQA certificate is authorized by ...

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Date Signature

Resources & Literature

www.euroflow.org (SOPs/protocols, reagents, Lyric setup files)

1: Glier H, Novakova M, Te Marvelde J, Bijkerk A, Morf D, Thurner D, Rejlova K, Lange S, Finke J, van der Sluijs-Gelling A, Sedek L, Flores-Montero J, Böttcher S, Fernandez P, Ritgen M, van Dongen JJM, Orfao A, van der Velden VHJ, Kalina T. ***Comments on EuroFlow standard operating procedures for instrument setup and compensation for BD FACS Canto II, Navios and BD FACS Lyric instruments.*** J Immunol Methods. 2019 Dec;475:112680. doi: 10.1016/j.jim.2019.112680. Epub 2019 Oct 23. PMID: 31655051.

2: Kalina T. ***Reproducibility of Flow Cytometry Through Standardization: Opportunities and Challenges.*** Cytometry A. 2020 Feb;97(2):137-147. doi: 10.1002/cyto.a.23901. Epub 2019 Oct 8. PMID: 31593368.

3: Kalina T, Brdickova N, Glier H, Fernandez P, Bitter M, Flores-Montero J, van Dongen JJM, Orfao A. ***Frequent issues and lessons learned from EuroFlow QA.*** J Immunol Methods. 2019 Dec;475:112520. doi: 10.1016/j.jim.2018.09.008. Epub 2018 Sep 17. PMID: 30253.

4: Nováková M, Glier H, Brdičková N, Vlková [illegible] Santos AH, Lima M, Roussel M, Flores-Montero J, Szczepanski T, Böttcher S, van der Velden VHJ, Fernandez P, Mejstříková E, Burgos L, Paiva B, van Dongen JJM, Orfao A, Kalina T. ***How to make use of the standardized EuroFlow 8-color protocols possible for instruments of different manufacturers.*** J Immunol Methods. 2019 Dec;475:112388. doi: 10.1016/j.jim.2017.11.007. Epub 2017 Nov 30. PMID: 29154914.

5: Kalina T, Flores-Montero J, Lécrevisse Q, Pedreira CE, van der Velden VH, Novakova M, Mejstrikova E, Hrusak O, Böttcher S, Karsch D, Sędek Ł, Trinquand A, Boeckx N, Caetano J, Asnafi V, Lucio P, Lima M, Helena Santos A, Bonaccorso P, van der Sluijs-Gelling AJ, Langerak AW, Martin-Ayuso M, Szczepański T, van Dongen JJ, Orfao A. ***Quality assessment program for EuroFlow protocols: summary results of four-year (2010-2013) quality assurance rounds.*** Cytometry A. 2015 Feb;87(2):145-56. doi: 10.1002/cyto.a.22581. Epub 2014 Oct 23. PMID: 25345353.

6: van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, Rawstron A, Asnafi V, Lécrevisse Q, Lucio P, Mejstrikova E, Szczepański T, Kalina T, de Tute R, Brüggemann M, Sedek L, Cullen M, Langerak AW, Mendonça A, Macintyre E, Martin-Ayuso M, Hrusak O, Vidriales MB, Orfao A; EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). ***EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes.*** Leukemia. 2012 Sep;26(9):1908-75. doi: 10.1038/leu.2012.120. Epub 2012 May 3. PMID: 22552007