PIDOT EQA certificate

Participation and performance certificate of the ESLHO-EuroFlow PIDOT EQA scheme

Round [YYYY X]

[Laboratory name]

[Organization name]

[City, Country]

[Additional info on certificate]

Code of the laboratory: A000

The laboratory at the University of Ghent, Ghent, BE, operates as the leading expert laboratory of the PIDOT EQA scheme, with Prof. Dr. Carolien Bonroy in the role of lead subject-matter expert. In addition, other EuroFlow subject-matter experts provide support with case selection, determination of consensus results, data analysis, performance evaluation, and reporting (see p. 15-16 for a complete list).

This PIDOT 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) A wet part individual report, in which participant results from the wet part of the test are provided in p-score metrics.

2) A wet part group report, which shows a graphical representation of the MedFI values as reported by the participant, in comparison with the values reported by other participants in the round.

3) A dry part report, in which individual participant results from the dry part are provided in comparison to the consensus reference and to summary statistics (median and min. - max. range values) of the other participants in the round.

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

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Wet part individual report

The EuroFlow PIDOT 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 PIDOT antibody panel in accordance with the relevant EuroFlow standard operating procedures (SOPs) in their routine diagnostics.

	p-score		
	A000	**A000**	**A000**
CD27 (unswitched B cells+PC)			
CD45RA (B cells)			
CD8 (CD8 T cells)			
IgD (PreGC B cells)			
CD16+CD56 (NK cells)			
CD4 (CD4 T cells)			
IgM (unswitched B cells+PC)			
CD19 (B cells)			
TCRgd (TCRgd T cells)			
CD3 (T cells)			
CD45 (T 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.13.

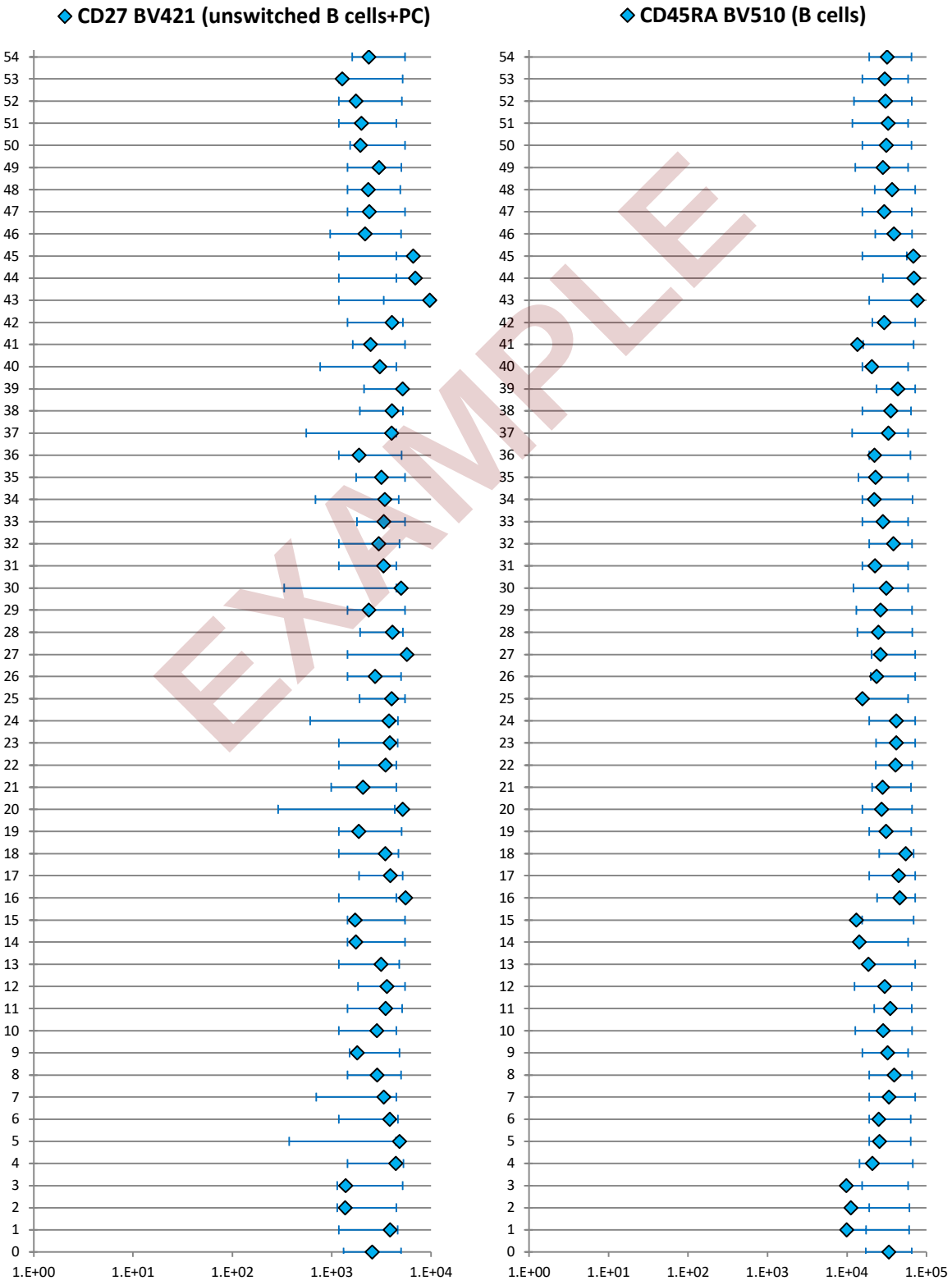
Summary: 23 out of 33 values in 3 samples are within the acceptable range

Performance rating: Unsuccessful

Wet part group report

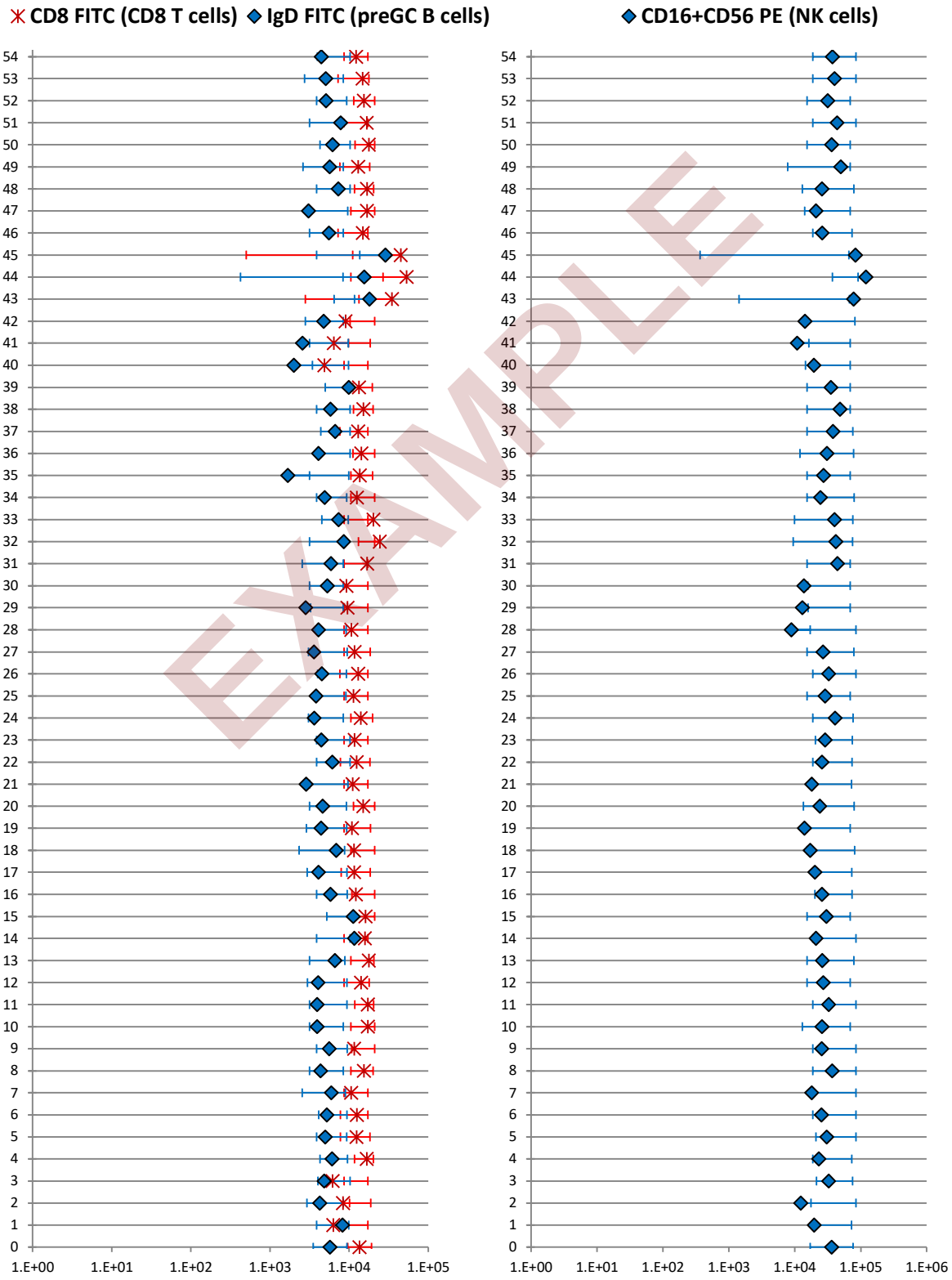
The MedFI values of the reported markers (x axis) are plotted against the file numbers in the current EQA round (y axis).
The file number 0 is the median of the reference data set. The error bars show the allowed distance from this median.

Your file numbers are 1,2,3.



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

Your file numbers are 1,2,3.



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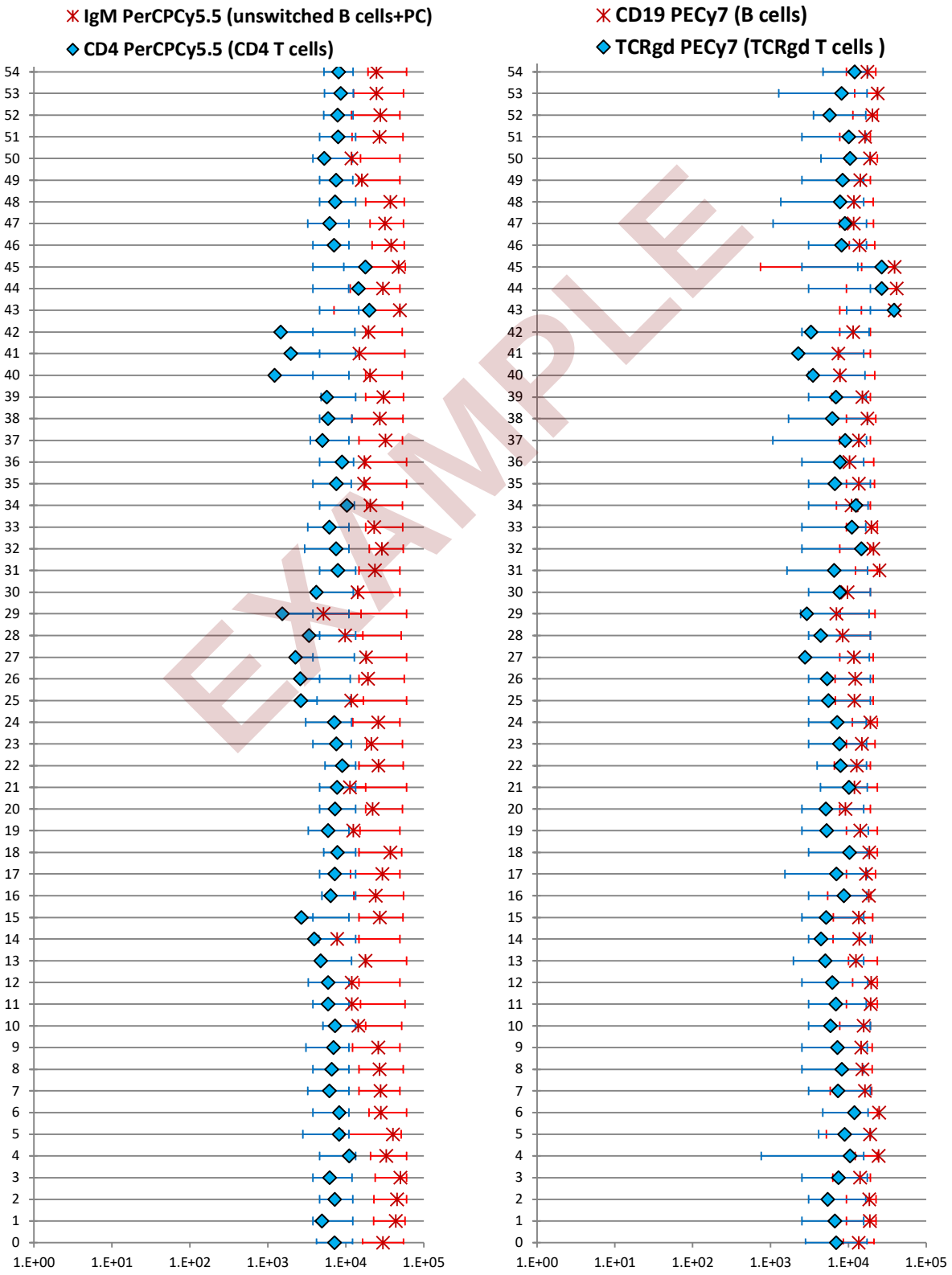
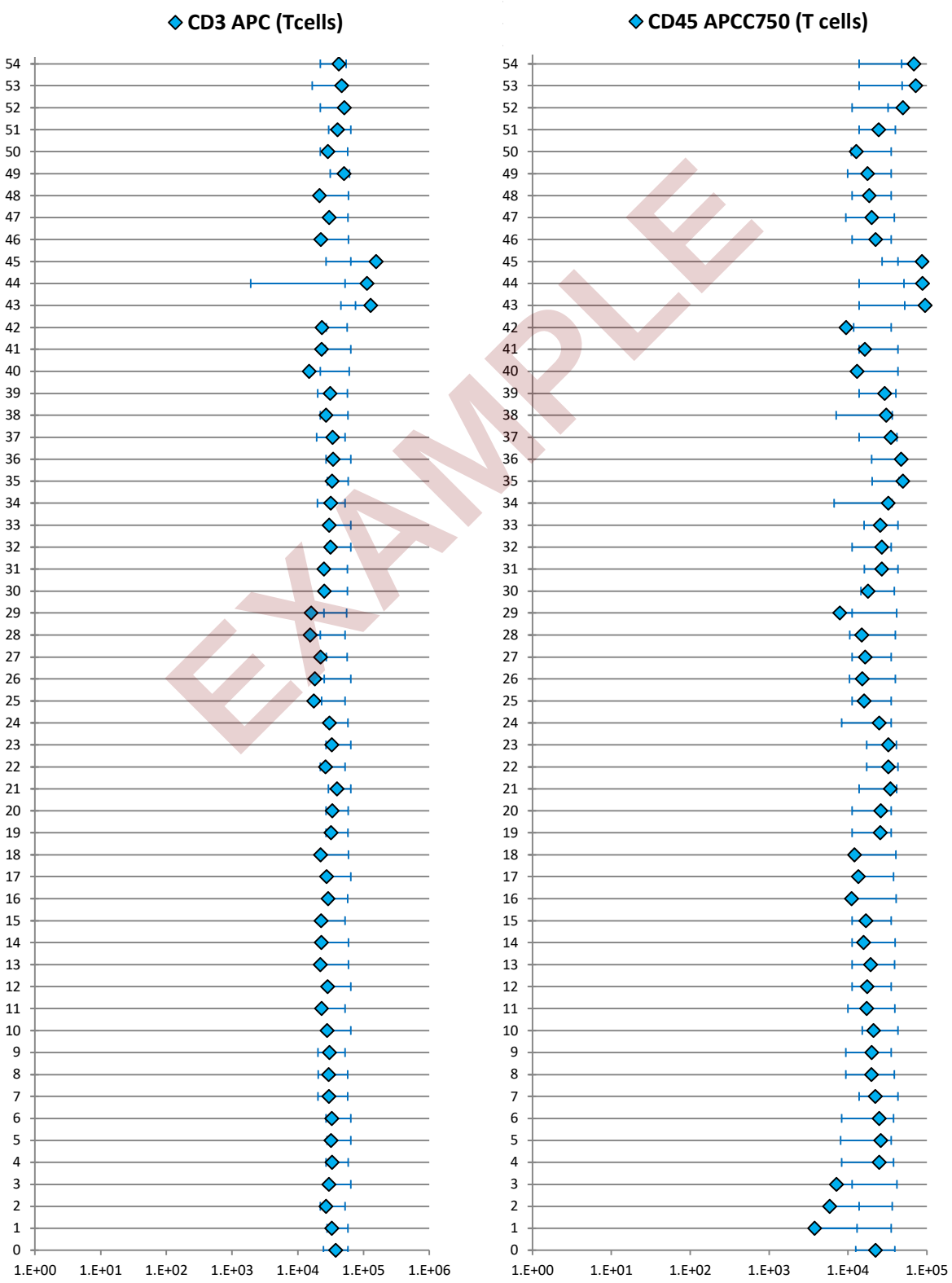


The MedFI values of the reported markers (x axis) are plotted against the file numbers in the current EQA round (y axis).
The file number 0 is the median of the reference data set. The error bars show the allowed distance from this median.

Your file numbers are 1,2,3.



Dry part report

Level 1: cell counts

Note: Reference = the result of the reference interpretation (median of 3 subject-matter experts)
Cell populations for which the reference is not provided, are to be compared to the peer group´s results.
All reports = median (minimum-maximum) of all participants´ reported values

File ID	case 1			case 2		
	A000	All reports	Reference	A000	All reports	Reference
B cells_abs (/µL)	111	124 (100 - 127)	123	325	343.5 (183 - 358)	345
B cells_% parent	6.5	6 (1.9 - 32.6)	6	28	17.4 (7.9 - 74.9)	17.7
PreGC B cells_abs (/µL)	20	114 (1 - 116)	109	254	324 (0 - 340)	329
PreGC B cells_% parent	80	91 (1 - 92)	90	95	94.7 (0 - 96)	94.2
memory B cells (IGMD+) + PC (unswitched)_abs (/µL)	5	3 (2 - 102)	4	13	14 (5 - 312)	10
memory B cells (IGMD+) + PC (unswitched)_ % parent	12	2 (0.039 - 93)	3.6	3.8	4 (0.35 - 99)	4
memory B cells (IGMD-) + PC (switched)_abs (/µL)	7	8 (5 - 9)	8.2	6	4 (2 - 6)	4.5
memory B cells (IGMD-) + PC (switched)_ % parent	58	6.45 (0.12 - 74.3)	6.6	21	1.14 (0.14 - 21.2)	1.3
T cells_abs (/µL)	1502	1818.5 (1416 - 1863)	1810	1025	1350.5 (867 - 1446)	1348
T cells_% parent	65	87.6 (28.4 - 91.2)	87.5	56	69.85 (49 - 75)	69.5
CD4+ T cells_abs (/µL)	1010	1151 (901 - 1177)	1138	150	188.5 (105 - 644)	189
CD4+ T cells_% parent	35	63.2 (17.9 - 68)	63.4	33	14 (5.9 - 48)	13.9
Naive CD4+ T cells_abs (/µL)	501	692 (498 - 734)	690	3	1.5 (0 - 9)	1.6
Naive CD4+ T cells_% parent	52	60.1 (10.6 - 65)	60.6	5	0.725 (0 - 6.1)	0.8
TM/CM CD4+ T cells_abs (/µL)	325	378.5 (304 - 413)	No reference is given for this subpopulation	158	174.5 (98 - 187)	No reference is given for this subpopulation
TM/CM CD4+ T cells_% parent	25	32.4 (5.9 - 39)	No reference is given for this subpopulation	94	93.55 (2.3 - 97.11)	No reference is given for this subpopulation
EM CD4+ T cells_abs (/µL)	59	72 (58 - 78)	No reference is given for this subpopulation	58	8.5 (3 - 161)	No reference is given for this subpopulation
EM CD4+ T cells_ % parent	6	6.27 (1.1 - 7)	No reference is given for this subpopulation	24	4.3 (0.22 - 25)	No reference is given for this subpopulation

Note: Reference = the result of the reference interpretation (median of 3 subject-matter experts)
Cell populations for which the reference is not provided, are to be compared to the peer group´s results.
All reports = median (minimum-maximum) of all participants´ reported values

File ID	case 1			case 2		
	A000	All reports	Reference	A000	All reports	Reference
TD CD4+ T cells_abs (/µL)	6	8.5 (0 - 15)	No reference is given for this subpopulation	1	0 (0 - 1)	No reference is given for this subpopulation
TD CD4+ T cells_ % parent	1.1	0.7 (0 - 1.5)	No reference is given for this subpopulation	0.66	0 (0 - 3.16)	No reference is given for this subpopulation
CD8+ T cells_abs (/µL)	468	498 (383 - 515)	496	56	115.5 (12 - 700)	32
CD8+ T cells_ % parent	28	27.5 (7.8 - 29.2)	27.6	46	7.075 (0.97 - 53)	2.4
Naive CD8+ T cells_abs (/µL)	399	387 (301 - 400)	377	25	0 (0 - 31)	0.3
Naive CD8+ T cells_ % parent	83	77.7 (6 - 84)	76.3	21	0.955 (0 - 25.8)	0.9
TM/CM CD8+ T cells_abs (/µL)	24	43 (23 - 56)	No reference is given for this subpopulation	85	30 (9 - 494)	No reference is given for this subpopulation
TM/CM CD8+ T cells_ % parent	9	8.5 (0.66 - 11)	No reference is given for this subpopulation	65	48.2 (0.95 - 80)	No reference is given for this subpopulation
EM CD8+ T cells_abs (/µL)	5	5.5 (3 - 7)	No reference is given for this subpopulation	9	10 (2 - 124)	No reference is given for this subpopulation
EM CD8+ T cells_ % parent	1.2	1 (0.084 - 2)	No reference is given for this subpopulation	20	9.3 (0.36 - 20)	No reference is given for this subpopulation
TD CD27+ CD8+T cells_abs (/µL)	35	17 (1 - 64)	No reference is given for this subpopulation	55	38.5 (0 - 286)	No reference is given for this subpopulation
TD CD27+ CD8+T cells_ % parent	6	4 (0 - 12.6)	No reference is given for this subpopulation	68.3	4.75 (0 - 77.5)	No reference is given for this subpopulation
TD CD8+ T cells_abs (/µL)	48	36 (16 - 52)	No reference is given for this subpopulation	161	2 (0 - 172)	No reference is given for this subpopulation
TD CD8+ T cells_ % parent	8.1	7 (0.75 - 10.3)	No reference is given for this subpopulation	33	3.5 (0.067 - 40)	No reference is given for this subpopulation
DN T cells_abs (/µL)	44	47.5 (32 - 52)	40.2	66	186 (0 - 309)	1.9
DN T cells_ % parent	2.3	2.235 (0.79 - 3)	2.2	25	11 (0 - 47.2)	0.3
TCRgd+ T cells_abs (/µL)	122	118 (96 - 124)	118	1158	801 (86 - 1185)	1124
TCRgd+ T cells_ % parent	6	6.5 (1.8 - 7)	6.5	45	57.4 (6 - 87)	83
NK cells_abs (/µL)	132	132.5 (38 - 147)	135	149	221.5 (133 - 266)	245
NK cells_ % parent	7.5	6.4 (2.1 - 8.83)	6.5	11.2	11.65 (8.2 - 13.5)	12.6

Level 2: cell count interpretation

Note: The Reference interpretation is the consensus of 3 experts.

File ID	case 1		case 2	
	A000	Reference	A000	Reference
B cells	Normal	Normal	Decreased	Decreased
PreGC B cells	Normal	Normal	Decreased	Decreased
memory B cells (IGMD+) + PC (unswitched)	Decreased	Decreased	Decreased	Decreased
memory B cells (IGMD-) + PC (switched)	Decreased	Decreased	Decreased	Decreased
T cells	Normal	Normal	Normal	Normal
CD4+ T cells	Normal	Normal	Normal	Normal
Naive CD4+ T cells	Normal	Normal	Normal	Normal
CD8+ T cells	Decreased	Decreased	Decreased	Decreased
Naive CD8+ T cells	Absent	Decreased	Absent	Decreased
DN T cells	Normal	Normal	Decreased	Decreased
TCRgd+ T cells	Normal	Normal	Increased	Increased
NK cells	Increased	Increased	Normal	Normal

Level 3: combined interpretation of the T and B cell maturation patterns

Note: The Reference interpretation is the consensus of 3 experts.

File ID	case 1		case 2	
B cell compartment	A000	Reference	A000	Reference
Normal peripheral B cell maturation, with no relevant deviations				
B cells absent, B cell maturation is not interpretable	x	x		
B cell lymphopenia, with normal B cell maturation				
B cell lymphopenia, with disturbed B cell maturation			x	x
Disturbed B cell maturation, with weak/absent memory compartment (switched + unswitched memory B cells)			x	x
Disturbed B cell maturation, with weak/absent unswitched memory compartment				
Disturbed B cell maturation, with weak/absent switched memory compartment				
T cell compartment	A000	Reference	A000	Reference
Normal peripheral T cell maturation, with no relevant deviations				
T cells absent, T cell maturation is not interpretable				
T cell lymphopenia, with normal T cell maturation				
T cell lymphopenia with disturbed T cell maturation	x	x	x	x
Disturbed T cell maturation (CD4+ and/or CD8+), with enriched naive compartment				
Disturbed T cell maturation (CD4+ and/or CD8+), with enriched memory compartment			x	
Decreased CD4+ (naive) T cell compartment			x	x
Decreased CD4+ (total) T cell compartment			x	x
Decreased CD8+ (naive) T cell compartment			x	x
Decreased CD8+ (total) T cell compartment			x	x
Increased double negative T cells				

Level 4: compatibility of immunophenotype with common PIDs		
Note: The Reference interpretation for qualitative data is the consensus of 3 experts.		
File ID	**case 1**	
	A000	**Reference**
SCID (T-B+/T-B-)	Yes	Possible
SCID with maternal engraftment	Possible	No
CID	No	No
XLA	No	No
CVID(-like)	No	Yes/Possible
HIgM	No	No
ALPS	No	No
Other		

Level 5: interpretation for the clinician
case 1
A000
Suspicion of SCID
REFERENCE
Note: The interpretations of all experts are presented to demonstrate the variability in their conclusions
Expert #1:
Interpretation of expert 1
Expert #2:
Interpretation of expert 2
Expert #3:
Interpretation of expert 3

Level 4: compatibility of immunophenotype with common PIDs		
Note: The Reference interpretation for qualitative data is the consensus of 3 experts.		
File ID	**case 2**	
	A000	**Reference**
SCID (T-B+/T-B-)	No	No
SCID with maternal engraftment	Possible	No
CID	Possible	Possible
XLA	No	No
CVID(-like)	Yes	No
HIgM	No	No
ALPS	No	No
Other		

Level 5: interpretation for the clinician
case 2
A000
Suspicion of CVID
REFERENCE
Note: The interpretations of all experts are presented to demonstrate the variability in their approaches.
Expert #1:
Interpretation of expert 1
Expert #2:
Interpretation of expert 2
Expert #3:
Interpretation of expert 3

General

The objective of the EuroFlow PIDOT EQA scheme is to evaluate the technical quality of the sample preparation and measurement on the flow cytometer by applying the PIDOT antibody panel (wet part) and to evaluate the ability to analyze and interpret provided fcs files of patients with PID (dry part).

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.

Wet part

The wet part tests the technical performance of the participant's instrument setup, sample preparation, measurement, and analysis of the PIDOT tube.
No samples were provided by ESLHO. The reported results include quantitative data including the MedFIs of major cell populations following PIDOT analysis of locally collected blood samples of 3 healthy controls.

Data analysis and performance

Your reported MedFI values were compared with the reference data set (Neirinck et al., 2024, 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 fluorescence intensities in the reference dataset and D^{max} is the maximal allowed difference from qaMedFI. D^{max} 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 D^{max}.
The absolute value of the p-score equals or exceeds the value '1' when the maximum allowed difference from the reference data set is exceeded. In such case, the particular result is considered incorrect. Based on the calculation of D^{max}, it is expected that 90 - 95% of the p-scores fall within the acceptable range.
The 'wet part overall score' is defined as the percentage of acceptable p-scores for each laboratory across all marker subset combinations per round. An overall score of 91% and above (at least 30 correct values out of 33 reported values) is considered as **successful with a perfect score**. Scorings higher than 76% and lower than 91% are considered as **successful with an acceptable score** (at least 26 correct values). Scorings equal to or below 76% are considered **unsuccessful** (less than 26 correct values). In summary, performance in the PIDOT EQA 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 three reported values for a given marker are incorrect, this indicates a systematic error in that marker.

Dry part

The dry part tests the analysis and interpretation of actual clinical data.
2 FCS files of well-defined (PID) cases provided by ESLHO are analysed by the participants. Participants report their quantitative and qualitative results,which are organized at several levels:

- Level 1: the cell counts (% and cells/µL) of 19 cell populations obtained following data analysis (quantitative data).
- Level 2: the cell count interpretations of the cell populations as either 'absent', 'decreased', 'normal', and 'increased' after comparison with age-matched reference ranges (qualitative data)
- Level 3: the combined interpretation of the T and B cell maturation stages (qualitative data).
- Level 4: the combined interpretation of the most compatible PID subtype with the immunophenotype (qualitative data).
- Level 5: the interpretation for a clinician (qualitative data).

Data analysis and participant performance

Participant outcomes are compared to the consensus reference interpretation, which is based on the interpretation of three subject-matter experts who analyzed the files independently.
For Level 1 quantitative data, the median of the experts´ values is used. A comparison to the median, minimum and maximum cell counts reported by all participating laboratories in a given round is also provided. This allows participants to evaluate their results in comparison to the peer group.
For Level 2, 3, and 4 qualitative data, the consensus interpretation of the experts is used. If no consensus is reached by the experts, the reference result for that particular question states "no consensus".
The median reference value for the non-mandatory subpopulations in levels 1 and 2 is not provided.
For Level 5, the reference interpretation of the three experts is provided to demonstrate the variability in their conclusions.

Task division

Participant-specific results are only known to you as EQA participant, ESLHO, and to the appropriate experts laboratories (e.g., for preparing the certificates). The following persons are involved in the design and operation of the PIDOT EQA scheme:

ESLHO

Name	Organization/Institute	Role	Tasks
Prof. Dr. Jacques J. M. van Dongen	ESLHO, Zutphen, NL	EQA Program Coordinator	Coordinator with final responsibility. Review and authorization of the MM MRD EQA certificate
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer (lead)	Overall responsible for organization and operation of the PIDOT scheme by ESLHO.
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer	Supports in the organization and operation of the PIDOT scheme. Back-up for review and authorization of the PIDOT EQA certificate

Lead expert laboratory

Name	Organization/Institute	Role	Tasks
Prof. Dr. Carolien Bonroy	Ghent University Hospital, Ghent, BE	Lead subject-matter expert	Pre-round: Case collection, clinical quality check of fcs data, case selection, PID case interpretation Round: Reference lab Post-round: Round summary report
Malicorne Buysse	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Case collection, technical/clinical quality check of fcs data, case selection, PID case interpretation Round: Reference lab Post-round: Round summary report
Julie D'Hondt	Ghent University Hospital, Ghent, BE	Subject-matter expert	Round: Reference lab Post-round: Round summary report
Dr. Mattias Hofmans	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: PID case interpretation Round: Reference lab Post-round: Round summary report
Pauline Breughe	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Technical quality check of fcs data

Additional expert laboratories

Name	Organization/Institute	Role	Tasks
Dr. Naděžda Brdičková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Supports preparation of the rounds. Post-round: Cleaning and analysis of the submitted results, preparation of the EQA certificate, support in performance evaluation and reporting.
Prof. Dr. Tomáš Kalina	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of consensus results
Dr. Ester Mejstříková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of consensus results

Prof. Dr. Martín Pérez	University of Salamanca, Salamanca, ES	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of consensus results

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