ELISPOT PROFICIENCY PANEL 2014

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ELISPOT PROFICIENCY PANEL 2014

This report summarizes the results of the Elispot Proficiency Panel 2014. The report provides individual test results for each participating laboratory that participated in the Elispot proficiency panel 2014, as well as an anonymized overview of the other participants' test results.

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Immudex has taken over the MHC Multimer and Elispot proficiency panels from the CIC (Cancer Immunotherapy Consortium of the Cancer Research Institute, USA) and the CIMT (Association for Cancer Immunotherapy, Europe). The proficiency panel services offered by Immudex are open to any laboratory with a need to perform accurate and reproducible quantification of antigen-specific T cells, independent on affiliation, geographic location, or field of interest.

The report is provided using the European numeration.

The proficiency panels conducted by Immudex are non-profit services offered with the intent of testing and ensuring a high level of proficiency and reliability among the researchers and clinicians that perform the immune monitoring assays. It is the hope and expectation that better immune monitoring assays will lead to better and more efficient immunotherapies.

Next Elispot and MHC Multimer proficiency panels to be held first half of 2015

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Contents

INFORMATION ON PARTICIPANTS, PROTOCOLS, REAGENTS, CELL SAMPLES

- 80 laboratories participated in the proficiency panel.
- Each participating laboratory was assigned a confidential participant Identification Number (Lab Id), only known by the laboratory itself and Immudex.
- Each participant received two vials of PBMCs (human peripheral blood mononuclear cells), termed 13081 and 13096, respectively, and three vials with reagents, Reagent 1 (CMV peptide pool), Reagent 2 (CEF peptide pool) and Reagent 3 (Negative control – PBS/DMSO). Reagent 1 is the PepMix HCMVA (pp65) (JPT Product Code. PM-PP65) in PBS buffer/DMSO; Reagent 2 is the PepMix CEF Pool (extended) (JPT Product Code PM-CEF-E) in PBS buffer/DMSO; Reagent 3 is a negative control (no peptide) PBS buffer/DMSO.
- All vials were shipped in liquid nitrogen. A temperature logger was included in the shipment, allowing observation of vial temperature from packaging to delivery.
- Each laboratory performed the Elispot assay according to their own preferred operating procedure.
- Instructions (see Appendix 1) including Harmonization Guidelines, were provided to all participants.

Prior to the shipping of the PBMCs to the participants, the PBMCs were pretested by two labs at separate locations, in order to verify the uniformity of the PBMC vials. Thus, the Elispot assay was performed on a total of 6 vials, using both the CMV peptide pool, the CEF peptide pool and the Negative control with no peptides. The observed variability between different PBMC vials of the same cell sample, as regards cell viability and number of antigen-specific T cells, was insignificant.

PBMC samples were thoroughly pretested prior to shipping and sample temperature was monitored from packaging to delivery

The assays performed included a range of frequencies of antigen-specific T cells, from about 1 in 10.000 PBMCs ("Low responder") to more than 1 in 400 PBMCs ("Very High responder").

ANALYSES PERFORMED BY THE PARTICIPANTS

Each participant received detailed instructions for carrying out the proficiency test; see Instructions (Appendix 1).

The participants were asked to determine the number of antigen-specific T cells of each of PBMC 13081 and 13096, as follows:

- Number of CMV-specific spots per 200.000 PBMC
- Number of CEF-specific spots per 200.000 PBMC
- Number of spots per 200.000 PBMC with Negative control reagent (no peptide)
- Number of spots with medium alone, no cells

All measurements should be done in triplicate.

PRESENTATION OF DATA

The results obtained are shown in Figures 1-4 and Appendices 2-3.

The 80 participants' data comprised significant outliers for all four PBMC/antigen combinations tested. It was therefore decided to use the median, rather than the average, of the reported results (including all outliers) to represent the "average" result.

The median, rather than the average, of the reported results represents the "average" result

Thus, in the following the median of the background-corrected results for each PBMC/antigen combination represents the "average value" for all the participants for that particular PBMC/peptide combination. It should be emphasized that the "average value" (the median) is not necessarily the "true" value, as no golden standard exists for the Elispot assay. Nevertheless, the median of all the participants' results for a particular PBMC/antigen combination was used to calculate the Relative Accuracy of a given participant's result (see below).

PROFICIENCY TESTING RESULTS

The results obtained by the 80 participants of the Elispot Proficiency Panel 2014 are shown in Figures 1-4 and Appendices 2-3.

Figures 1A, 2A and 4A show the number of antigen-specific spots per 200.000 PBMCs (triplicate analysis; three red diamonds), and the number of negative-control spots per 200.000 PBMCs (triplicate analysis; three black dots).

Figures 1B, 2B and 4B show the background-corrected, mean number of antigen-specific spots per 200.000 PBMCs (one red diamond).

Figures 1C, 2C and 4C show the Relative Accuracy. The Relative Accuracy is defined as the result determined by the individual

participant, divided by the median result of all participants for that PBMC/antigen combination (background spots subtracted).

Relative Accuracies of 0,66–1,50 are here considered "in the average range" and are represented by filled black columns; Relative Accuracies of 0,50–0,65 or 1,51–2,00 are considered "near average" and are represented by hatched columns; Relative Accuracies below 0,50 or above 2,00 are considered "far from average" and are represented by open columns. The data is presented in order of increasing relative accuracy from left to right.

Figure 3 shows the number of spots determined for the PBMC/antigen combination 13096/CMV. This is a "Very High responder" and for many of the participants the number of spots was too numerous to count. Consequently, a number of laboratories commented that the reported result was underestimated, while other laboratories reported the results "0" or "1" to indicate that this was above the upper limit for their assay. As a result, a meaningful median of all the participants' results could not be determined and consequently, Relative Accuracy was not determined. This measurement (PBMC 13096 with CMV peptide pool) was therefore not included in the calculation of Overall Proficiency (see page 17).

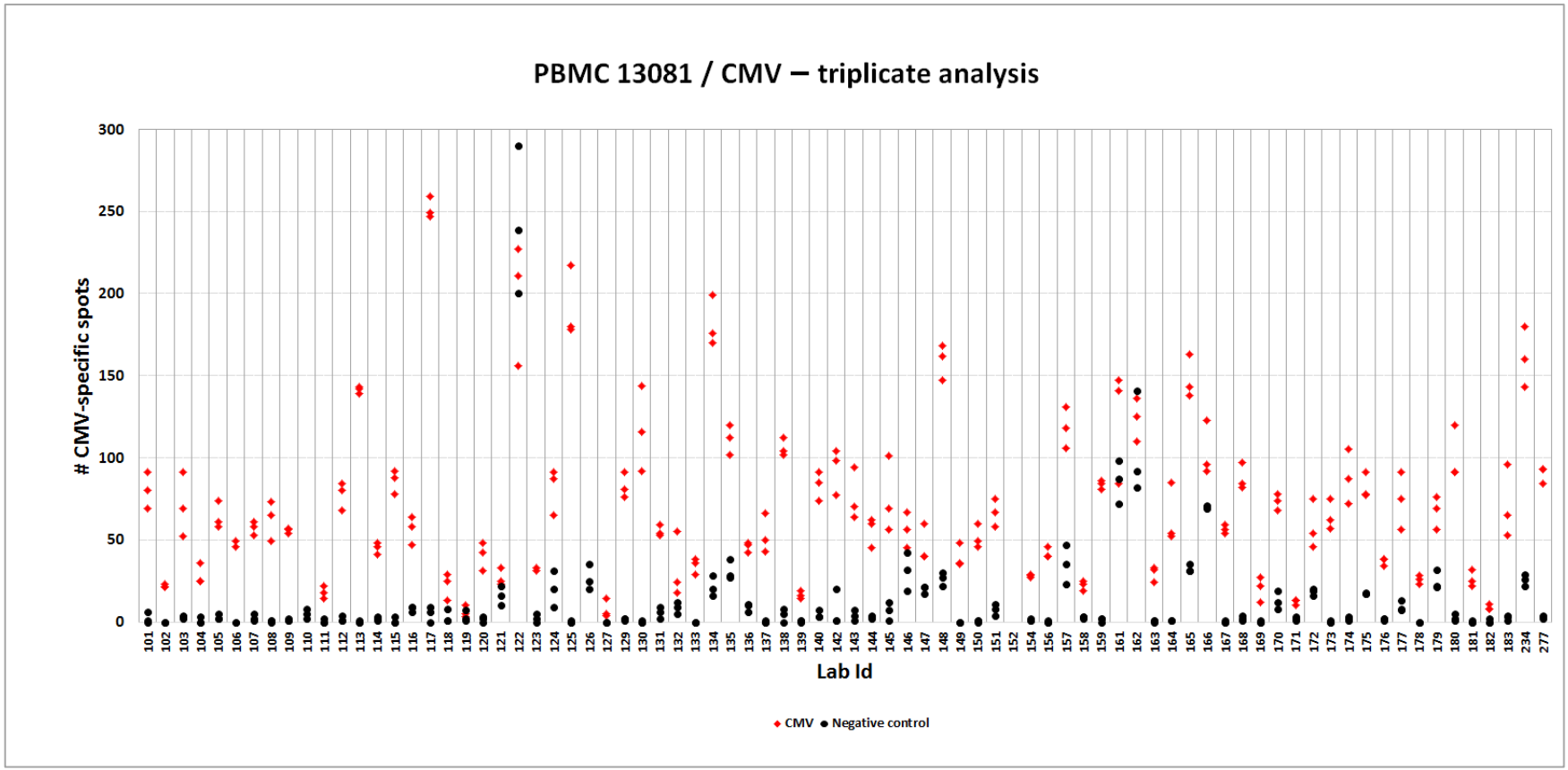

Figure 1A. CMV-specific spots and background spots for PBMC 13081.

The number of spots per 200.000 PBMCs for PBMC 13081/CMV (triplicate analysis; 3 red diamonds) or PBMC 13081/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 152 reported values higher than 300 for both the Negative control and CMV. Lab Id 126 and 110 reported values higher than 300 for CMV (see appendix 2). Lab Id 106 only reported duplicate values for the Negative control and CMV. Lab Id 123 only reported duplicate values for CMV.

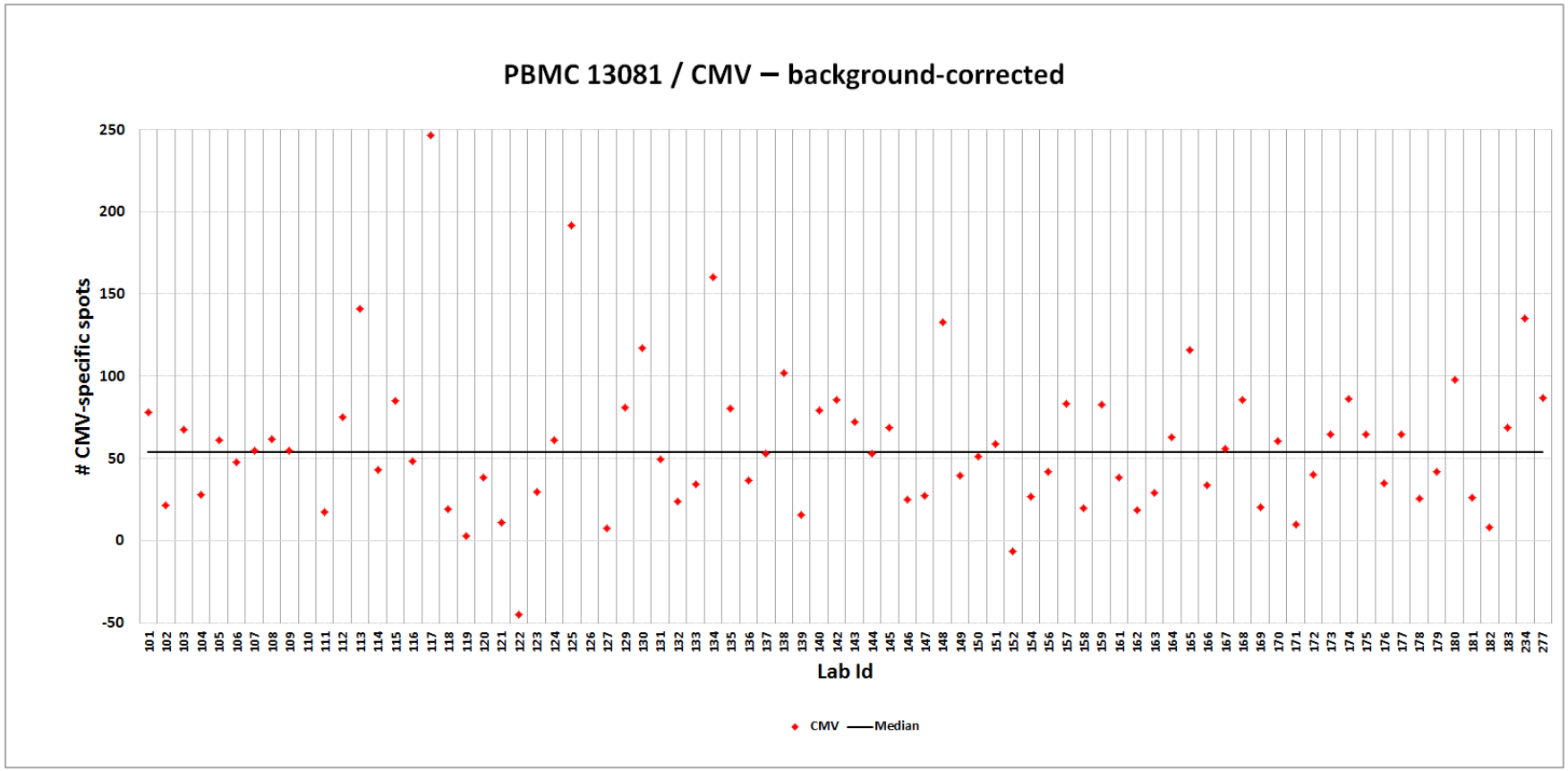

Figure 1B. Background-corrected number of CMV-specific spots for PBMC 13081.

The number of background-corrected, CMV-specific spots per 200.000 PBMCs, based on the triplicate analysis, is shown (red diamond). The mean number of spots per 200.000 PBMCs for the Negative control was subtracted from the mean number of spots obtained with the CMV peptide pool, to give the background-corrected value. The median (54 CMV-specific spots) is indicated with a black horizontal line. Lab Id 110 and 126 reported value for CMV of 344 and 502, respectively.

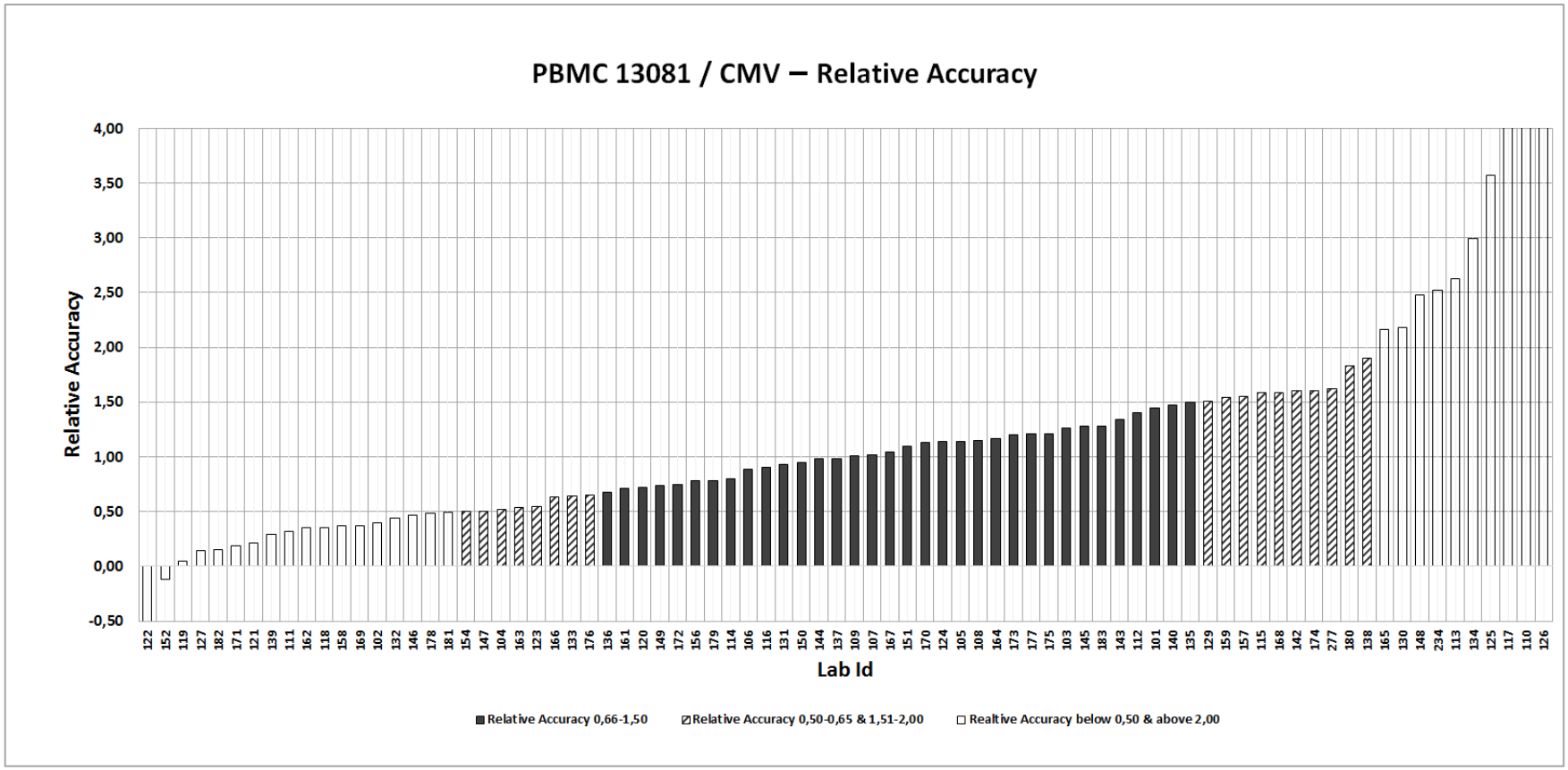

Figure 1C. Relative Accuracy for the 13081/CMV combination.

The Relative Accuracy, equaling the result divided by the median (54) of all results, for background-corrected, CMV-specific spots is shown. Relative Accuracy for Lab Id 122, 117, 110 and 126 is -0,84, 4,60, 6,42 and 9,36, respectively. 34 out of 80 participants had a Relative Accuracy between 0,66-1,50 and are therefore considered "in the average range".

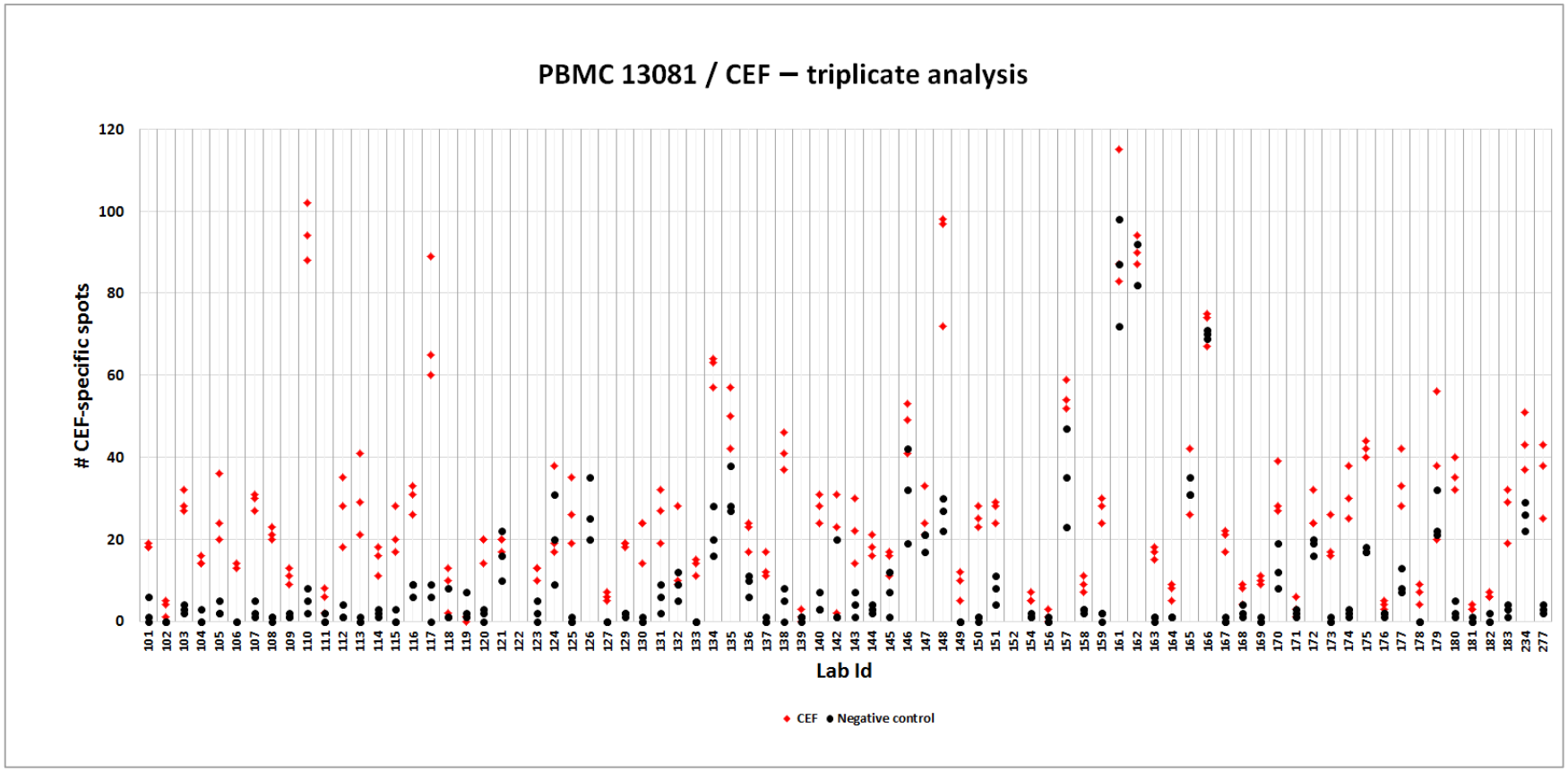

Figure 2A. CEF-specific spots and background spots for PBMC 13081.

The number of spots per 200.000 PBMCs for PBMC 13081/CEF (triplicate analysis; 3 red diamonds) or PBMC 13081/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 152 and 122 reported values higher than 120 for the Negative Control. Lab Id 152, 122 and 126 reported values higher than 120 for CEF. Lab Id 165 reported one value higher than 120 for CEF (see appendix 2). Lab Id 106 only reported duplicate values for the Negative control and CEF.

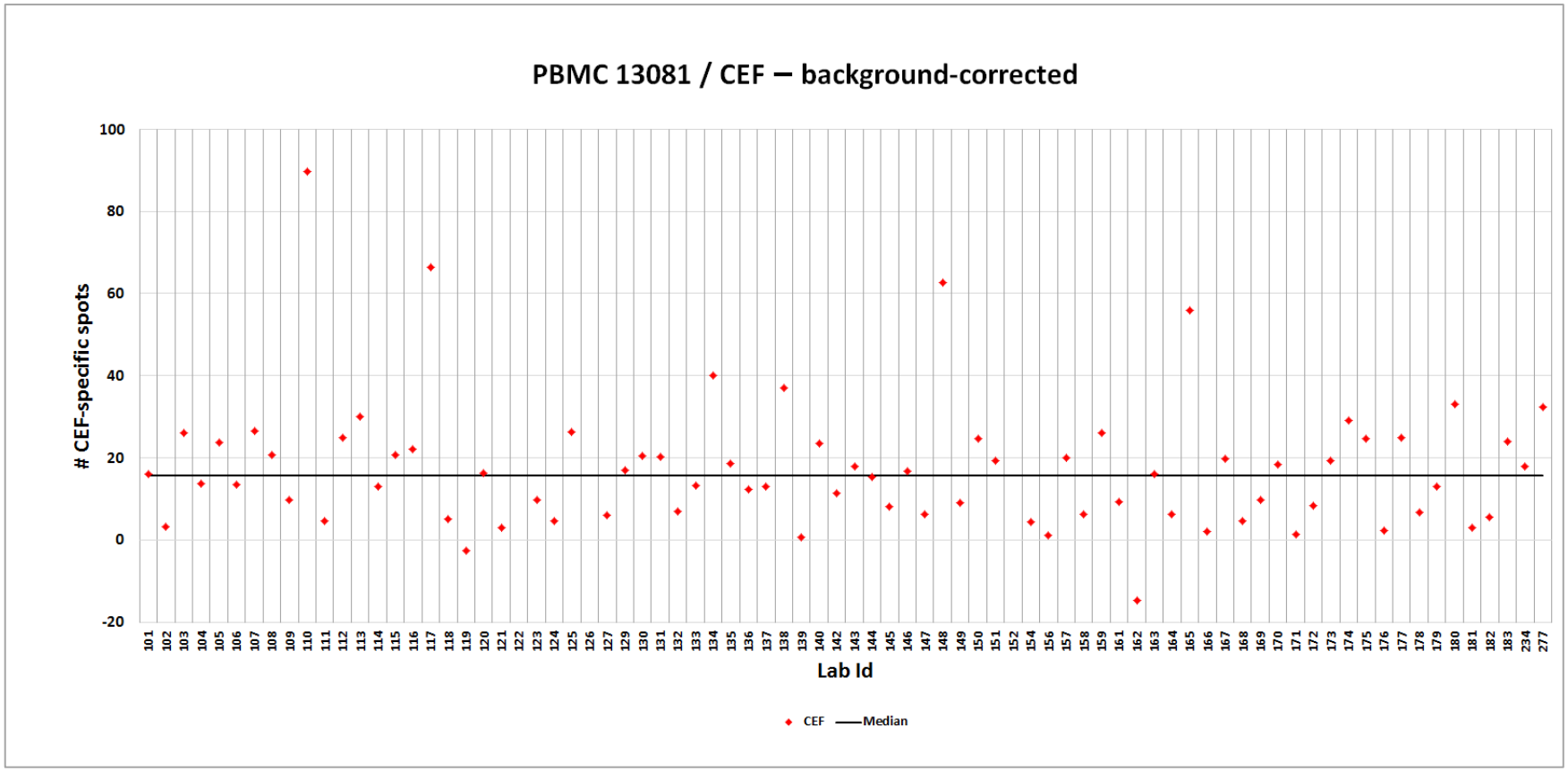

Figure 2B. Background-corrected number of CEF-specific spots for PBMC 13081.

The number of background-corrected, CEF-specific spots per 200.000 PBMCs, based on the triplicate analysis, is shown (red diamond). The mean number of spots per 200.000 PBMCs for the Negative control was subtracted from the mean number of spots obtained with the CEF peptide pool, to give the background-corrected value. The median (16 CEF-specific spots) is indicated with a black horizontal line. Lab Id 122, 152 and 126 reported value for CEF of -67, -51 and 212, respectively.

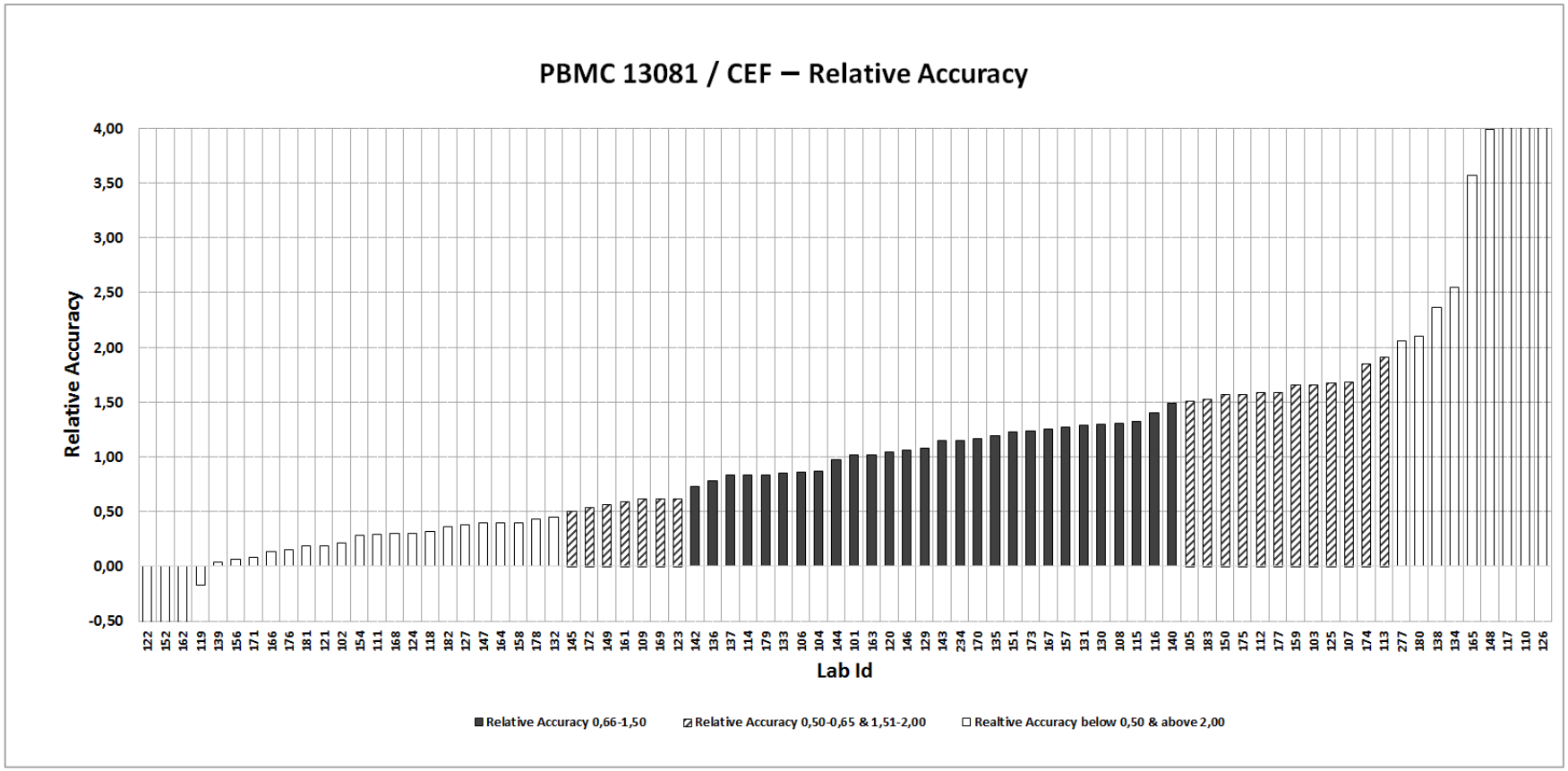

Figure 2C. Relative Accuracy for the 13081/CEF combination.

The Relative Accuracy, equaling the result divided by the median (16) of all results, for background-corrected CEF-specific spots is shown. Relative Accuracy for Lab Id 122, 152, 162, 117, 110, and 126 is –4,25, -3,25, -0,94, 4,22, 5,71 and 13,48, respectively. 28 out of 80 participants had a Relative Accuracy between 0,66-1,50 and are therefore considered "in the average range".

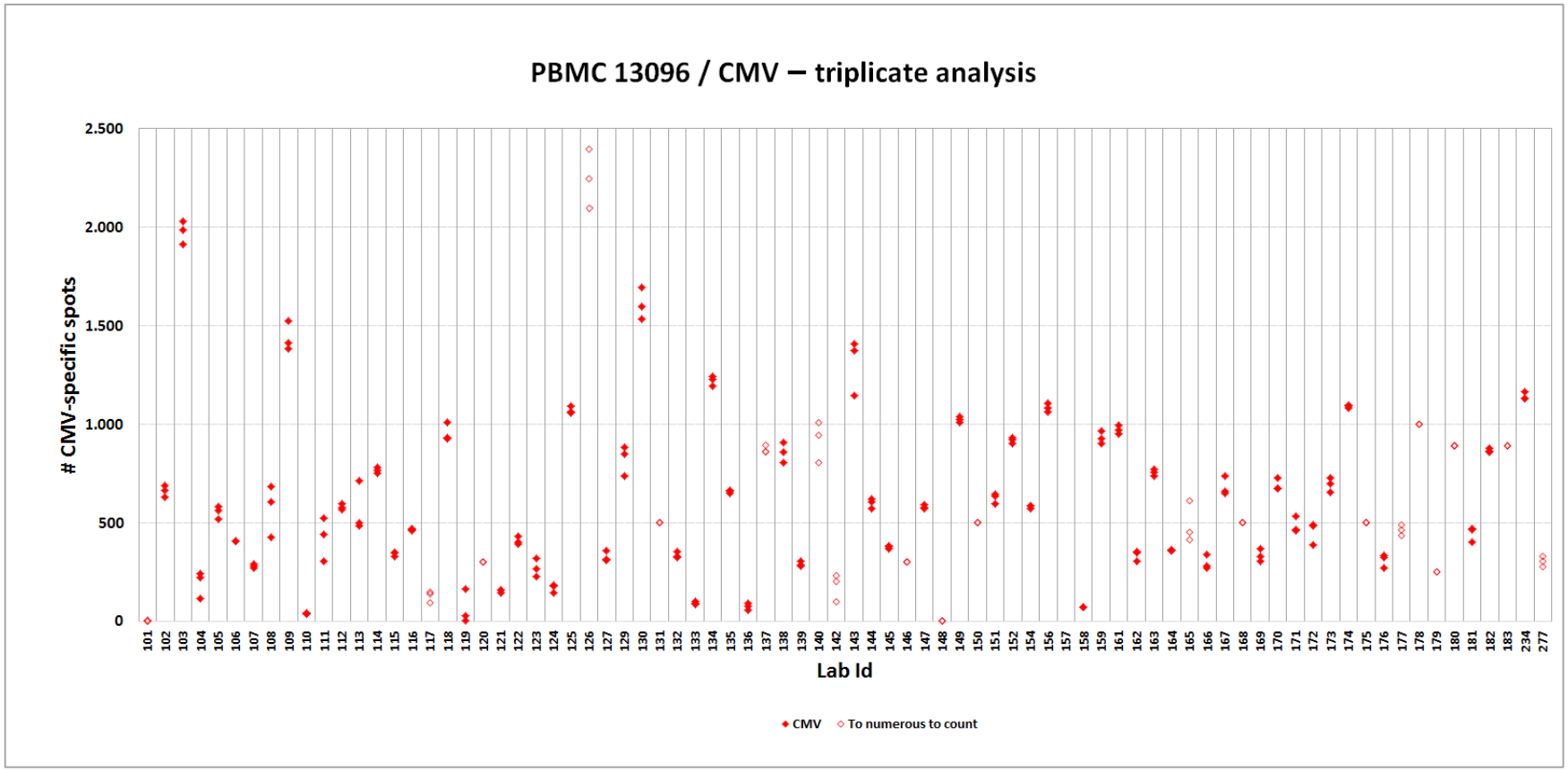

Figure 3. CMV-specific spots for PBMC 13096.

The number of spots per 200.000 PBMCs for PBMC 13096/CMV (triplicate analysis; 3 red diamonds) is shown. PBMC 13096/Negative control (triplicate analysis) is not shown; however, the PBMC 13096/Negative control data can be seen in Figure 4A. Lab Id 157 has value for CMV above 2.500 (see appendix 3). 21 laboratories reported that the number of spots exceeded the maximum capacity of their procedure/instruments, and consequently reported a minimum number or the number 0 or 1 to indicate this. For these laboratories, the number of spots per 200.000 PBMCs, obtained for PBMC 13096, with the CMV peptide pool (triplicate analysis) are indicated with red diamonds without filling.

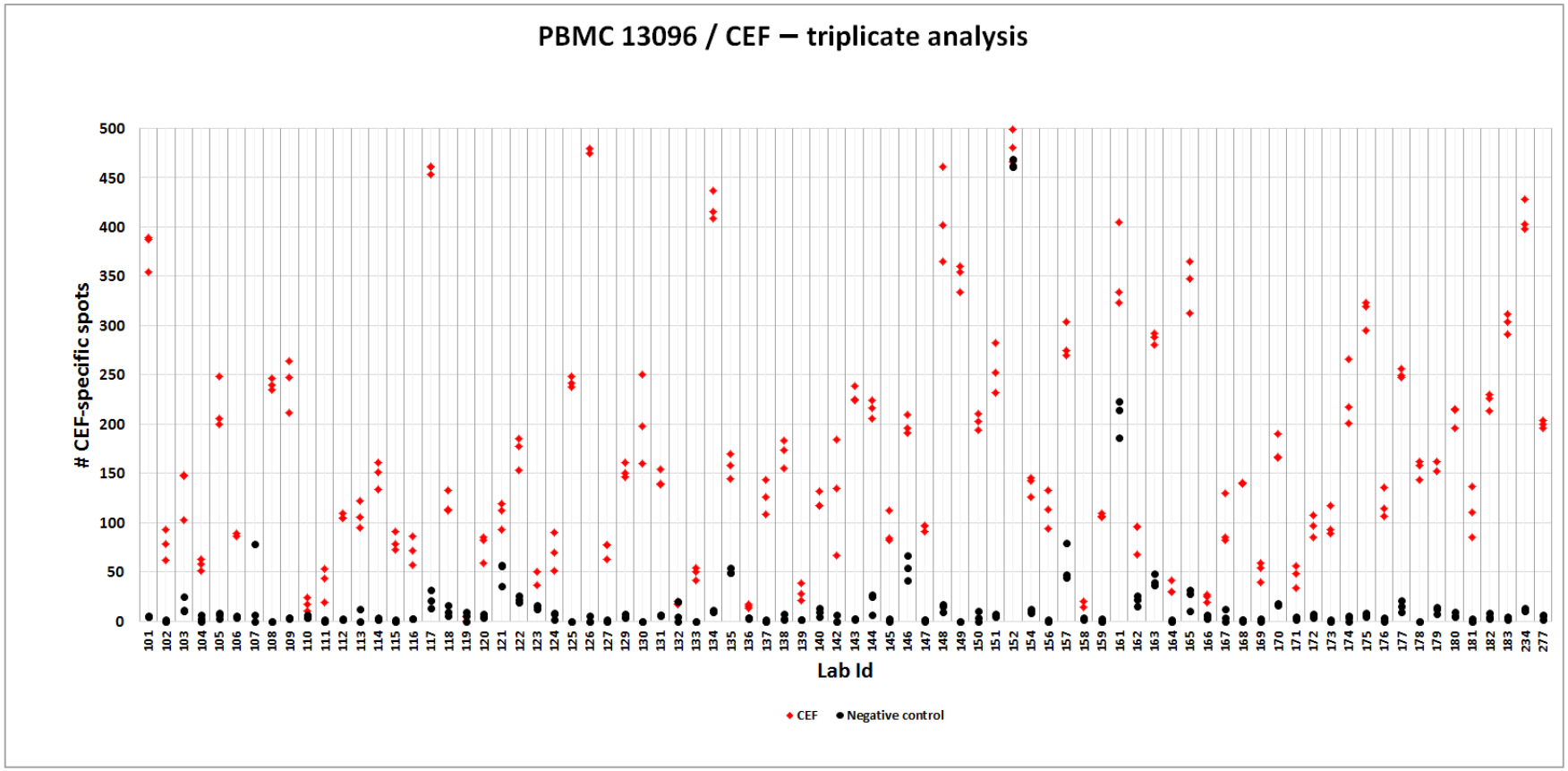

Figure 4A. CEF-specific spots and background spots for PBMC 13096.

The number of spots per 200.000 PBMCs for PBMC 13096/CEF (triplicate analysis; 3 red diamonds) or PBMC 13096/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 107 reported values higher than 500 for CEF. Lab Id 126 reported one value higher than 500 for CEF (see appendix 3). Lab Id 106 and 158 reported only duplicate for the Negative control and CEF. Lab Id 123 and 179 reported only duplicate for CEF.

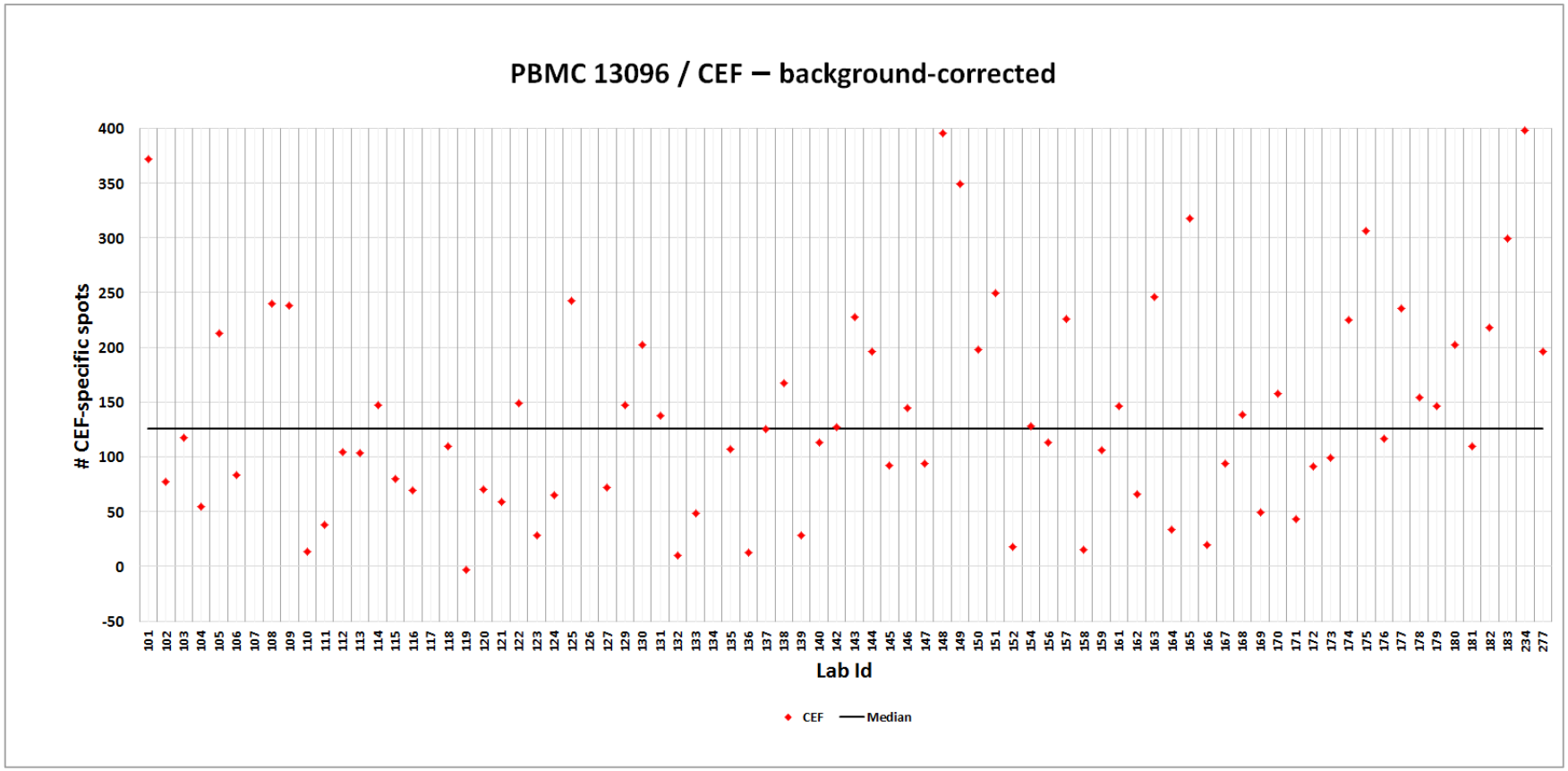

Figure 4B. Background-corrected number of CEF-specific spots for PBMC 13096.

The number of background-corrected, CEF-specific spots per 200.000 PBMCs, based on the triplicate analysis, is shown (red diamond). The mean number of spots per 200.000 PBMCs for the Negative control was subtracted from the mean number of spots obtained with the CEF peptide pool, to give the background-corrected value. The median (126 CEF-specific spots) is indicated with a black horizontal line. Lab Id 107, 117, 126 and 134 reported value for CEF of 1048, 436, 490 and 410, respectively.

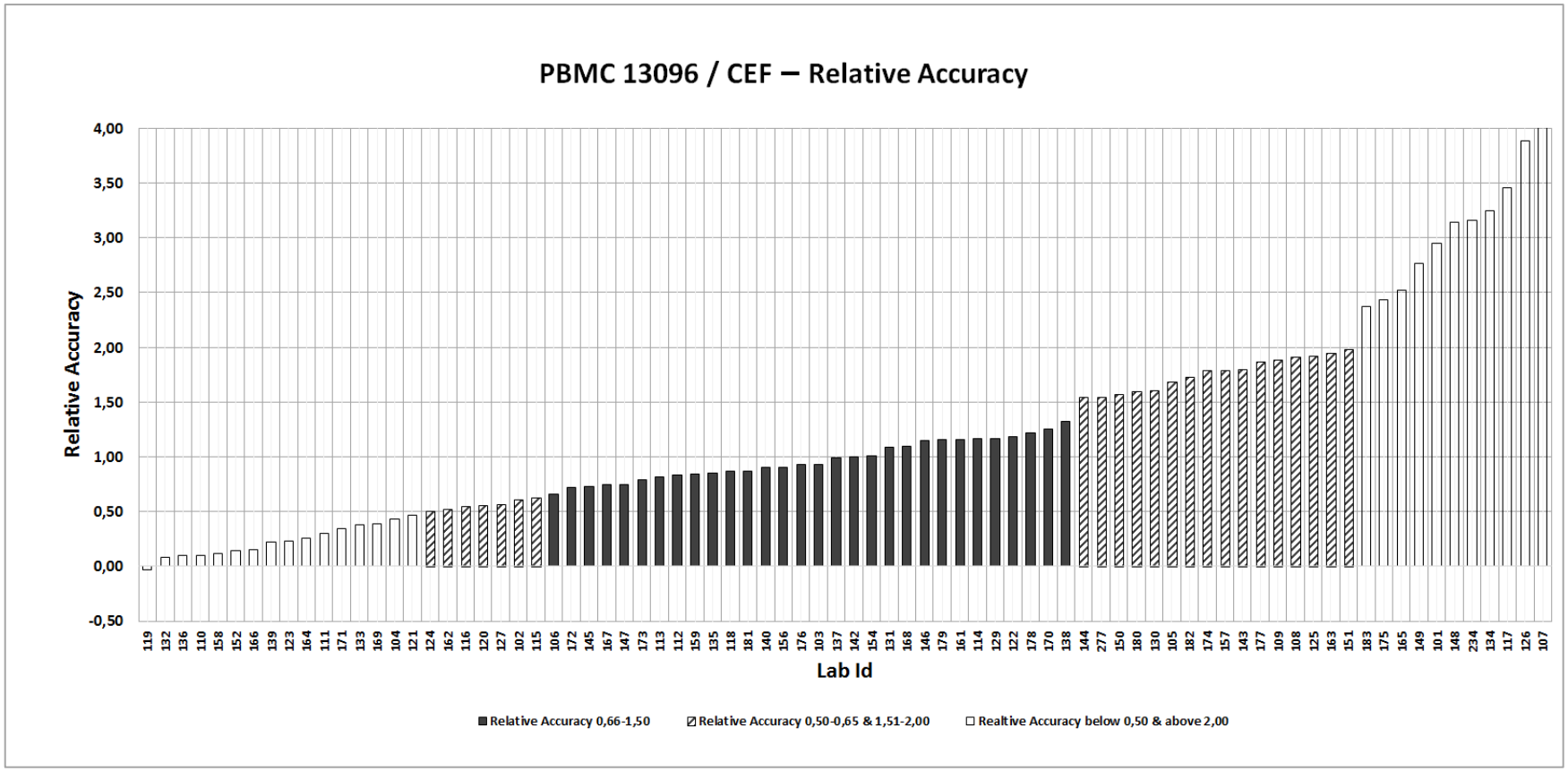

Figure 4C. Relative Accuracy for the 13096/CEF combination.

The Relative Accuracy, equaling the result divided by the median (126) of all results, for background-corrected CEF-specific spots is shown. Relative Accuracy for Lab Id 107 is 8,31. 30 out of 80 participants had a Relative Accuracy between 0,66-1,50 and are therefore considered "in the average range".

OVERALL PROFICIENCY

In order to describe the Overall Proficiency of each participating laboratory in enumerating the antigen-specific cells, a score was assigned to each laboratory for each of the measurements performed. However, because of the issues that many laboratories had with "excess number of spots" on the 13096/CMV combination, the measurement on 13096/CMV was not included in the calculation of the individual laboratory's Overall Proficiency. Thus, only the following measurements were included in the determination of Overall Proficiency: 13081/CMV, 13081/CEF and 13096/CEF.

The score "3" was assigned to results in the average range (i.e. Relative Accuracy between 0,66 and 1,50), the score "2" was assigned to results near average (i.e. Relative Accuracy 0,50-0,65 or 1,51-2,00), and finally, the score "1" was assigned to results far from average (i.e. Relative Accuracy below 0,50 or above 2,00).

Overall Proficiency is defined by the average score obtained over the three measurements. Thus, a laboratory with an overall proficiency of "3" is in the average range on all three measurements and has the highest possible score, and a laboratory with an average score of "1" is far from average on all three measurements and has the lowest possible score.

Overall Proficiency is shown in Figure 5. As can be seen, 10 out of 80 laboratories (13% and first 10 laboratories from the left) are in the average range on all three measurements, and thus have the highest possible Overall Proficiency score of "3".

13% of participants had results in "the average range" for 3 out of 3 measurements.

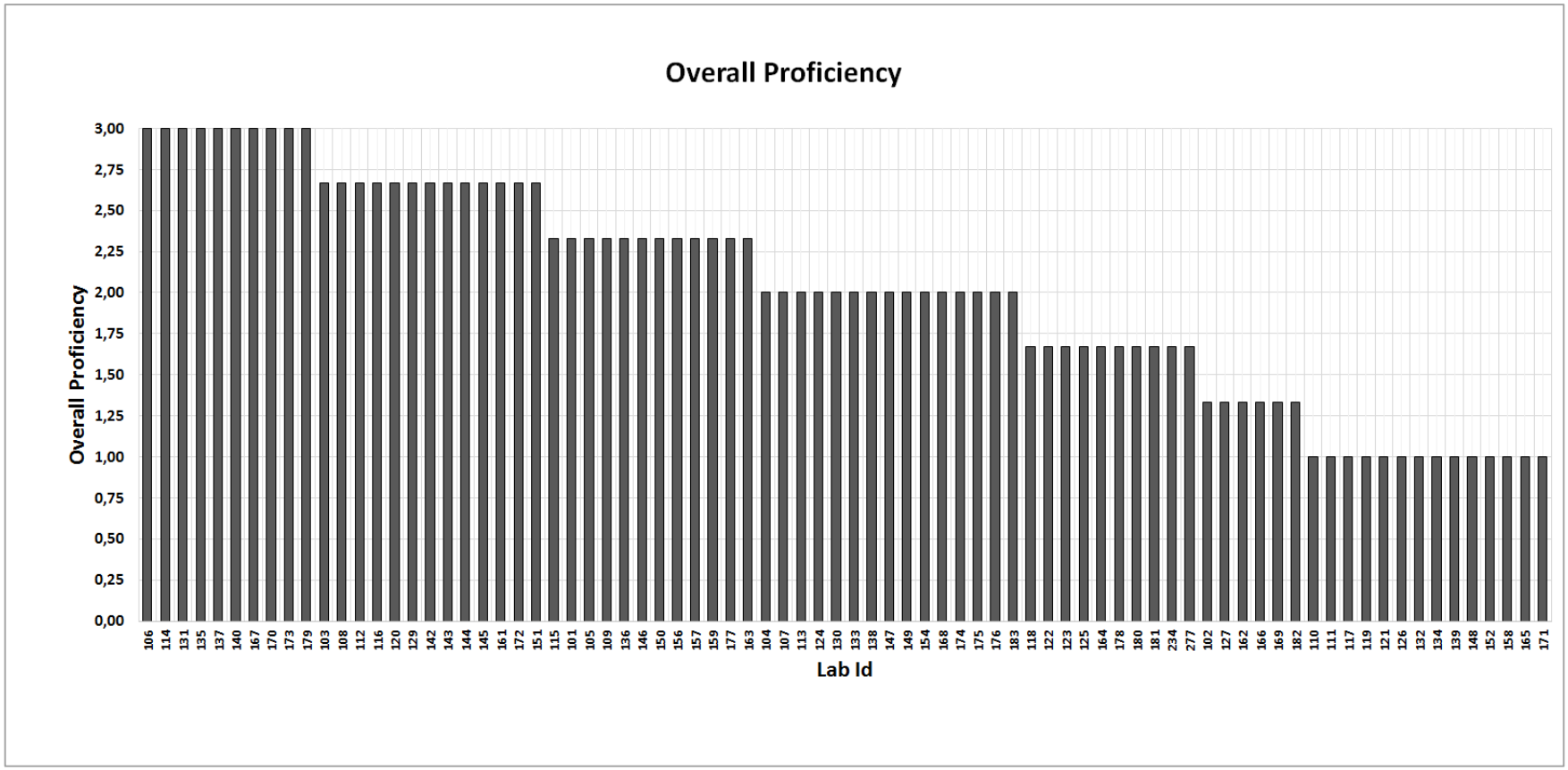

Figure 5. Overall Proficiency. *The laboratories' proficiency in performing the Elispot measurements is shown. An Overall Proficiency of "3" represents the highest possible proficiency score; an Overall Proficiency of "1" represents the lowest possible Overall proficiency score. A score of 3" indicates that this laboratory was "in average" on all three measurements. A score of "1" indicates that this laboratory was "far from average" on all three measurements.*

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GENERAL COMMENTS

The proficiency panel series was initiated by the CIC and CIMT in 2006. One of the goals of past proficiency panels was to harmonize procedures across laboratories. Harmonization is now considered finalized, and the responsibility of conducting the Elispot proficiency panel was handed over to Immudex in 2013.

The current Elispot proficiency panel is therefore not a harmonization panel, but rather a proficiency testing service. Consequently, harmonization and standardization is not addressed in this report.

A survey was carried out in connection with the proficiency panel execution. The full set of reported data and information will be published separately.

General observations and conclusions.

The Elispot assays performed in this proficiency panel involved "Low responders" (1 antigen-specific cell per 10.000 PBMCs) to Very High responders (1 antigen-specific cell per 400 PBMCs).

- For a given measurement, about 40 % of the participants had a Relative Accuracy of between 0,66 and 1,50, and were defined as being "in the average range". In other words, for a given measurement, about 40% of the participants were close to the median value of all participants.

For each measurement, ~40% of the participants obtained a value close to average

- 13% of the participants were "in the average range" for three out of three measurements.

13% of the participants were close to average for 3 out of 3 measurements

- For a given measurement, the 75% of the participants that were closest to the "average" (median value) had results differing from 6 to 10 fold. For example, for the PBMC 13081/CEF antigen combination, the 75% of participants (i.e. 60 out of 80 participants) that were closest to the median value, detected from 3 to 29 antigen-specific cells per 200.000 PBMC.

Among the 75% of participants closest to the "average", the lowest and highest number of antigen-specific T cells detected differed 6-10 fold

- The number of laboratories with Relative Accuracies between 0,66 and 1,50 (and therefore "in the average range") was approximately the same for Low, Medium and High responders. In other words, the accuracy of the Elispot assay does not seem to get higher with a higher frequency of antigen-specific T cells, at least not for the examined frequencies.

ACKNOWLEDGEMENTS

We thank Jenny Fohlstedt at Mabtech, Mads Hjortsø at Rigshospitalet and Marij Schoenmaekers-Welters for performing the pretesting of PBMCs and Sylvia Janetzki, Cecile Gouttefangeas, Cedrik Britten, Sjoerd van der Burg, Sine Reker and Leah Shifra Price for providing helpful advice.

ABOUT IMMUDEX

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex provides MHC Dextramer products for the monitoring of antigen-specific T cells, as well as provides MHC multimer and Elispot immune monitoring proficiency panel services. Immudex recognizes the need for accuracy and reproducibility in scientific and clinical research, and patient care, and has a number of research- and clinical products on the market. The goal is to enable simple, accurate and reliable monitoring of antigen-specific cellular immunity, and to promote the routine use of these technologies in diagnostics and research, and in all steps of immunotherapeutics development.

www.immudex.com

APPENDIX 1: INSTRUCTIONS

FOR THE ELISPOT PROFICIENCY PANEL 2014

General introduction to the Elispot proficiency panel:

All participants will receive two pre-tested PBMC samples. All participants must use the Elispot assay to determine the spot count per well as a result of stimulation with HCMVA (pp65), CEF (extended) and negative control for both PBMC samples using predefined peptide/negative reagents.

PLEASE READ ALL THE BELOW INSTRUCTIONS CAREFULLY BEFORE THAWING AND PREPARING THE PBMCs.

If you have any questions, please contact the organizer

Charlotte Halgreen
Coordinator of Proficiency Panels
email: ProficiencyPanel@immudex.com
Phone: +45 3917 9772

Material and Reagents:

Cell samples:
Each participant receives two vials each of which comprising a different PBMC sample; lot 13081 and lot 13096, respectively. Each vial contains app.10 million cells in 1mL.
Store cell vials in liquid nitrogen upon arrival and until use.

Reagents:
Each participant will receive three vials; Reagent 1 (HCMVA (pp65) peptide pool), Reagent 2 (CEF (extended) peptide pool), and Reagent 3 (Negative control - PBS/DMSO).
Store reagent vials in liquid nitrogen upon arrival and until use.

Cell samples and Reagents are shipped in a liquid nitrogen container. Instructions for the unloading of samples and return of the shipping container will be included. Please return the liquid nitrogen shipping container promptly.

General procedure for the Elispot proficiency panel:

While we recommend consideration of previously established Elispot harmonization guidelines, (please see Appendix A: "Assay Harmonization Guidelines"), please use your currently established SOP for this panel.

Use your own SOP (protocol) for Direct Human IFNγ Elispot Assay, as well as antibodies, plates, enzyme, substrate, equipment, medium and other miscellaneous chemicals and tools to perform the assay.

Please follow the instructions below as outlined.

Instructions for the Elispot proficiency panel:

1. One 96-well plate is required for this assay. Coat columns 3-5 of the plate according to your own protocol. You will need to coat 3x8 = 24 wells in total.

2. Thaw both vials of PBMC. Count and record total cell number and the number of viable cells, and calculate the percentage of viable cells, after thawing. If a resting step is performed, please count and record total cell number and the number of viable cells, and calculate the percentage of viable cells *both* before and after the resting step.

3. All Reagent vials (Reagent 1, Reagent 2, and Reagent 3) contain 75µl and must prior to use be diluted 1:10 to obtain a total volume of 750µl with the medium you use for the assay.

4. The PBMC samples and Reagents must be plated exactly according to the scheme below as the data will be reported and analyzed in this format. Please use columns 3-5 for the assay.

	1-2	3	4	5	6-12
A		**No cells Medium**	**No cells Medium**	**No cells Medium**	
B		PBMC lot 13081 Reagent 1	PBMC lot 13081 Reagent 1	PBMC lot 13081 Reagent 1	
C		PBMC lot 13081 Reagent 2	PBMC lot 13081 Reagent 2	PBMC lot 13081 Reagent 2	
D		PBMC lot 13081 Reagent 3	PBMC lot 13081 Reagent 3	PBMC lot 13081 Reagent 3	
E		PBMC lot 13096 Reagent 1	PBMC lot 13096 Reagent 1	PBMC lot 13096 Reagent 1	
F		PBMC lot 13096 Reagent 2	PBMC lot 13096 Reagent 2	PBMC lot 13096 Reagent 2	
G		PBMC lot 13096 Reagent 3	PBMC lot 13096 Reagent 3	PBMC lot 13096 Reagent 3	
H		**No cells Medium**	**No cells Medium**	**No cells Medium**	

5. Plate 200,000 cells/well for all samples in 50µl medium/well. Plate Reagents at 50µl/well. The final volume of cells and Reagent should be 100µl.
   Add medium only (no cells or Reagent) to A3-5 and H3-5). This will enable assessment of false positive spots.

4. Perform the assay according to your own SOP.

Reporting of data

Use this link » to record experimental details, data and your "Elispot plate reader file" (image of spots in wells)

All documents, and report forms can be found on the proficiencypanel - Elispot home page link ».

If you experience any problems, please contact the organizer:

Charlotte Halgreen
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Appendix A

Assay harmonization guidelines

Initial Elispot Harmonization Guidelines to Optimize Assay Performance (based on previously published recommendations based on the CIC/CRI and CIMT Elispot panel programs)

A. Use only pretested and optimized serum or serum-free media allowing for low background: high signal ratio.

B. Establish laboratory SOP for ELISPOT testing procedures, including:

    B1. Counting method for apoptotic cells for determining adequate cell dilution for plating,

    B2. Duration of resting period (i.e. overnight) of cells before plating and incubation.

C. Test each condition at least in triplicates.

D. Add optimal cell number per well for sufficient antigen presentation and highest signal to noise ratio.

E. Establish SOP for plate reading, including:

    E1. Human auditing during reading process,

    E2. Adequate adjustments for technical artefacts.

F. Only allow trained personnel, which is trained per laboratory SOP, to conduct assays.

APPENDIX 2: PBMC 13081 REPORTED NUMBER OF SPOTS

PBMC 13081 / Negative Control / CMV pool / CEF pool

Lab Id	Well D3-5 / Negative Control			Well B3-5 / CMV			Well C3-5 / CEF		
101	1	6	0	69	80	91	19	18	18
102	0	0	0	23	21	21	4	5	1
103	4	3	2	69	91	52	28	32	27
104	3	0	0	25	36	25	16	14	14
105	2	5	2	61	58	74	36	24	20
106	0	0	ND	49	46	ND	14	13	ND
107	5	2	1	58	61	53	27	30	31
108	0	1	1	65	73	49	23	21	20
109	2	1	1	54	56	57	13	9	11
110	8	5	2	358	351	338	88	102	94
111	2	0	0	18	22	14	8	2	6
112	1	4	1	68	80	84	35	18	28
113	1	0	0	143	139	142	41	21	29
114	2	1	3	48	41	46	11	16	18
115	3	0	0	92	78	88	28	20	17
116	9	9	6	58	47	64	33	26	31
117	0	9	6	249	247	259	65	60	89
118	8	1	1	25	13	29	13	10	2
119	1	2	7	5	3	10	0	1	1
120	2	3	0	42	31	48	14	20	20
121	10	22	16	33	23	25	20	20	17
122	290	239	200	211	227	156	199	159	171
123	0	5	2	ND	31	33	10	13	13
124	31	20	9	65	91	87	17	38	19
125	0	1	0	180	178	217	19	35	26
126	20	35	25	480	575	530	210	270	235
127	0	0	0	5	4	14	5	7	6
129	1	2	2	91	76	81	18	19	19
130	0	0	1	92	116	144	24	14	24
131	9	6	2	54	59	53	32	19	27
132	5	12	9	55	24	18	28	9	10
133	0	0	0	29	38	36	15	14	11
134	16	20	28	176	170	199	64	63	57
135	38	27	28	112	120	102	42	57	50
136	6	11	10	47	48	42	24	17	23
137	0	1	0	66	50	43	17	12	11
138	8	0	5	104	112	102	37	46	41
139	1	0	1	16	14	19	0	3	1
140	3	7	3	91	85	74	28	24	31
142	20	1	1	77	98	104	23	31	2

Lab Id	Well D3-5 / Negative Control			Well B3-5 / CMV			Well C3-5 / CEF		
143	1	7	4	70	94	64	30	14	22
144	4	3	2	60	45	62	18	21	16
145	7	1	12	69	56	101	17	16	11
146	19	42	32	45	67	56	41	53	49
147	21	17	21	40	60	40	21	33	24
148	22	30	27	162	147	168	72	97	98
149	0	0	0	36	35	48	10	12	5
150	1	1	0	49	60	46	23	25	28
151	8	4	11	58	75	67	28	24	29
152	851	835	760	821	813	793	781	808	704
154	2	1	1	27	29	28	5	7	5
156	0	1	0	40	46	40	3	0	1
157	23	35	47	106	131	118	52	54	59
158	3	3	2	23	25	19	7	11	9
159	2	2	0	84	86	81	28	30	24
161	87	98	72	141	147	84	87	83	115
162	82	141	92	136	110	125	90	94	87
163	1	0	1	24	32	33	17	15	18
164	1	1	1	54	85	52	5	9	8
165	35	31	31	163	138	143	197	26	42
166	71	70	69	123	92	96	67	74	75
167	0	0	1	54	56	59	21	22	17
168	1	4	2	97	84	82	4	8	9
169	0	0	1	12	27	22	10	9	11
170	19	12	8	78	74	68	27	39	28
171	3	2	1	10	13	13	6	3	1
172	19	20	16	46	75	54	24	32	24
173	0	1	0	57	62	75	17	26	16
174	2	1	3	72	105	87	25	38	30
175	18	17	17	91	78	77	40	42	44
176	1	2	2	38	38	34	4	3	5
177	13	8	7	75	91	56	42	28	33
178	0	0	0	28	23	26	7	9	4
179	32	22	21	69	56	76	38	56	20
180	2	5	1	91	120	91	40	32	35
181	1	0	0	25	22	32	4	3	3
182	2	0	0	8	11	8	6	6	7
183	1	4	3	96	65	53	19	29	32
234	29	26	22	143	160	180	51	37	43
277	3	4	2	84	93	93	25	43	38

APPENDIX 3: PBMC 13096 REPORTED NUMBER OF SPOTS

PBMC 13096 / Negative Control / CMV pool / CEF pool

Lab Id	Well G3-5 / Negative Control			Well E3-5 CMV			Well F3-5 CEF		
101	5	4	4	0	0	0	387	389	354
102	1	0	0	689	663	629	78	93	62
103	11	10	25	2030	1914	1985	148	147	103
104	6	2	0	223	115	242	51	58	63
105	6	8	2	519	580	561	248	206	200
106	3	5	ND	407	408	ND	89	86	ND
107	0	78	6	280	271	287	1118	1055	1055
108	0	0	0	423	605	681	235	240	246
109	2	3	3	1383	1412	1523	247	264	211
110	6	3	3	38	37	42	24	17	10
111	0	0	1	440	522	302	19	53	43
112	2	1	1	576	567	595	104	104	109
113	12	0	0	713	498	484	122	105	95
114	3	1	1	753	764	778	161	134	151
115	0	0	1	346	330	347	78	72	91
116	2	2	2	468	463	461	71	57	86
117	32	13	21	147	93	138	453	461	461
118	5	16	9	1008	927	930	113	112	133
119	5	0	9	3	29	163	4	0	0
120	7	3	4	300	300	300	85	82	59
121	56	57	35	158	142	157	93	119	112
122	26	19	22	430	390	403	185	177	153
123	16	12	15	320	227	265	50	ND	36
124	8	7	1	143	180	176	51	90	69
125	0	0	0	1055	1090	1061	248	238	241
126	0	0	5	2395	2245	2095	480	475	520
127	0	1	0	313	310	355	77	63	77
129	4	3	7	883	847	737	161	150	146
130	0	0	0	1533	1595	1692	198	160	250
131	5	6	6	500	500	500	154	138	139
132	20	4	0	351	330	321	17	17	20
133	0	0	0	88	99	86	50	54	41
134	9	10	11	1239	1195	1229	409	437	415
135	49	49	54	660	647	662	170	158	144
136	3	2	3	57	88	75	17	13	15
137	0	0	1	859	860	893	126	108	143
138	1	7	2	905	804	857	183	173	155
139	1	1	1	282	304	279	21	28	38
140	4	13	9	804	1007	943	132	117	117
142	0	0	6	201	98	231	184	135	67

Lab Id	Well G3-5 / Negative Control			Well E3-5 / CMV			Well F3-5 / CEF		
143	2	1	2	1142	1373	1406	225	239	224
144	6	25	27	619	572	606	216	206	224
145	0	2	1	377	381	365	112	84	82
146	54	67	41	300	300	300	191	196	209
147	1	0	1	578	571	592	91	97	97
148	15	9	17	1	1	1	365	461	402
149	0	0	0	1035	1008	1023	334	360	354
150	10	3	0	500	500	500	194	203	210
151	7	4	6	636	597	645	282	252	232
152	461	469	462	932	920	901	481	499	466
154	8	12	10	584	571	583	145	142	126
156	1	0	0	1064	1081	1106	113	133	94
157	44	79	47	10000	10000	10000	270	304	275
158	1	3	ND	72	72	ND	14	20	ND
159	0	2	0	902	927	965	106	105	109
161	186	223	214	950	994	971	405	334	323
162	22	26	15	350	345	302	96	96	68
163	36	48	39	772	736	757	288	292	280
164	0	1	0	356	357	364	41	30	30
165	32	28	10	611	451	413	347	365	312
166	5	2	6	337	277	272	19	25	27
167	0	3	12	736	649	656	85	82	130
168	1	0	1	500	500	500	140	139	139
169	1	0	2	329	303	366	54	39	59
170	18	17	16	726	674	673	167	190	166
171	1	4	3	531	464	460	48	56	34
172	7	5	3	483	385	488	107	85	97
173	0	1	0	696	653	725	89	93	117
174	0	3	5	1082	1097	1092	266	201	217
175	4	6	8	500	500	500	295	319	323
176	2	3	0	324	270	332	136	106	114
177	9	21	15	488	434	462	247	256	249
178	0	0	0	999	999	999	158	162	143
179	14	12	7	250	250	ND	152	162	ND
180	4	5	9	890	890	890	214	196	215
181	2	0	0	470	465	403	110	137	85
182	4	2	8	856	862	879	230	213	226
183	1	3	4	890	890	890	304	311	291
234	11	10	13	1164	1130	1128	398	403	428
277	1	5	6	329	276	303	196	200	204