ELISPOT PROFICIENCY PANEL 2015

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ELISPOT PROFICIENCY PANEL 2015

This report summarizes the results of the Elispot Proficiency Panel 2015. The report provides individual test results for each participating laboratory that participated in the Elispot proficiency panel 2015, 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, independent on geographic location or field of interest, with a need to perform accurate and reproducible quantification of antigen-specific T cells.

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 MHC Multimer and Elispot proficiency panels to be held in the fall 2016 and spring 2017, respectively

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Contents

INFORMATION ON PARTICIPANTS, PROTOCOLS, REAGENTS, CELL SAMPLES

- 42 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 13089 and 13110, 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 4.000 PBMCs ("Low responder") to more than 1 in 600 PBMCs ("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 13089 and 13110, 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 42 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 42 participants of the Elispot Proficiency Panel 2015 are shown in Figures 1-4 and Appendices 2-3.

Figures 1A, 2A, 3A 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, 3B and 4B show the background-corrected, mean number of antigen-specific spots per 200.000 PBMCs (one red diamond).

Figures 1C, 2C, 3C 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 0,66–1,5 are here considered "in the average range" and are represented by filled black columns; Relative Accuracies of 0,50–0,65 or 1,6–2,0 are considered "near average" and are represented by hatched columns; Relative Accuracies below 0,50 or above 2,0 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.

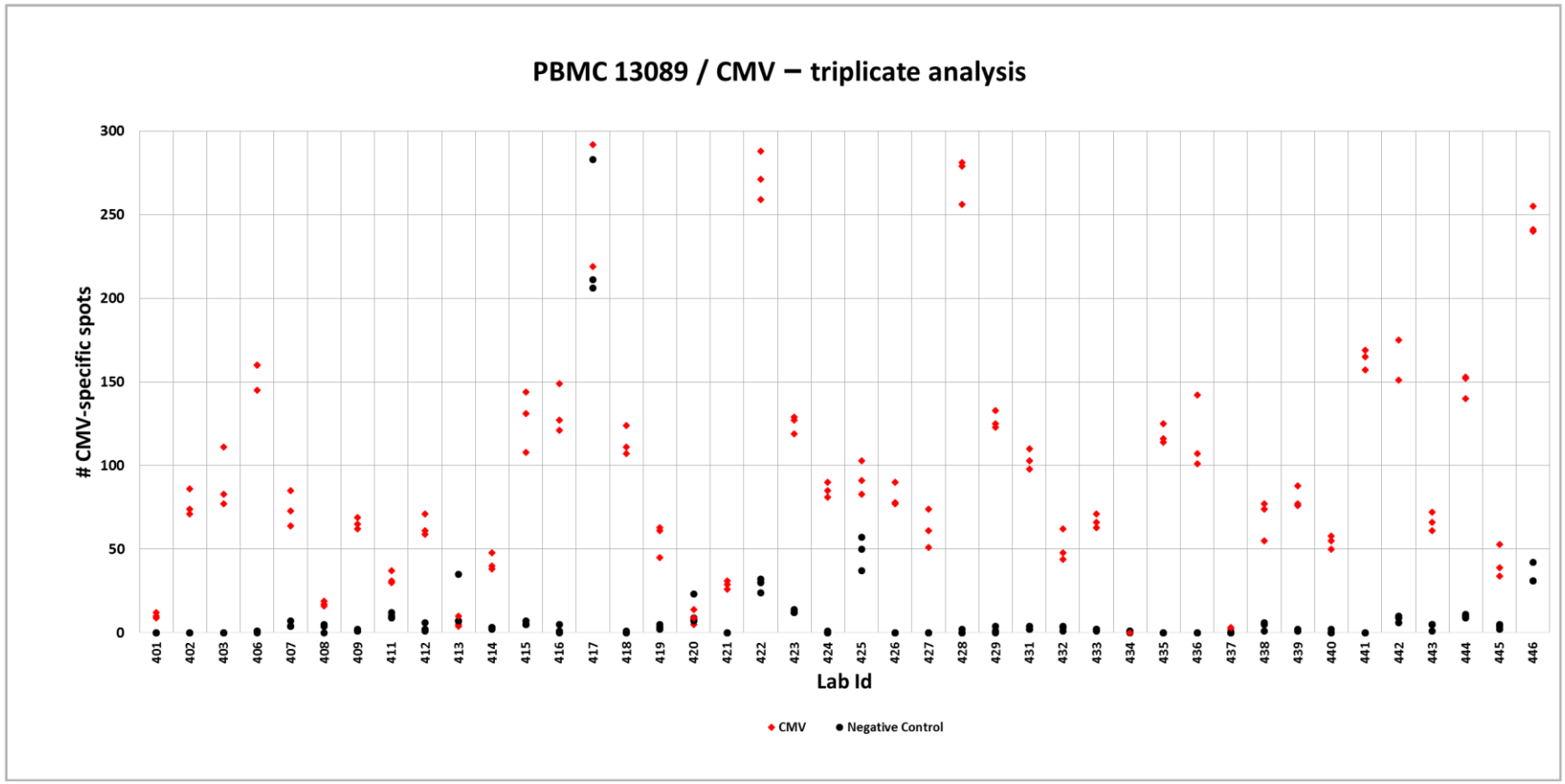

Figure 1A. CMV-specific spots and background spots for PBMC 13089.

The number of spots per 200.000 PBMCs for PBMC 13089/CMV (triplicate analysis; 3 red diamonds) or PBMC 13089/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 417 reported one value higher than 300 for CMV (see appendix 2). Lab Id 442 only reported duplicate values for CMV.

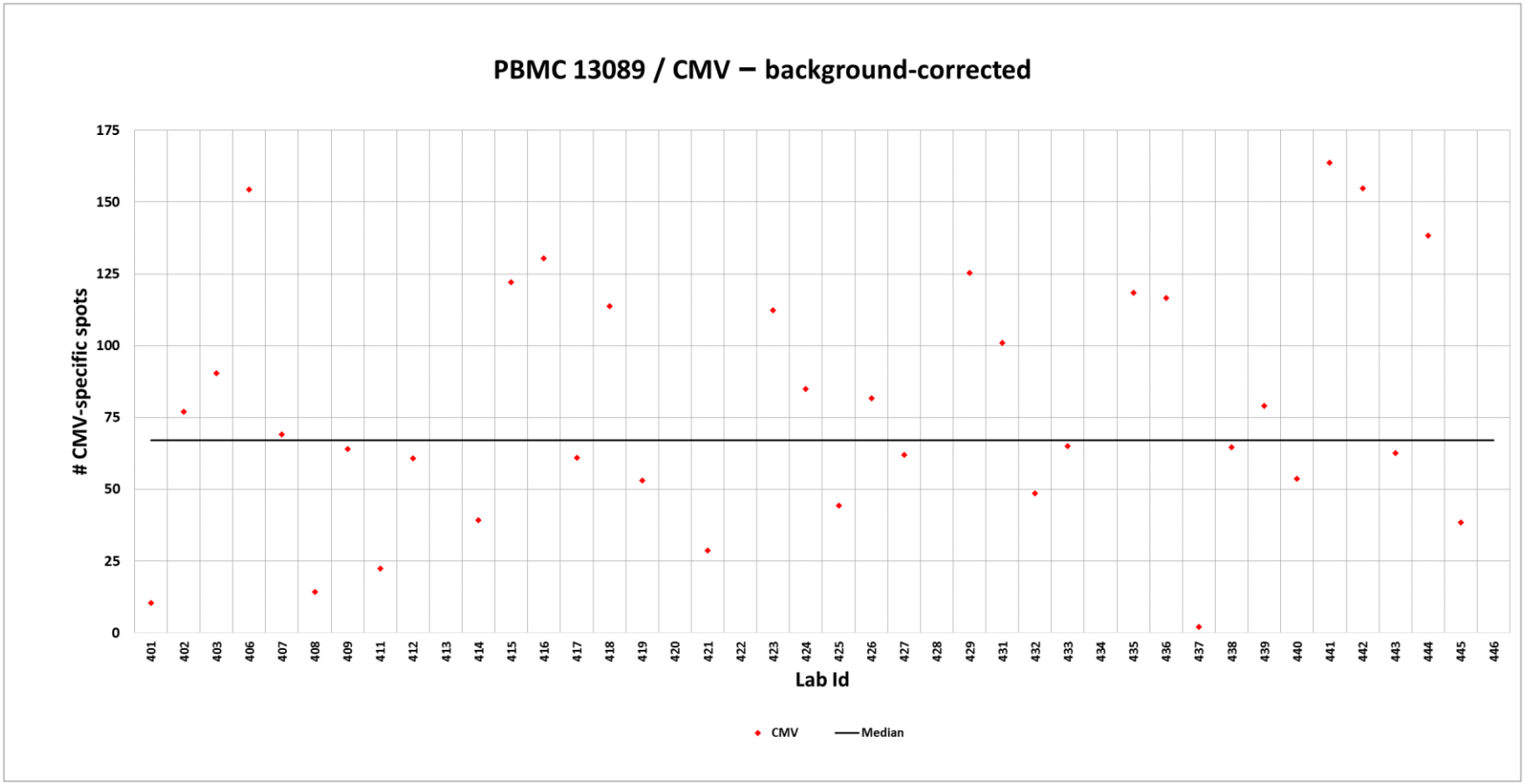

Figure 1B. Background-corrected number of CMV-specific spots for PBMC 13089.

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 (67 CMV-specific spots) is indicated with a black horizontal line. Lab Id 413, 420, 422, 428, 434 and 446 reported value for CMV of -10, -3,7, 244, 271, -0,67 and 211, respectively.

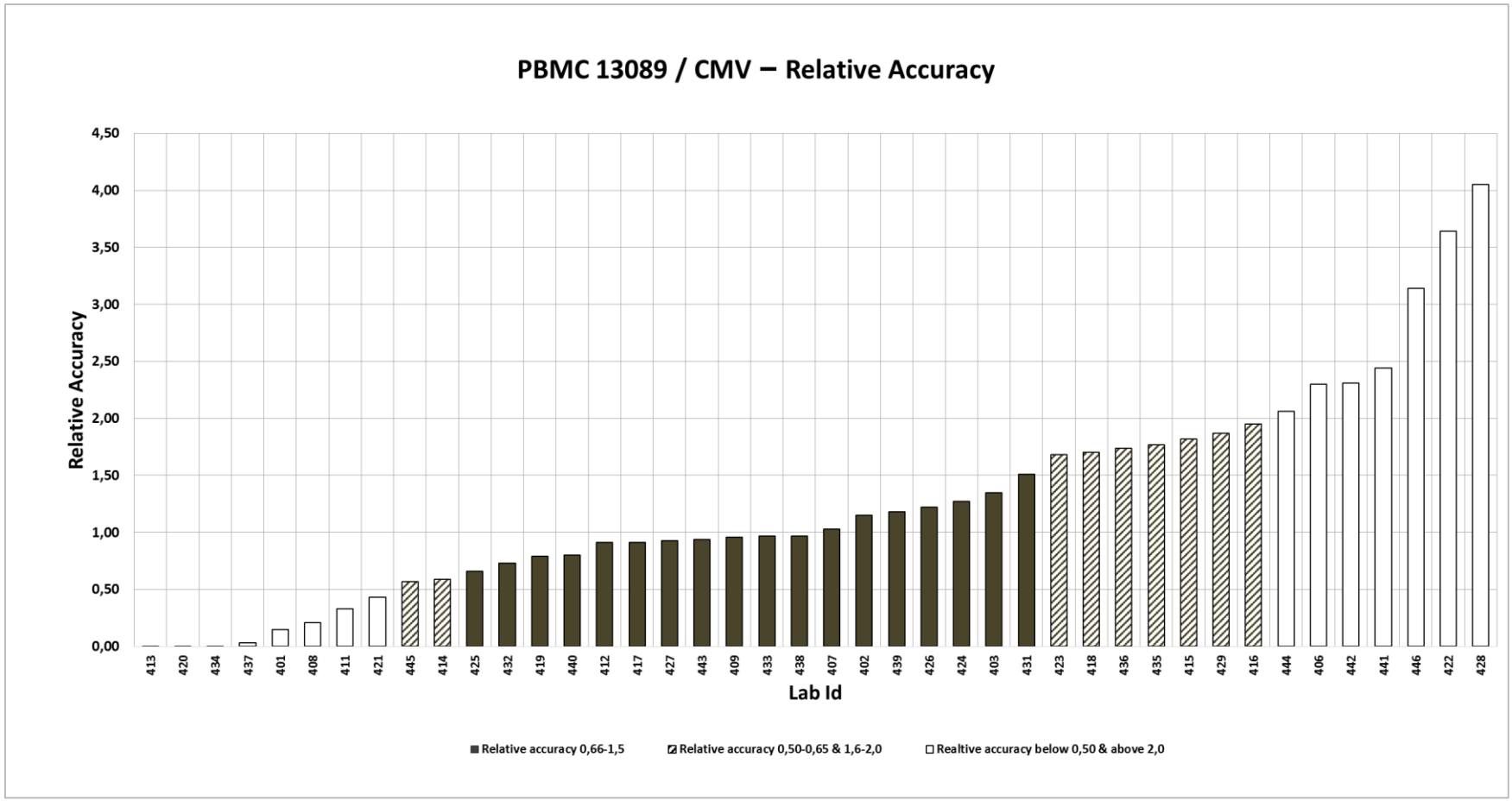

Figure 1C. Relative Accuracy for the 13089/CMV combination.

The Relative Accuracy, equaling the result divided by the median (67) of all results, for background-corrected, CMV-specific spots is shown. Relative Accuracy for Lab Id 413, 420, 434 and 437 is -0,15, -0,05, -0,01 and 0,03, respectively. 18 out of 42 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".

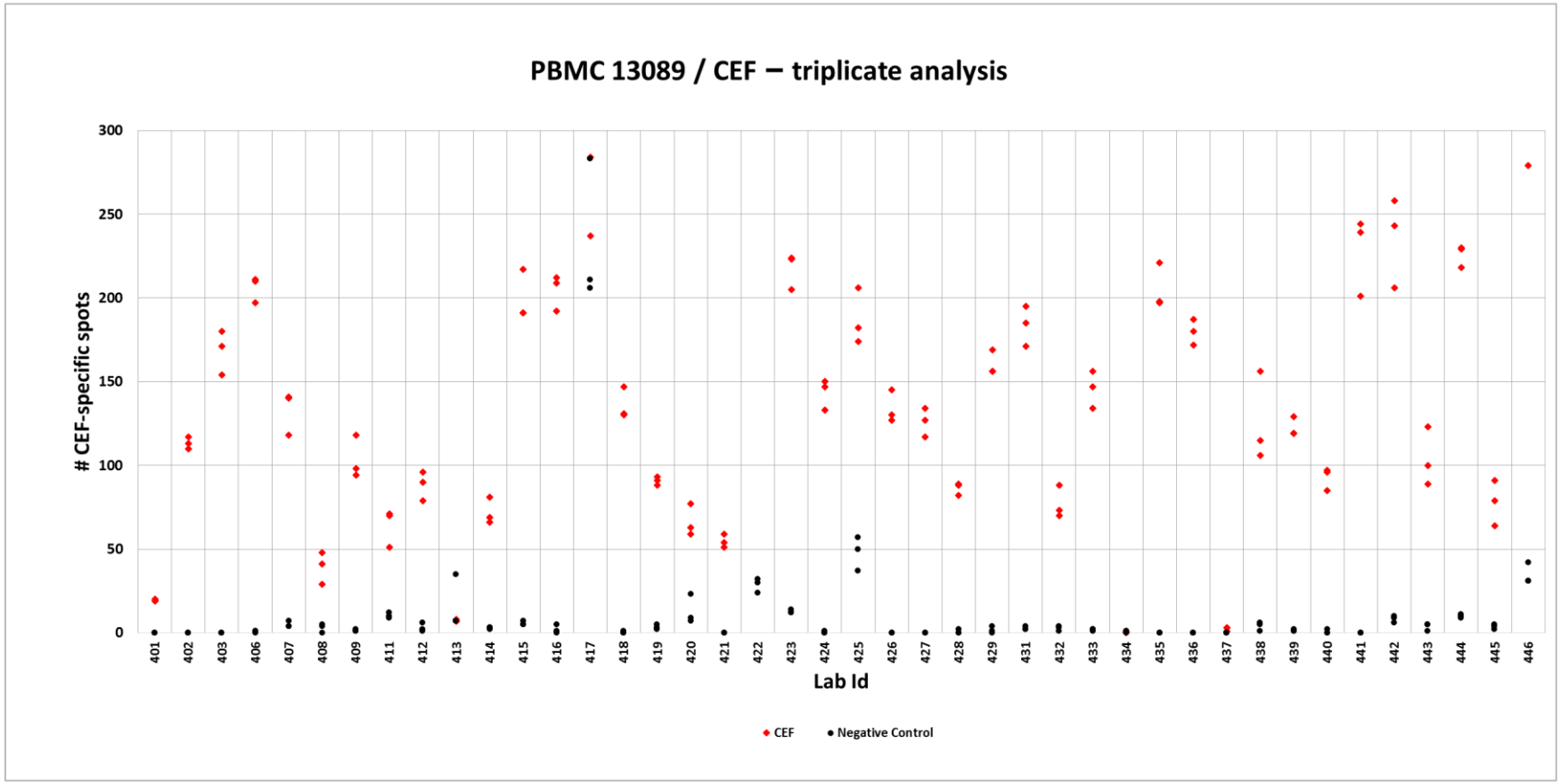

Figure 2A. CEF-specific spots and background spots for PBMC 13089.

The number of spots per 200.000 PBMCs for PBMC 13089/CEF (triplicate analysis; 3 red diamonds) or PBMC 13089/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 422 reported values higher than 300 for CEF. Lab Id 417 reported one value higher than 300 for CEF. Lab Id 446 reported two values higher than 300 for CEF (see appendix 2).

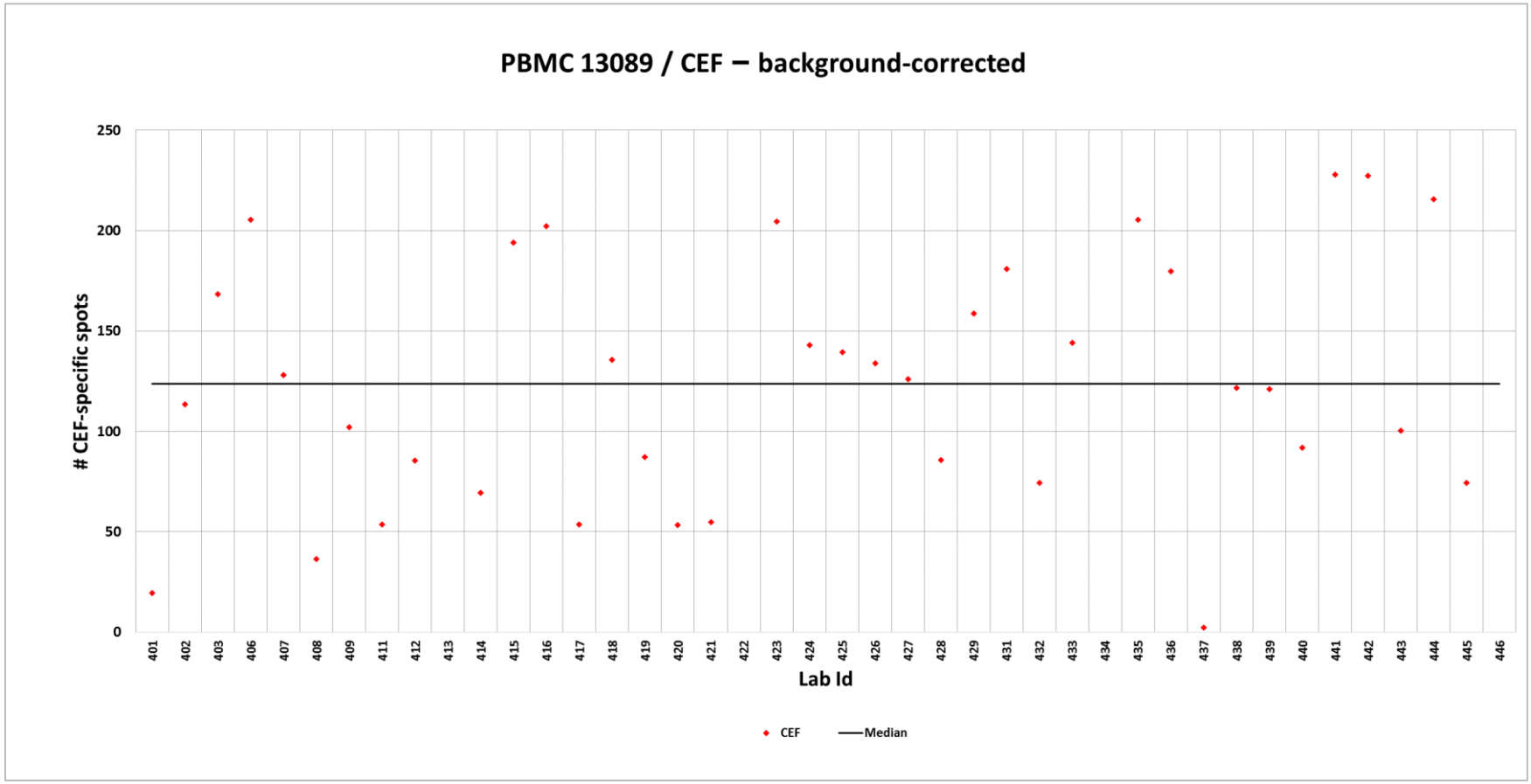

Figure 2B. Background-corrected number of CEF-specific spots for PBMC 13089.

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 (124 CEF-specific spots) is indicated with a black horizontal line. Lab Id 413, 434, 422 and 446 reported value for CEF of -9, -0,3, 339 and 273, respectively.

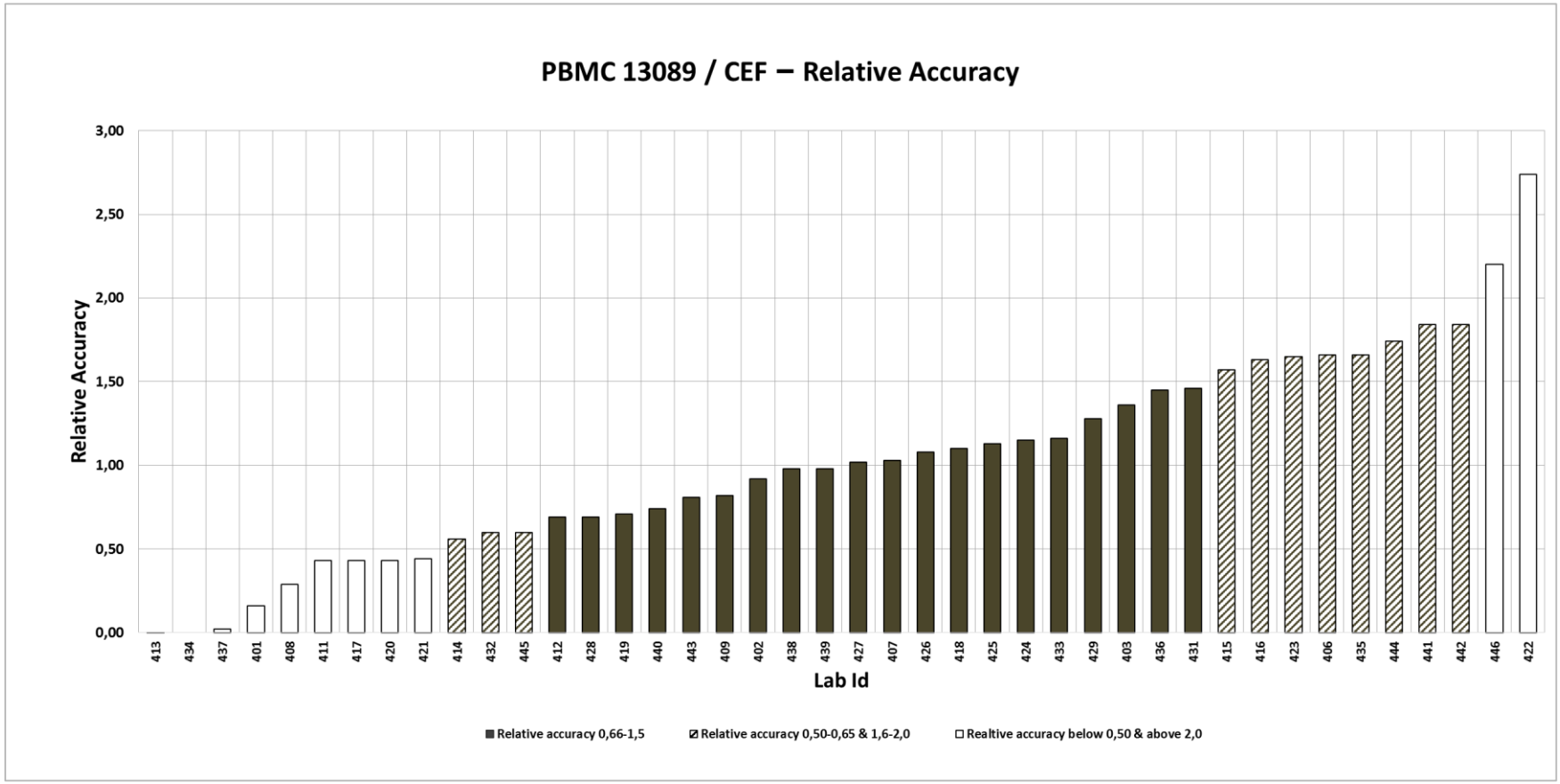

Figure 2C. Relative Accuracy for the 13089/CEF combination.

The Relative Accuracy, equaling the result divided by the median (124) of all results, for background-corrected CEF-specific spots is shown. Relative Accuracy for Lab Id 413, 434 and 437 is –0,07, 0, and 0,02, respectively. 20 out of 42 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".

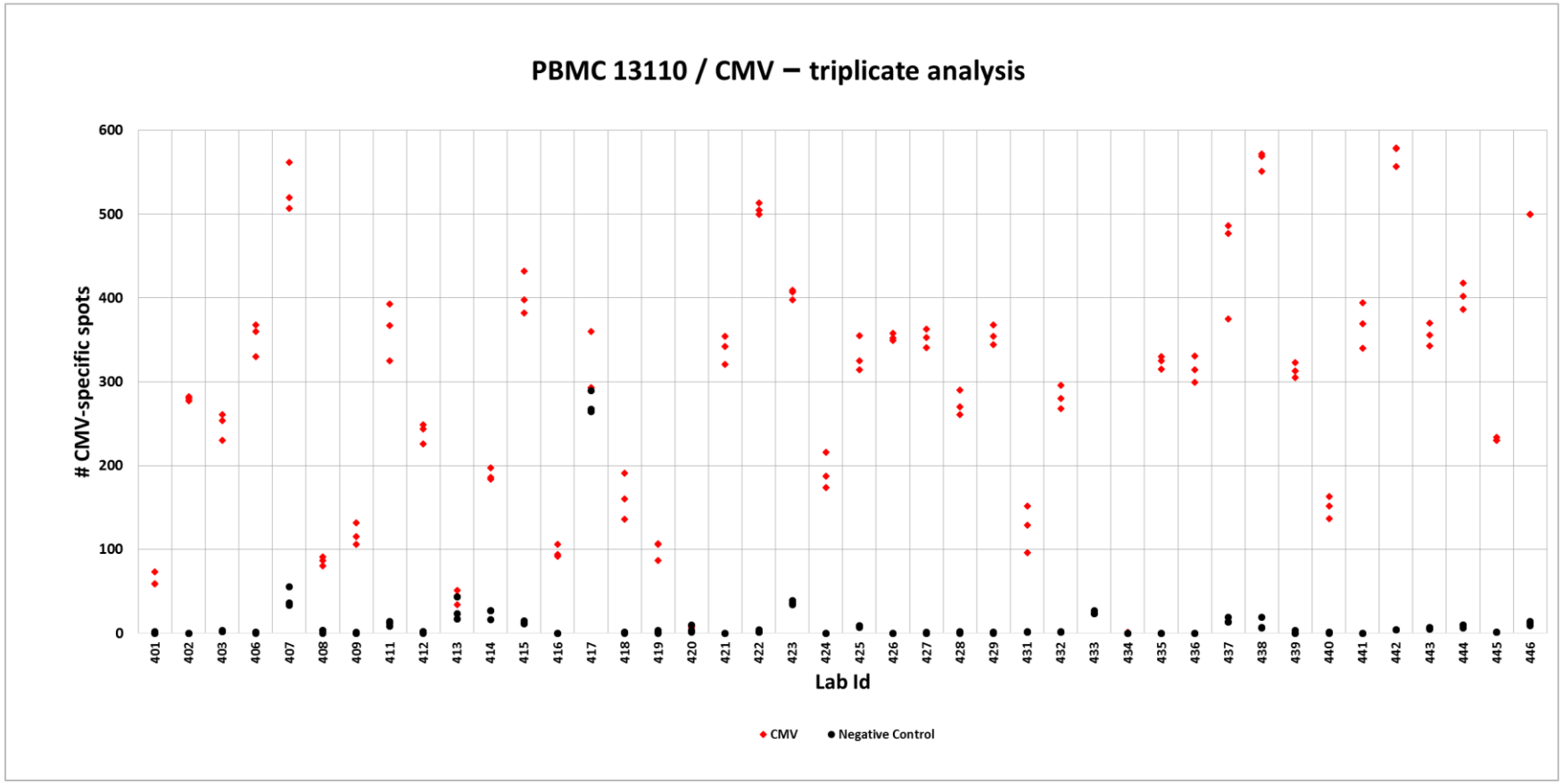

Figure 3A. CMV-specific spots for PBMC 13110.

The number of spots per 200.000 PBMCs for PBMC 13110/CMV (triplicate analysis; 3 red diamonds) or PBMC 13110/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 433 reported values higher than 600 for CMV (see appendix 3).

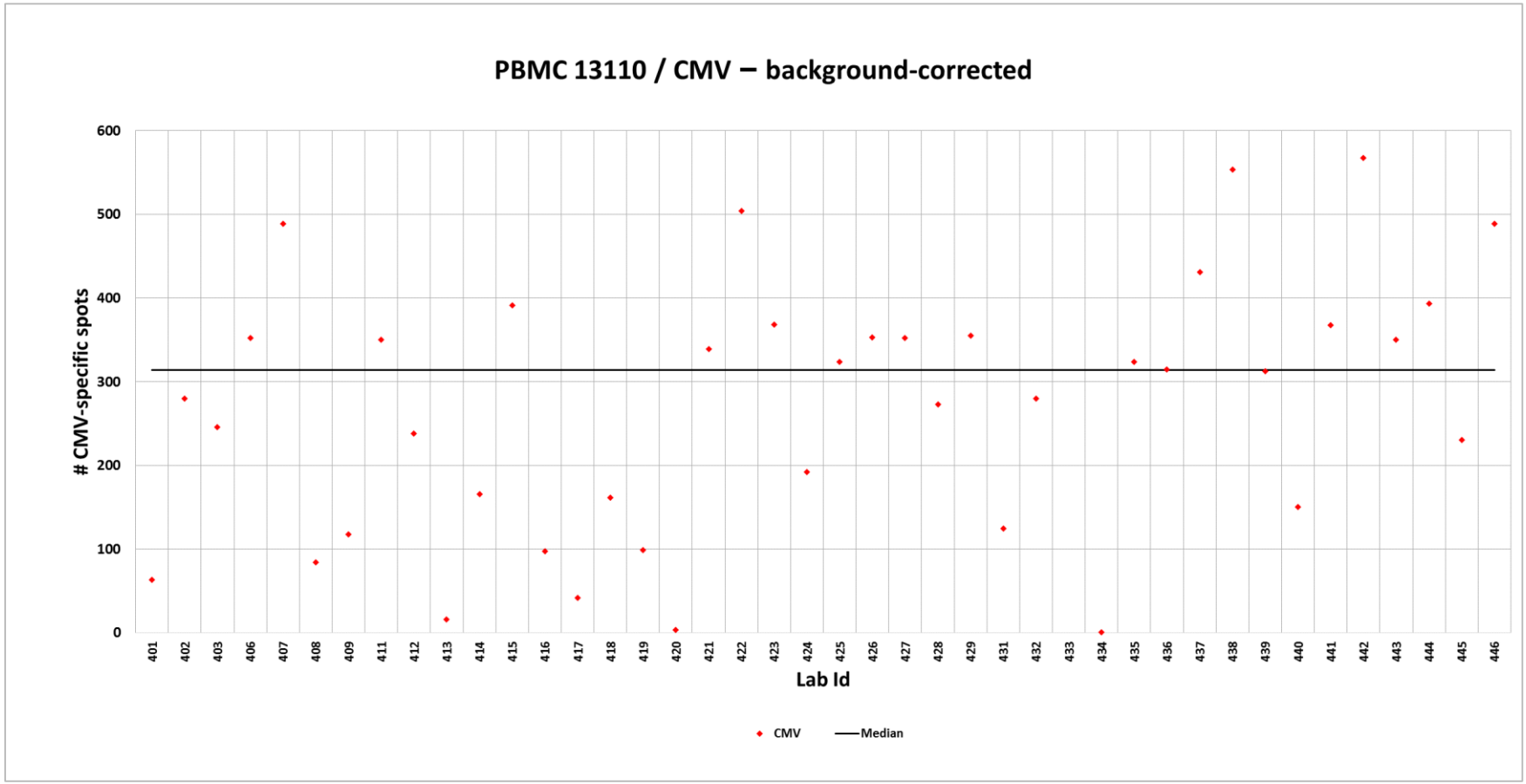

Figure 3B. Background-corrected number of CMV-specific spots for PBMC 13110.

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 (314 CMV-specific spots) is indicated with a black horizontal line. Lab Id 433 reported value for CMV of 795.

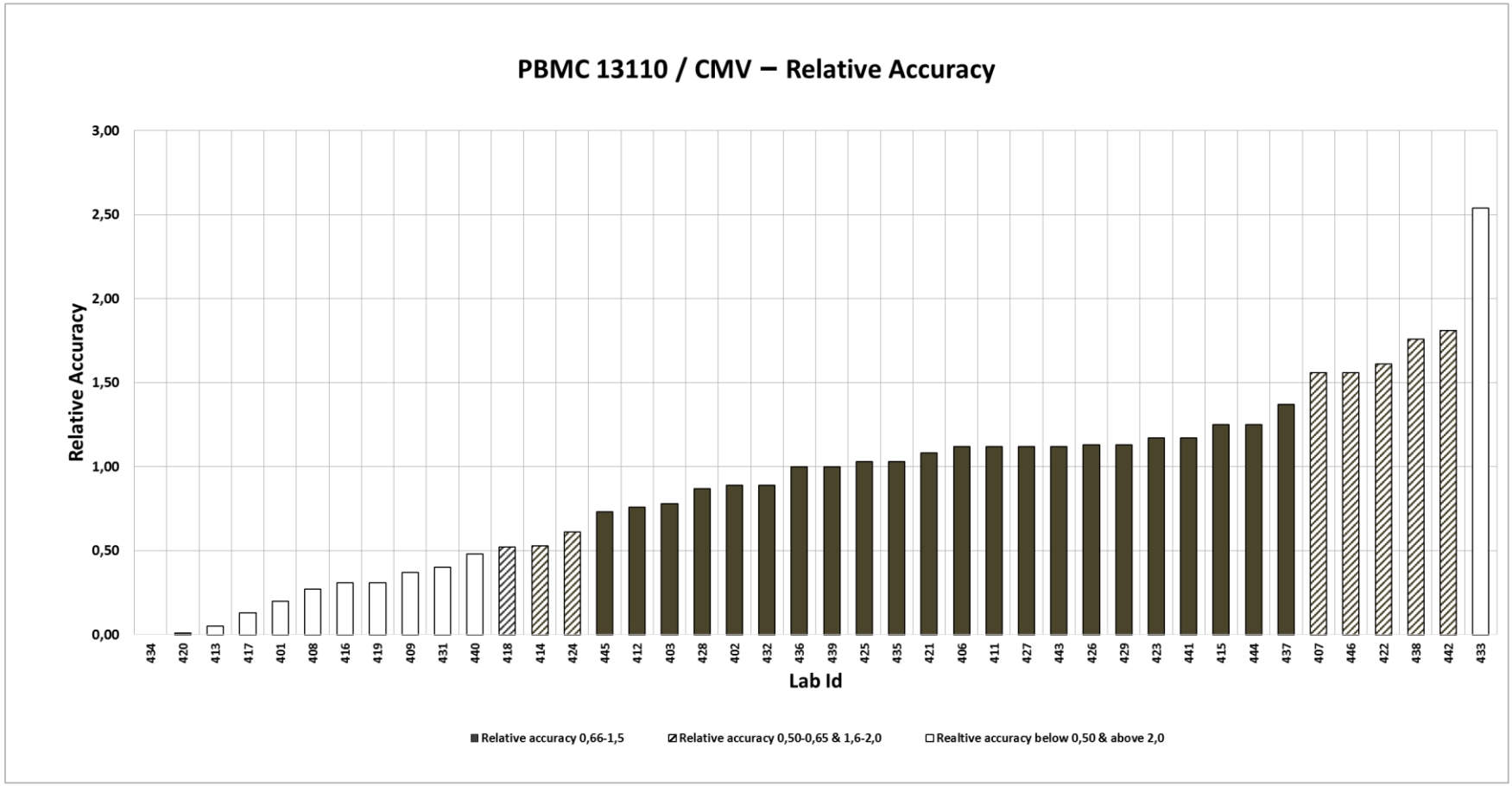

Figure 3C. Relative Accuracy for the 13110/CMV combination.

The Relative Accuracy, equaling the result divided by the median (314) of all results, for background-corrected, CMV-specific spots is shown. Relative Accuracy for Lab Id 434 and 420 is 0 and 0,01, respectively. 22 out of 42 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".

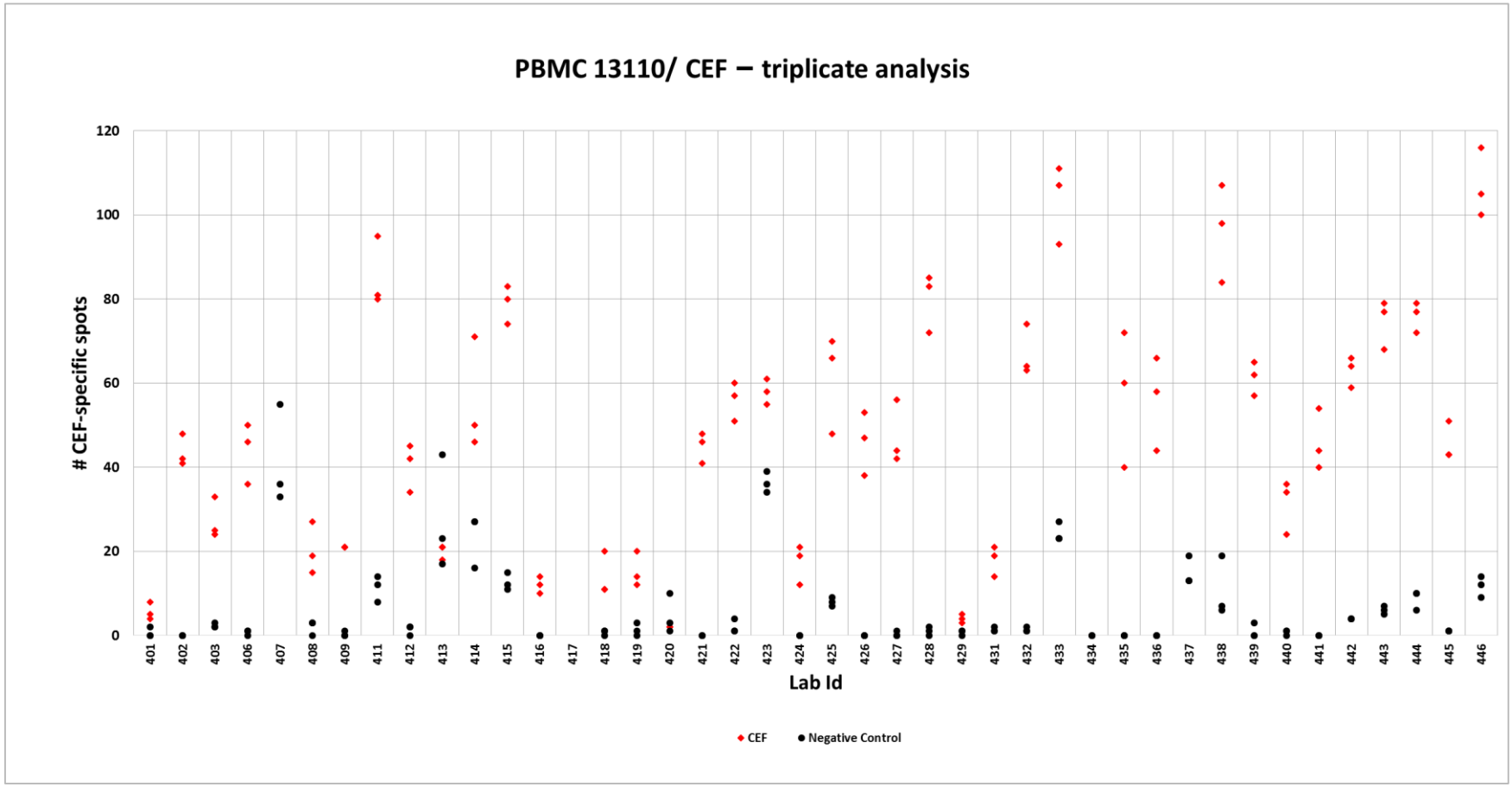

Figure 4A. CEF-specific spots and background spots for PBMC 13110.

The number of spots per 200.000 PBMCs for PBMC 13110/CEF (triplicate analysis; 3 red diamonds) or PBMC 13096/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 417 reported values higher than 120 for both the Negative control and CEF. Lab Id 407 and 437 reported values higher than 120 for CEF (see appendix 3).

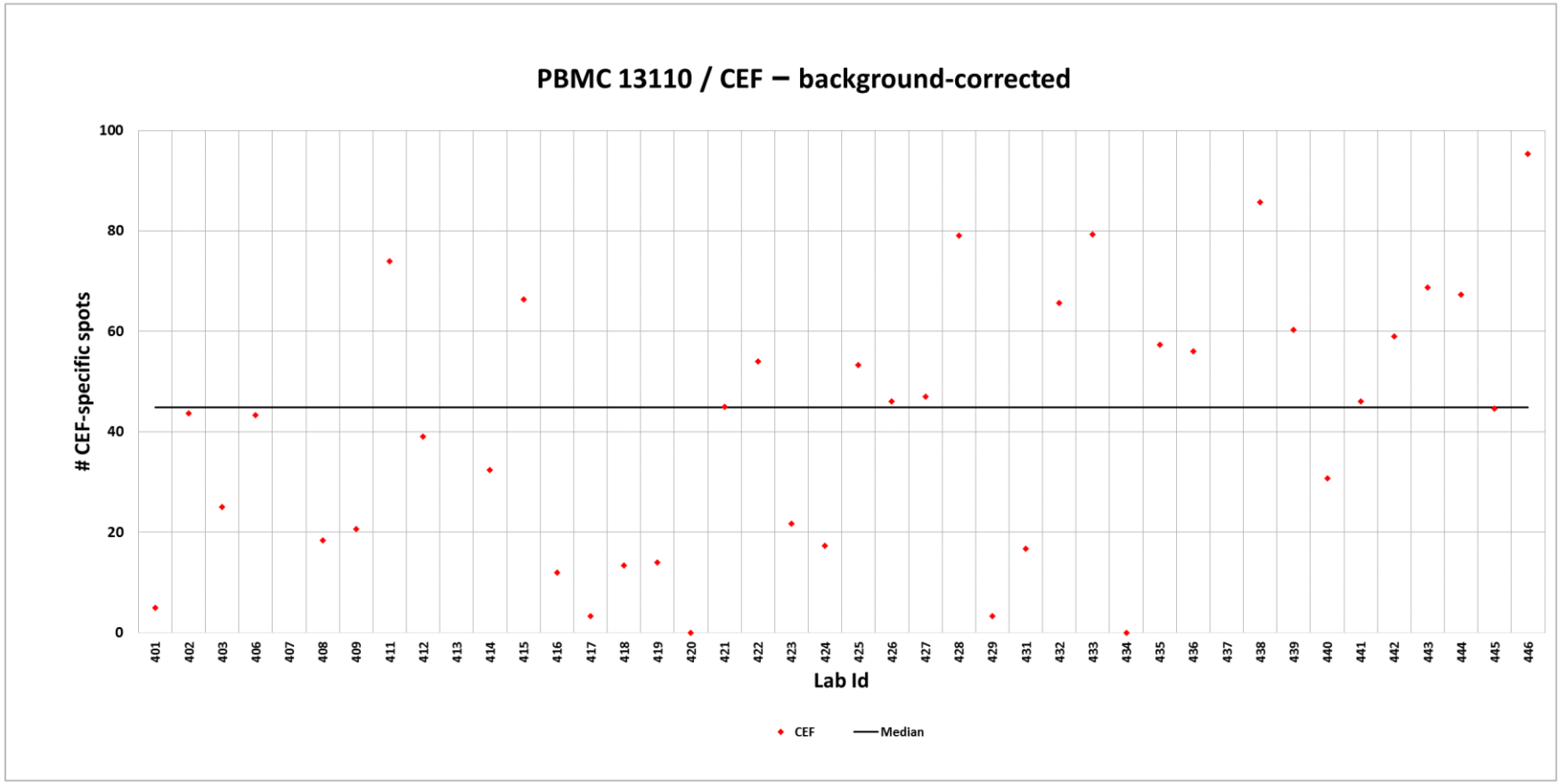

Figure 4B. Background-corrected number of CEF-specific spots for PBMC 13110.

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 (45 CEF-specific spots) is indicated with a black horizontal line. Lab Id 407, 413 and 437 reported value for CEF of 133, -7,7 and 135, respectively.

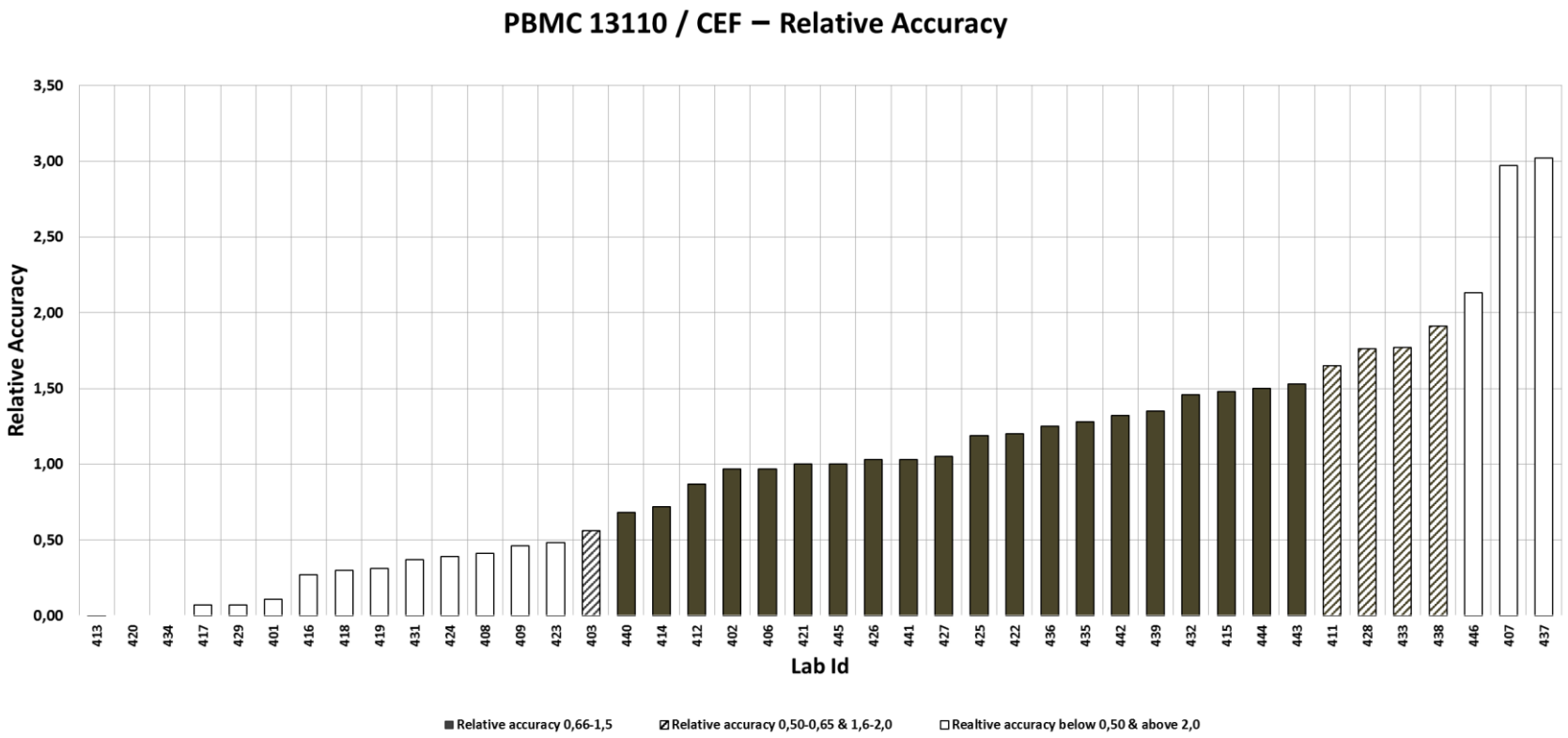

Figure 4C. Relative Accuracy for the 13110/CEF combination.

The Relative Accuracy, equaling the result divided by the median (45) of all results, for background-corrected CEF-specific spots is shown. Relative Accuracy for Lab Id 413, 420 and 434 is -0,17, 0 and 0, respectively. 20 out of 42 participants had a Relative Accuracy between 0,66-1,5 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.

The score "3" was assigned to results in the average range (i.e. Relative Accuracy between 0,66 and 1,5), the score "2" was assigned to results near average (i.e. Relative Accuracy 0,50-0,65 or 1,6-2,0), and finally, the score "1" was assigned to results far from average (i.e. Relative Accuracy below 0,50 or above 2,0).

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

Overall Proficiency is shown in Figure 5. As can be seen, 7 out of 42 laboratories (17% and first 7 laboratories from the left) are in the average range on all four measurements, and thus have the highest possible Overall Proficiency score of "3".

17% of participants had results in "the average range" for 4 out of 4 measurements.

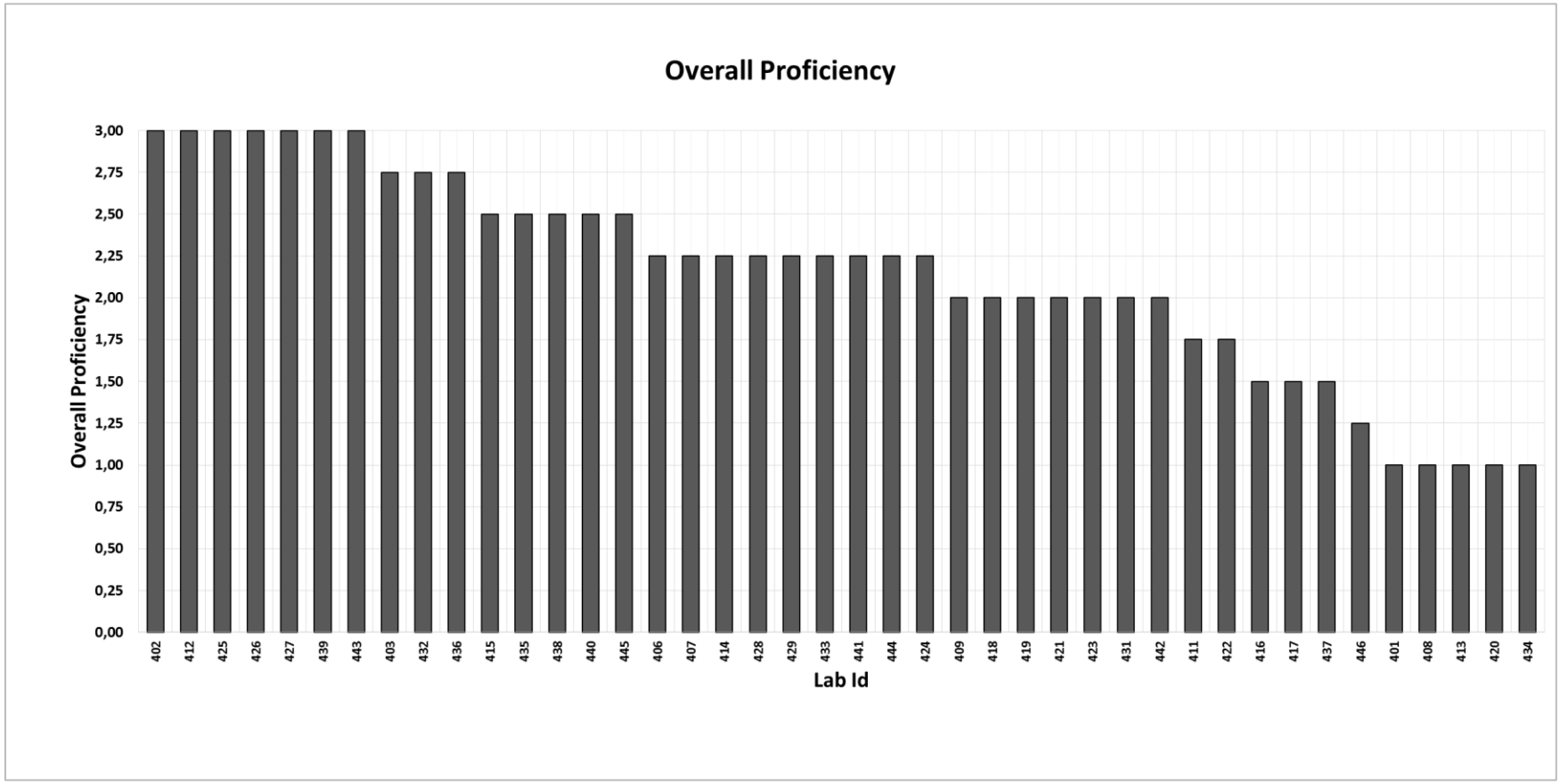

Figure 5. Overall Proficiency. *The laboratories' proficiency in performing the Elispot measurements is shown. An Overall Proficiency of "3" represents the highest possible proficiency score; an Overall Proficiency of "1" represents the lowest possible Overall proficiency score. A score of 3" indicates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

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 4.000 PBMCs) to High responders (1 antigen-specific cell per 600 PBMCs).

- For a given measurement, about 50% of the participants had a Relative Accuracy of between 0,66 and 1,5 and were defined as being "in the average range". In other words, for a given measurement, about 50% of the participants were close to the median value of all participants.

For each measurement, ~50% of the participants obtained a value close to average

- 17% of the participants were "in the average range" for four out of four measurements.

17% of the participants were close to average for 4 out of 4 measurements

- For a given measurement, the 75% of the participants that were closest to the "average" (median value) had results differing from 4 to 16 fold. For example, for the PBMC 13089/CEF antigen combination, the 75% of participants (i.e. 32 out of 42 participants) that were closest to the median value, detected from 54 to 205 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 4-16 fold

- The number of laboratories with Relative Accuracies between 0,66 and 1,5 (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 ImmuneCarta, Mabtech and Marij Schoenmaekers-Welters for quality control and Elispot assay testing of cell samples, JPT Peptide Technologies for providing peptides and Sylvia Janetzki for providing helpful advice.

ABOUT IMMUDEX

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex provides MHC Dextramers for the monitoring of antigen-specific T cells. Under an agreement with the US Cancer Immunotherapy Consortium (CIC) and the European Cancer Immunotherapy Consortium (CIMT), Immudex also provides MHC Multimer and Elispot proficiency panel services worldwide.

Immudex's MHC Dextramer® products are utilized for the quantification or sorting of antigen-specific T cells in life science research, in vitro diagnostics, as well as the development of immunotherapeutics and vaccines. The primary focus is research-use-only products for the immune monitoring of immunotherapy development, and monitoring of CMV cellular immunity in transplant and other immune-deficient patients. In Europe, the CE-marked Dextramer CMV Kit is approved for in vitro diagnostic use, for the quantification of CMV-specific T cells.

Our soon-to-be released DNA tagged Dextramer reagents enable massive multiplexing of antigen-specific T cell detection. To date quantitation of over 1000 CD8+ T cell specificities in a single, small blood sample has been achieved. To find out more about how Immudex is improving immune monitoring and enabling better treatment decisions please visit us at www.immudex.com.

*The Immudex CMV kit is for Research Use Only in the United States

APPENDIX 1: INSTRUCTIONS

FOR THE ELISPOT PROFICIENCY PANEL 2015

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

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Material and Reagents:

Cell samples:

Each participant receives two vials each of which comprising a different PBMC sample; lot 13089 and lot 13110, 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 13089 Reagent 1	PBMC lot 13089 Reagent 1	PBMC lot 13089 Reagent 1	
C		PBMC lot 13089 Reagent 2	PBMC lot 13089 Reagent 2	PBMC lot 13089 Reagent 2	
D		PBMC lot 13089 Reagent 3	PBMC lot 13089 Reagent 3	PBMC lot 13089 Reagent 3	
E		PBMC lot 13110 Reagent 1	PBMC lot 13110 Reagent 1	PBMC lot 13110 Reagent 1	
F		PBMC lot 13110 Reagent 2	PBMC lot 13110 Reagent 2	PBMC lot 13110 Reagent 2	
G		PBMC lot 13110 Reagent 3	PBMC lot 13110 Reagent 3	PBMC lot 13110 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.

Perform the assay according to your own SOP.

Reporting of data

Use this link » to record experimental details and data.

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
Coordinator of Proficiency Panels
mail: ProficiencyPanel@immudex.com
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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.

*For details see Nature Protocols 2015 (Guidelines for the automated evaluation of Elispot assays. (Janetzki, Sylvia et. al.; 2015. Nat Protoc. 2015))

APPENDIX 2: PBMC 13089 REPORTED NUMBER OF SPOTS

PBMC 13089 / Negative Control / CMV pool / CEF pool

Lab Id	Well D3-5 / Negative Control			Well B3-5 / CMV			Well C3-5 / CEF		
401	0	0	0	10	12	9	19	19	20
402	0	0	0	71	74	86	110	117	113
403	0	0	0	77	111	83	154	171	180
406	0	1	1	160	160	145	210	211	197
407	4	7	4	85	73	64	141	118	140
408	0	5	4	16	19	17	41	29	48
409	2	1	1	62	65	69	98	94	118
411	12	10	9	37	31	30	51	71	70
412	6	1	2	71	59	61	96	90	79
413	7	7	35	5	4	10	7	8	7
414	3	2	3	38	48	40	66	69	81
415	5	5	7	108	131	144	217	191	191
416	5	1	0	149	127	121	212	209	192
417	283	206	211	372	292	219	340	237	284
418	0	0	1	107	124	111	147	130	131
419	2	5	3	61	63	45	91	88	93
420	7	9	23	5	14	9	77	63	59
421	0	0	0	31	26	29	59	54	51
422	32	30	24	271	288	259	375	380	349
423	12	14	12	129	119	127	205	224	223
424	0	0	1	81	85	90	147	133	150
425	37	57	50	91	103	83	182	206	174
426	0	0	0	77	90	78	127	130	145
427	0	0	0	51	61	74	134	117	127
428	0	0	2	281	256	279	82	89	88
429	4	0	1	133	123	125	156	156	169
431	2	4	2	103	98	110	171	195	185
432	4	3	1	48	44	62	70	73	88
433	1	2	2	63	71	66	134	156	147
434	0	1	1	0	0	0	1	0	0
435	0	0	0	116	114	125	198	221	197
436	0	0	0	101	107	142	172	187	180
437	0	0	0	1	2	3	3	1	3
438	5	6	1	74	55	77	115	156	106
439	1	2	1	77	88	76	119	119	129
440	2	0	0	50	58	55	97	85	96
441	0	0	0	165	169	157	239	244	201
442	9	10	6	151	175	ND	206	243	258
443	5	5	1	61	72	66	123	100	89
444	9	10	11	140	153	152	229	218	230

Lab Id	Well D3-5 / Negative Control			Well B3-5 / CMV			Well C3-5 / CEF		
445	2	4	5	39	53	34	79	64	91
446	42	31	31	255	241	240	279	314	329

APPENDIX 3: PBMC 13110 REPORTED NUMBER OF SPOTS

PBMC 13110 / Negative Control / CMV pool / CEF pool

Lab Id	Well G3-5 / Negative Control			Well E3-5 CMV			Well F3-5 CEF		
401	0	2	0	59	73	59	8	5	4
402	0	0	0	282	280	277	41	48	42
403	2	3	2	254	230	261	25	33	24
406	1	0	1	360	330	368	46	36	50
407	55	36	33	507	562	520	189	167	168
408	0	3	3	80	91	87	15	27	19
409	1	0	0	115	132	106	21	21	21
411	12	14	8	325	393	367	80	81	95
412	0	2	2	244	226	249	34	45	42
413	43	17	23	45	34	51	18	21	21
414	16	27	27	184	186	197	71	46	50
415	12	11	15	398	382	432	80	83	74
416	0	0	0	94	106	92	10	14	12
417	289	264	267	360	293	293	317	282	231
418	1	0	1	136	160	191	11	20	11
419	0	1	3	107	106	87	14	20	12
420	10	3	1	8	9	7	10	2	2
421	0	0	0	342	321	354	41	46	48
422	1	4	1	500	513	505	60	51	57
423	36	39	34	398	409	407	61	55	58
424	0	0	0	216	187	174	21	12	19
425	7	9	8	355	325	314	48	66	70
426	0	0	0	349	352	358	38	47	53
427	0	1	0	353	341	363	44	56	42
428	2	0	1	261	270	290	83	72	85
429	1	1	0	354	368	344	3	5	4
431	2	1	1	96	152	129	14	21	19
432	1	2	1	296	268	280	64	74	63
433	23	27	23	787	843	829	111	93	107
434	0	0	0	0	0	1	0	0	0
435	0	0	0	330	325	315	40	60	72
436	0	0	0	314	299	331	66	44	58
437	13	13	19	486	375	477	142	179	130
438	7	6	19	569	572	551	98	107	84
439	3	0	0	305	313	323	65	62	57
440	0	1	1	137	152	163	34	24	36
441	0	0	0	369	340	394	44	40	54
442	4	4	4	578	579	557	66	59	64
443	5	7	6	343	370	356	79	68	77
444	6	10	10	402	386	418	77	79	72

Lab Id	Well G3-5 / Negative Control			Well E3-5 / CMV			Well F3-5 / CEF		
445	1	1	1	230	234	230	51	43	43
446	9	14	12	500	500	500	105	116	100