

# ELISPOT PROFICIENCY PANEL 2017

April 2018

# ELISPOT PROFICIENCY PANEL 2017

This report summarizes the results of the T cell Elispot Proficiency Panel 2017. The report provides individual test results for each participating laboratory that participated in the Elispot proficiency panel 2017, as well as an anonymized overview of the other participants' test results.

36 laboratories from 13 countries participated in the Elispot Proficiency Panel.

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, clinical developers 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.

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## **INFORMATION ON PARTICIPANTS, PROTOCOLS, REAGENTS, CELL SAMPLES**

- 36 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-PP65-2) in PBS buffer/DMSO; Reagent 2 is the PepMix CEF Pool (extended) (JPT Product Code PM-CEF-E-3) in PBS buffer/DMSO; Reagent 3 is a negative control (no peptide) comprising 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.

The PBMC batches used in the panel were pretested by two labs at separate locations, in order to verify the uniformity of the PBMC vials. Each pretest lab performed Elispot assay according to "Instruction for the Elispot assay" on a total of 6 vials, using both the CMV peptide pool, the CEF peptide pool and the Negative control with no peptides. See Pretest of PBMC batches (Appendix 4).

## **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 samples, 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 AND PROFICIENCY TESTING RESULTS

The results obtained by the 36 participants of the Elispot Proficiency Panel 2017 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).

The median of the results for each PBMC/peptide pool combination for all the participants were calculated for each PBMC/peptide pool combination. The median of all the participants' results for a particular PBMC/peptide pool combination is shown in Figures 1B, 2B, 3B and 4B respectively and was used to calculate the Relative Accuracy of a given participant's result.

The relative accuracy was calculated as the result obtained by a given participant, divided by the median of all participant's results.

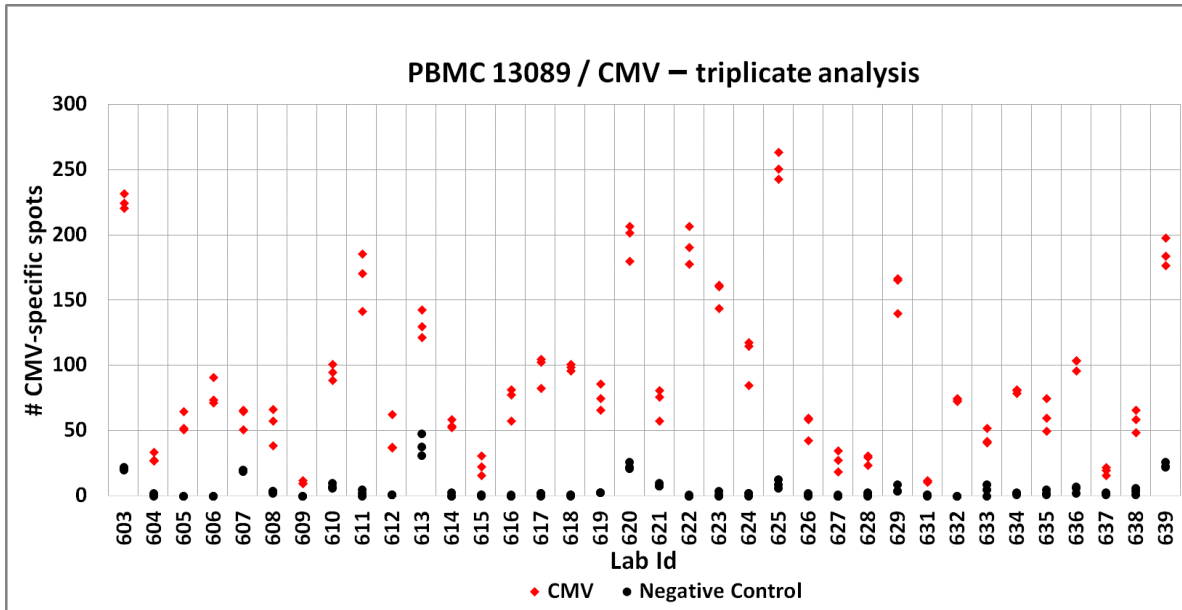
Figures 1C, 2C, 3C and 4C show the Relative Accuracy.

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.

Any result from 1,5 times lower to 1,5 times higher than the median, corresponding to a relative accuracy of 0,66-1,5 is considered "in the average range".

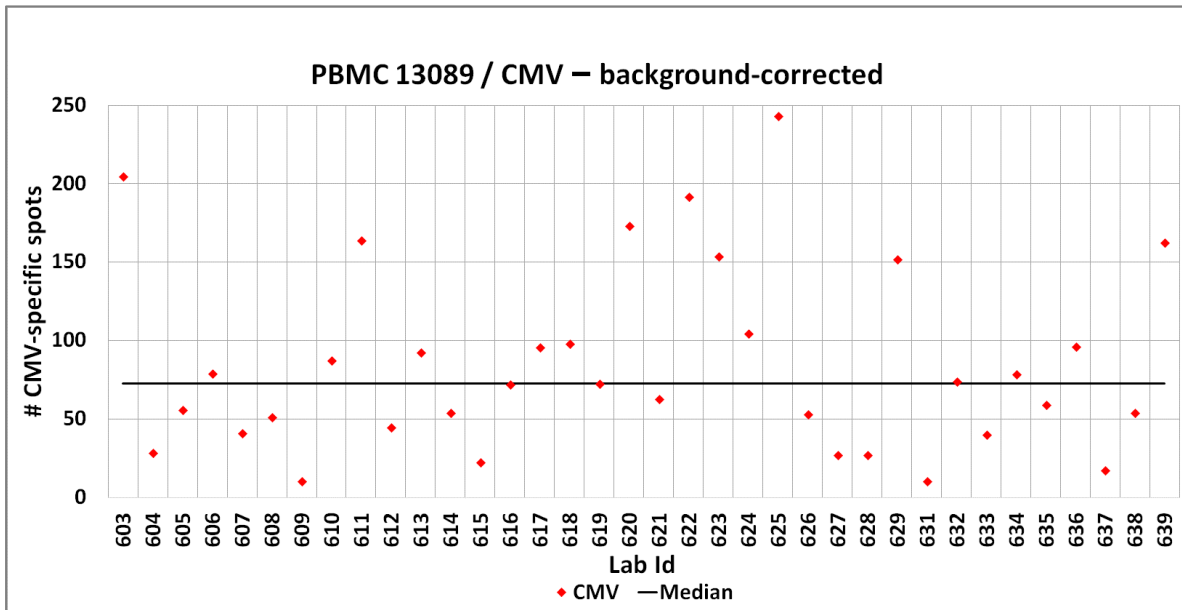
Any result from 1,6 to 2,0 times higher than the median, corresponding to a relative accuracy of 1,6-2,0, and any result from 0,50 to 0,65 times lower than the median, corresponding to a relative accuracy of 0,50-0,65, is considered "near the average range".

Any result below or above 2,0 times the median, corresponding to a relative accuracy of below 0,50 and above 2,0 is considered "far from average"



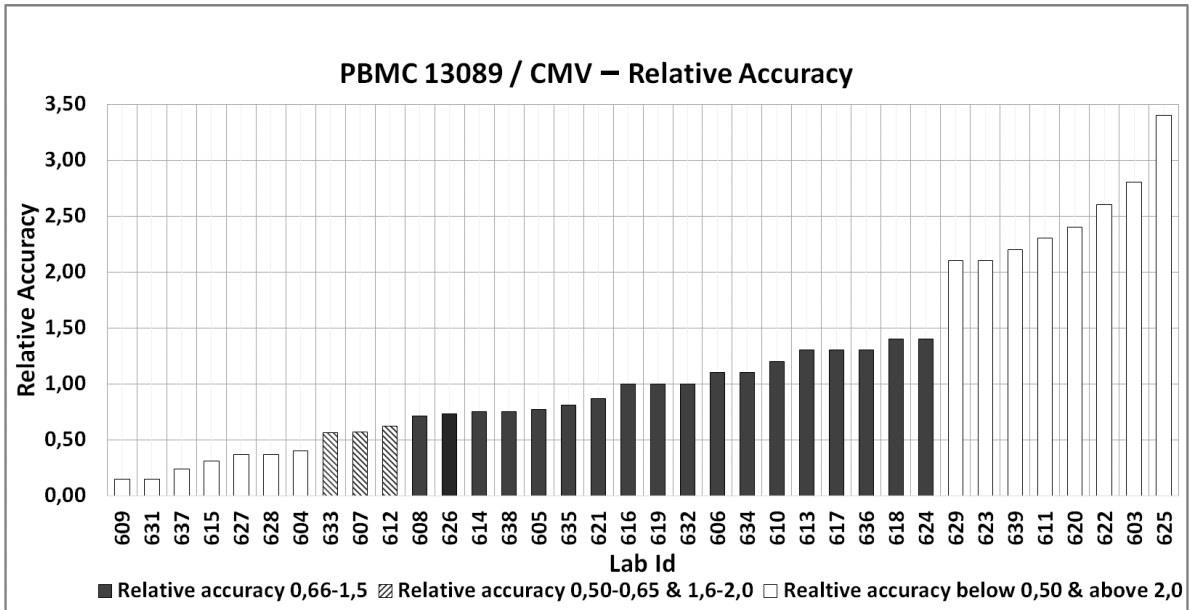
**Figure 1A. CMV-specific spots and background spots for PBMC 13089.**

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.



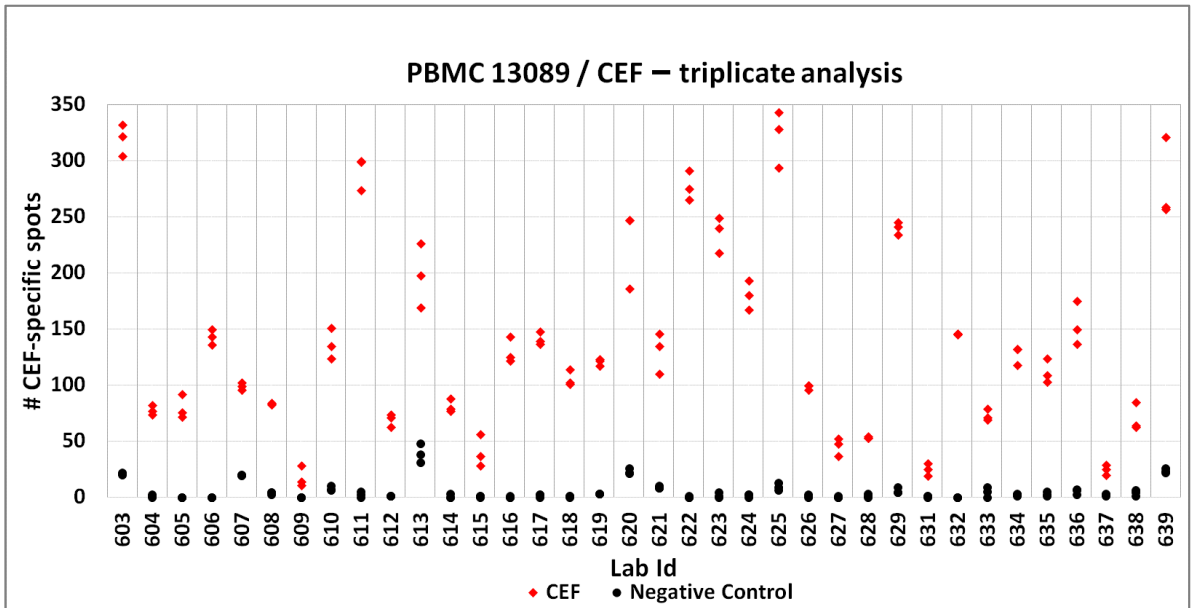
**Figure 1B. Background-corrected number of CMV-specific spots for PBMC 13089.**

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 (73 CMV-specific spots) is indicated with a black horizontal line.



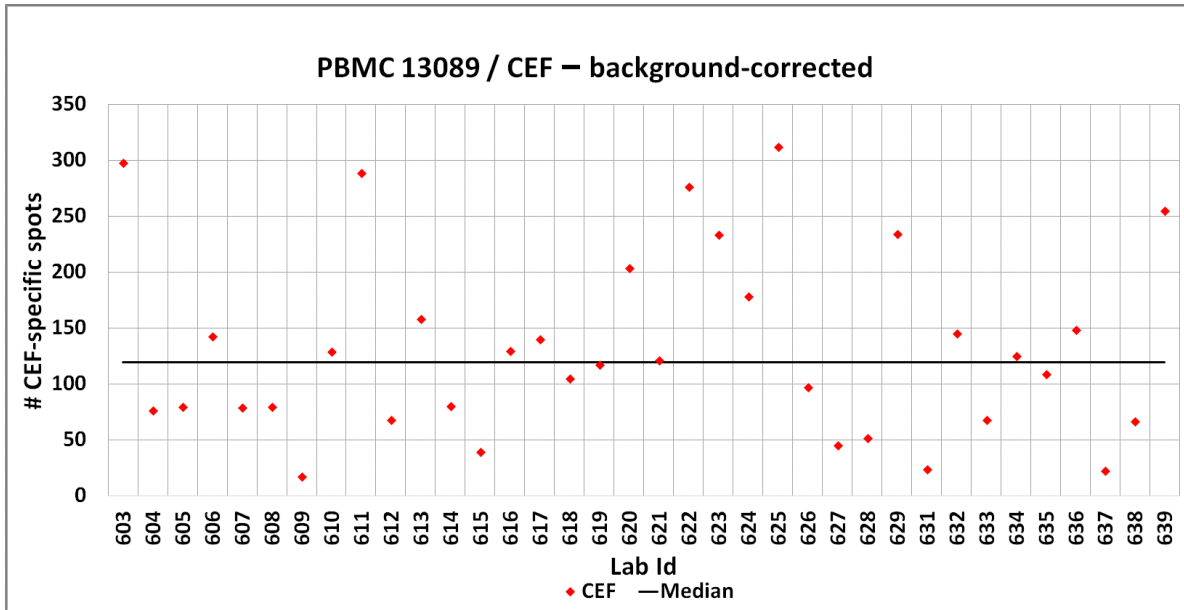
**Figure 1C. Relative Accuracy for the 13089/CMV combination.**

The Relative Accuracy, equaling the result divided by the median (73) of all results, for background-corrected, CMV-specific spots is shown. 18 out of 36 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".



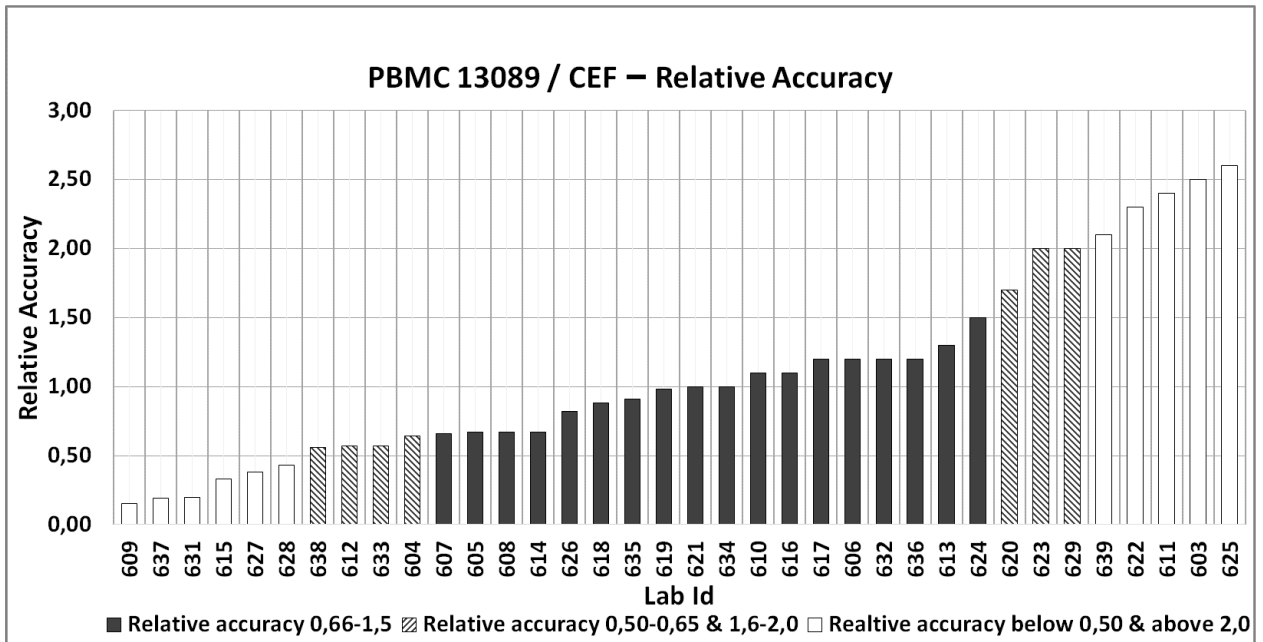
**Figure 2A. CEF-specific spots and background spots for PBMC 13089.**

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.



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

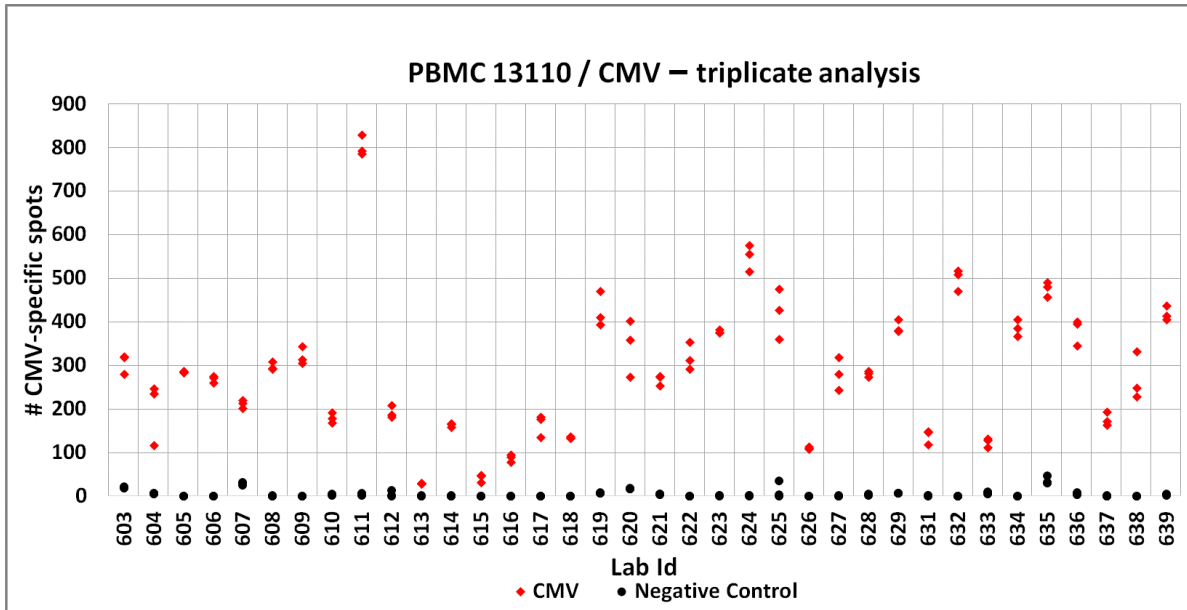
The number of background-corrected, CEF-specific spots per 200.000 PBMCs, based on the triplicate analysis, is shown (red diamond). The mean number of spots per 200.000 PBMCs for the Negative control was subtracted from the mean number of spots obtained with the CEF peptide pool, to give the background-corrected value. The median (120 CEF-specific spots) is indicated with a black horizontal line.



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

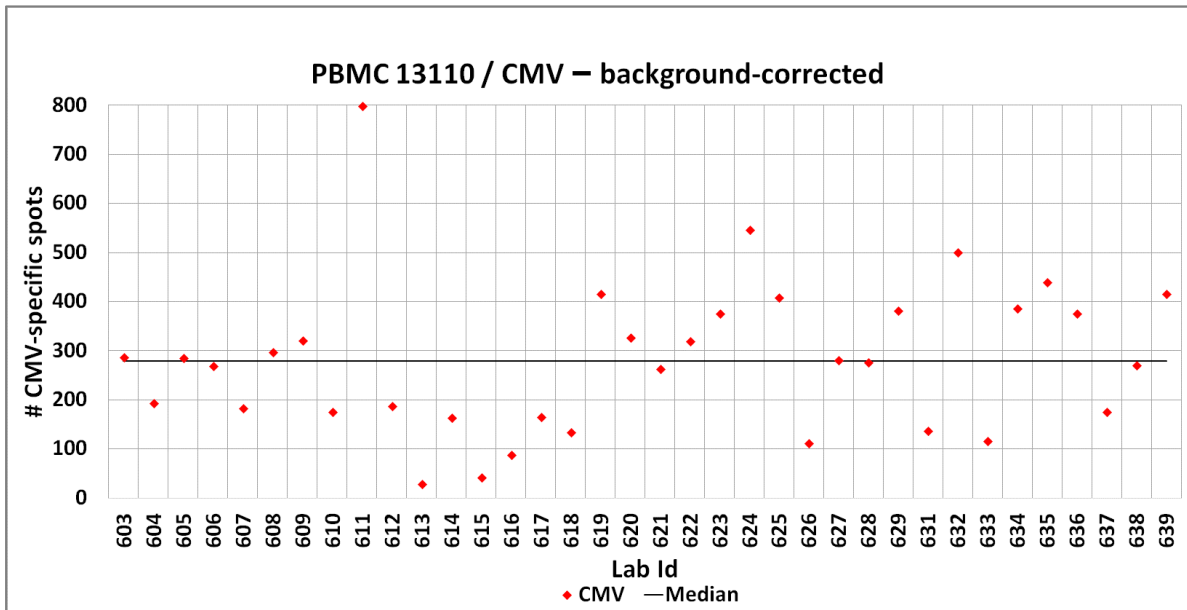
The Relative Accuracy, equaling the result divided by the median (120) of all results, for background-corrected CEF-specific spots is shown. 18 out of 36 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".





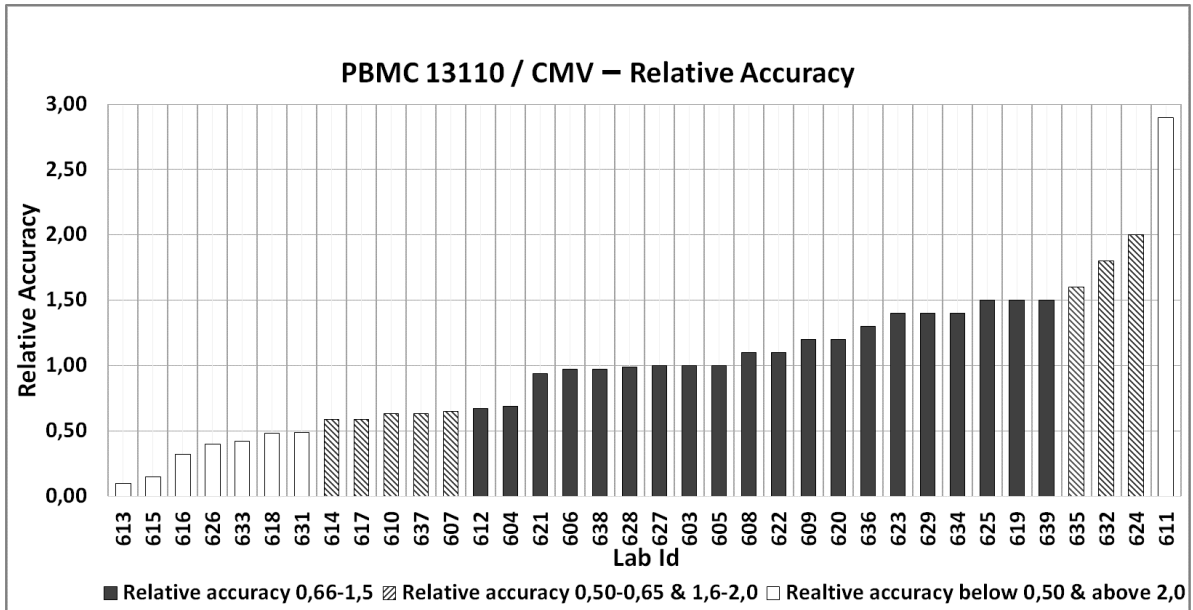
**Figure 3A. CMV-specific spots for PBMC 13110.**

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.



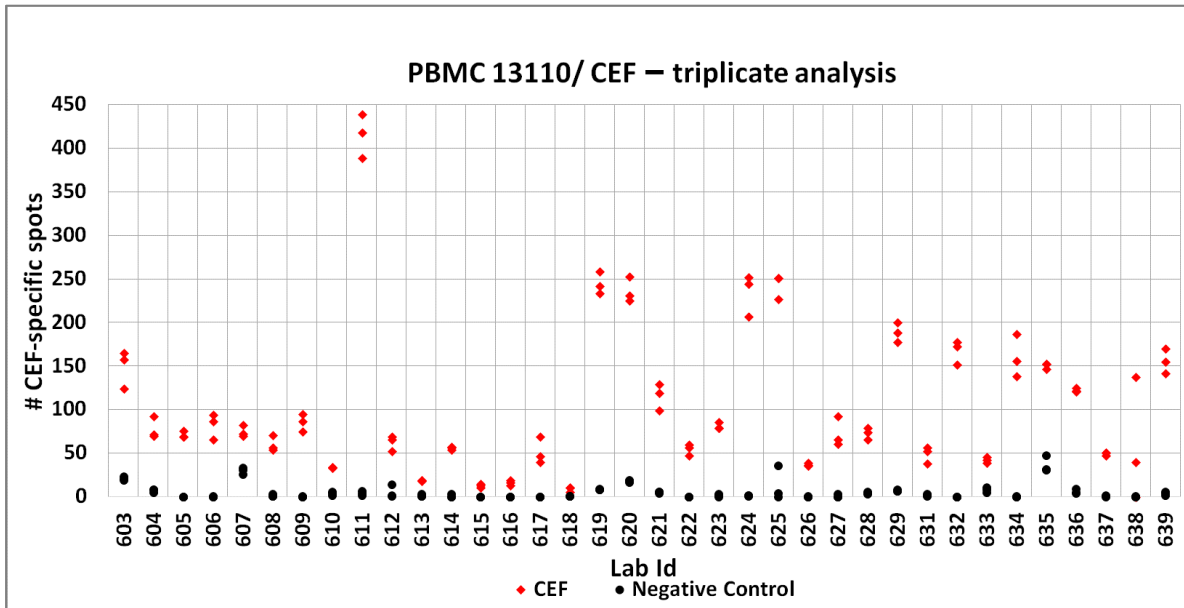
**Figure 3B. Background-corrected number of CMV-specific spots for PBMC 13110.**

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 (279 CMV-specific spots) is indicated with a black horizontal line.



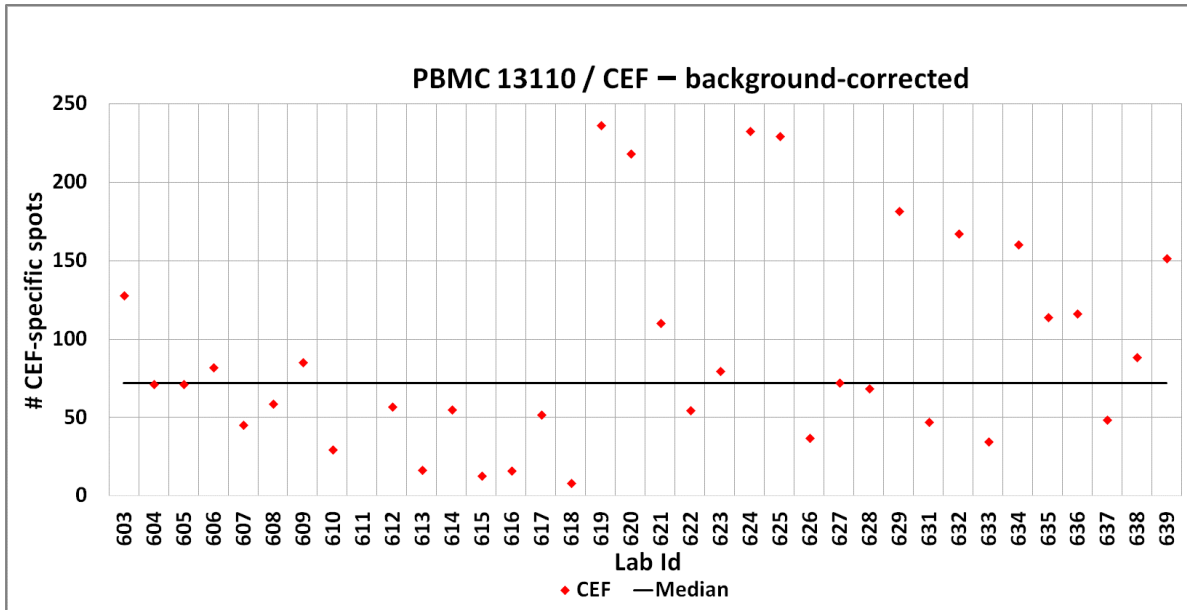
**Figure 3C. Relative Accuracy for the 13110/CMV combination.**

The Relative Accuracy, equaling the result divided by the median (279) of all results, for background-corrected, CMV-specific spots is shown. 20 out of 36 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".



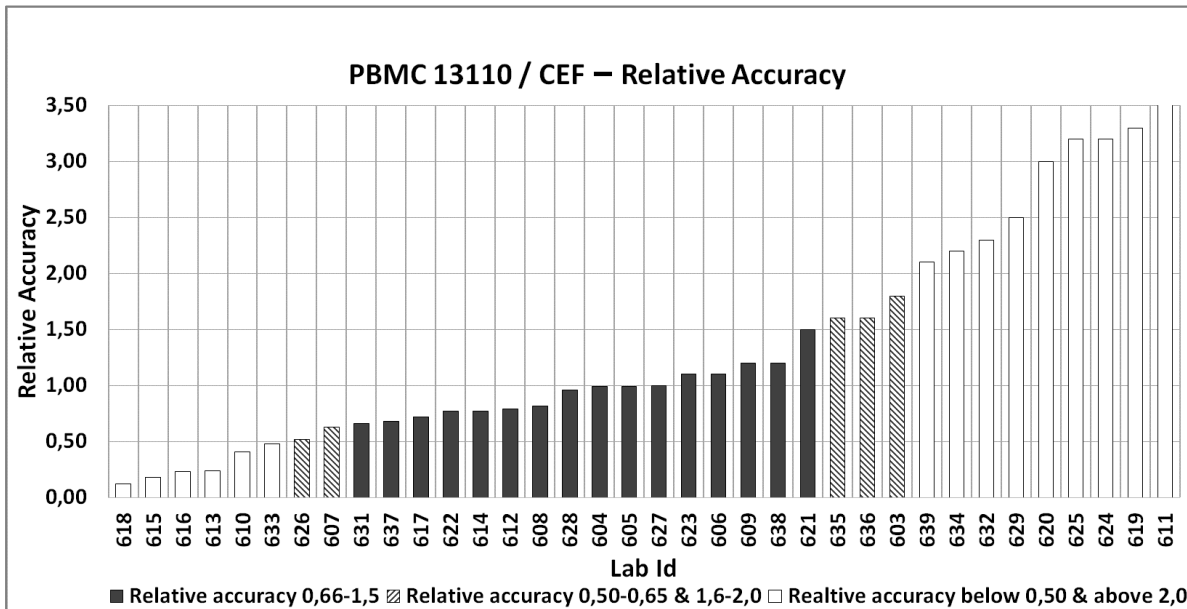
**Figure 4A. CEF-specific spots and background spots for PBMC 13110.**

The number of spots per 200.000 PBMCs for PBMC 13110/CEF (triplicate analysis; 3 red diamonds) or PBMC 13110/Negative control (triplicate analysis; 3 black dots) is shown. Lab Id 638 reported only duplicates.



**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 (72 CEF-specific spots) is indicated with a black horizontal line. Lab Id 611 reported value for CEF of 411. Lab Id 638 reported only duplicates.



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

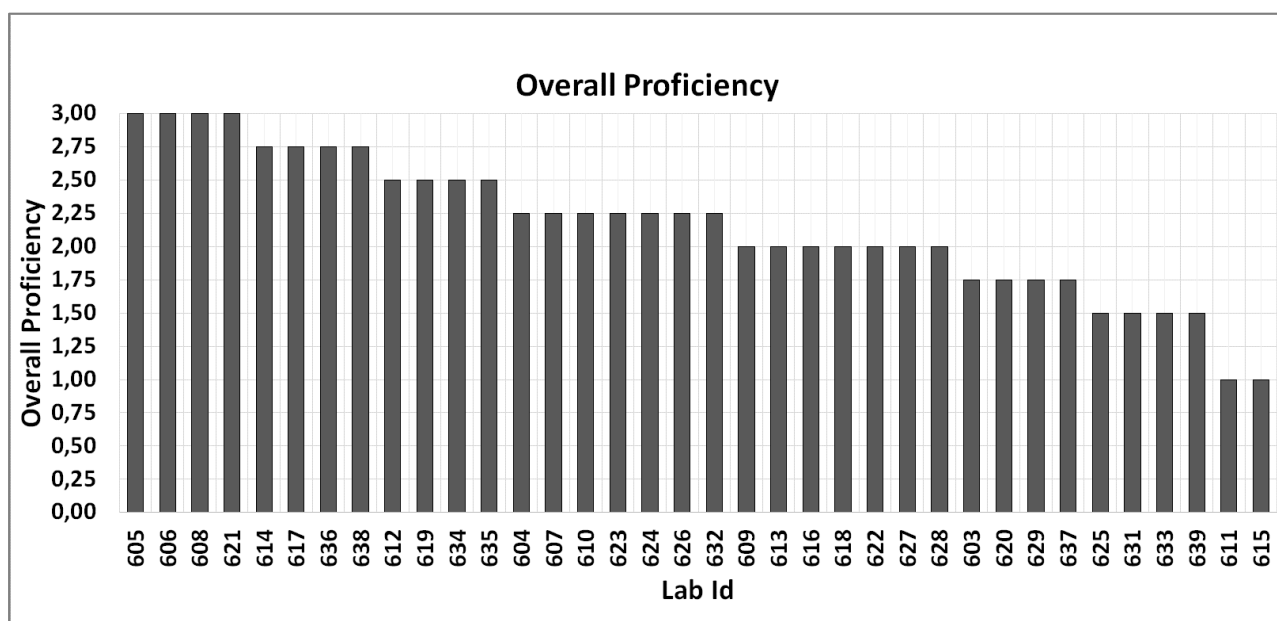
The Relative Accuracy, equaling the result divided by the median (72) of all results, for background-corrected CEF-specific spots is shown. Relative Accuracy for Lab Id 611 is 5,7. 16 out of 36 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range".

## 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.



**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.

## **ACKNOWLEDGEMENTS**

We thank Mabtech and ImmuneCarta for quality control and Elispot assay testing of cell samples, JPT Peptide Technologies for providing peptides, Cryoport for sponsoring shipping expenses at a reduced price and Sylvia Janetzki and Marij Schoenmaekers-Welters 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 T cell Elispot proficiency panel services worldwide.

Immudex's MHC Dextramer® products enable an easy and reliable identification of antigen-specific T cells (both CD4+ and CD8+) and are used in life science research, including *in vitro* diagnostics and the development of immunotherapeutics and vaccines. Immudex's extensive knowledge in detecting antigen-specific T cells has led to the development of more than 2000 different MHC Dextramer® specificities, allowing identification of antigen-specific T cells in multiple cancer types and in a range of infectious diseases.

Immudex's first *in vitro* diagnostics (IVD) product for monitoring CMV-specific T cell immunity in transplant patients is now available. The IVD product is CE marked in Europe and cleared by the FDA in US.

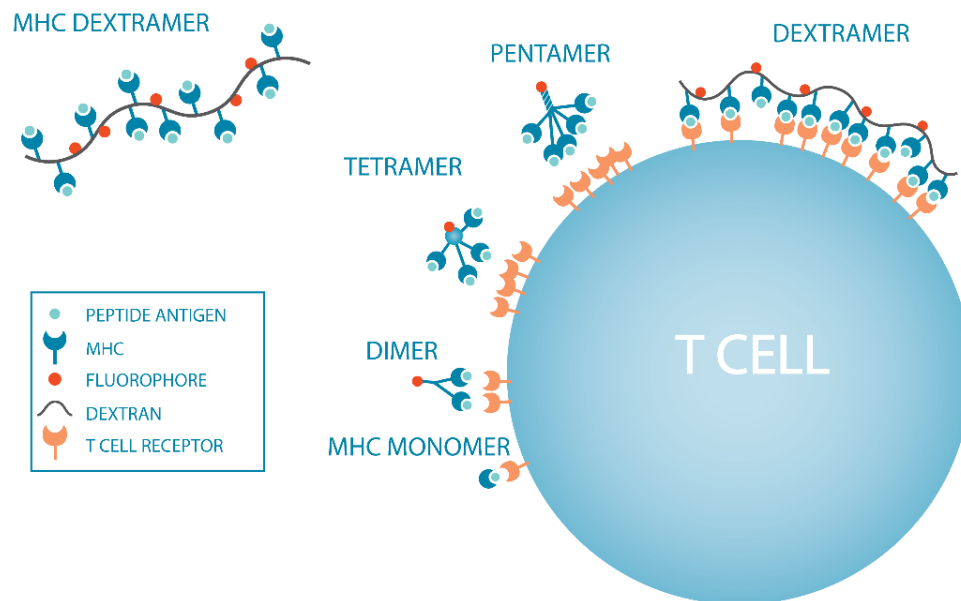


Figure 1 Schematic drawing of the MHC Dextramer® and the conventional MHC multimers binding to T-cell receptors (TCRs) on the surface of a T cell. MHC Dextramer® reagents are fluorescently labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a strong and stable interaction between the MHC Dextramer® reagents and the T cell, enabling detection of antigen-specific T cells with low affinity for the MHC-peptide complex.

**APPENDIX 1: INSTRUCTIONS**  
**FOR THE ELISPOT PROFICIENCY PANEL 2017**

**General introduction to the Elispot proficiency panel:**

All participants will receive two pre-tested Human Peripheral Blood Mononuclear Cell 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:**

Store PBMC vials and Reagent vials in vapor phase liquid nitrogen upon arrival and until use.

PBMC 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 1,5mL.

Reagents:

Each participant will receive three vials; Reagent-1 (HCMVA peptide pool), Reagent-2 (CEF peptide pool), and Reagent-3 (Negative control PBS/DMSO).

PBMC samples and Reagents are shipped in a liquid nitrogen container. Instructions for unloading 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:**

**Please use your currently established SOP (protocol) for Elispot analysis for this panel.**

**We recommend consideration of previously established Elispot harmonization guidelines, (please see Appendix A: "Assay Harmonization Guidelines").**

Use your own SOP for Direct Human IFN $\gamma$  Elispot Assay, including antibodies, plates, enzyme, substrate, equipment, medium and other miscellaneous chemicals and tools to perform the assay.

Please follow the instructions below.

**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  $3 \times 8 = 24$  wells in total (see plate setup next page).
2. Thaw both vials of PBMC. Count and record total cell number and the number of viable cells in each vial, 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 approximately 100µl and must prior to use be diluted 1:10 with the medium you use for the assay.
4. Plate PBMC samples and Reagents exactly as outlined in the scheme next page as the data will be reported and analyzed in this format. Please use columns 3-5 for the assay.

Plate 200,000 viable 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 100µl/well medium only (no cells or Reagent) to A3-5 and H3-5). This will enable assessment of false positive spots.

	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. 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 or have questions, please contact the organizer:

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

## 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		
603	22	20	21	221	232	225	304	332	322
604	1	0	2	27	34	28	82	77	74
605	0	0	0	52	65	51	76	72	92
606	0	0	0	74	91	72	150	143	136
607	20	20	19	51	65	66	99	102	96
608	4	2	4	67	58	39	83	84	83
609	0	0	0	12	10	10	28	14	11
610	6	10	7	101	89	95	135	151	124
611	0	5	2	171	186	142	274	299	300
612	1	1	1	63	38	37	71	63	74
613	38	48	31	143	130	122	169	226	198
614	3	0	0	54	53	59	88	77	79
615	1	0	1	23	16	31	56	28	37
616	0	1	0	58	78	82	143	125	122
617	2	0	2	103	83	105	139	148	137
618	0	1	1	101	99	96	114	102	101
619	3	3	3	66	75	86	123	117	122
620	21	26	22	202	207	180	247	186	247
621	8	10	8	76	58	81	110	146	135
622	0	0	1	178	191	207	275	291	265
623	0	1	4	144	161	162	218	249	240
624	2	0	2	85	115	118	167	180	193
625	6	9	13	264	243	251	328	294	343
626	0	1	2	60	59	43	96	100	100
627	0	0	1	19	28	35	48	52	37
628	1	3	0	24	30	31	53	53	54
629	4	9	4	166	140	167	234	245	241
631	1	0	1	11	12	11	25	19	30
632	0	0	0	75	74	73	146	145	146
633	5	9	0	52	41	42	79	71	69
634	1	3	2	81	79	82	132	118	132
635	5	1	2	75	50	60	109	124	103
636	2	6	7	96	104	104	150	175	137
637	3	2	1	16	22	20	25	20	29
638	1	4	6	59	49	66	64	63	85
639	22	23	26	184	177	198	259	321	257

**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		
603	19	21	23	319	322	282	165	158	124
604	8	8	5	118	248	236	72	93	70
605	0	0	0	288	287	284	69	69	76
606	0	1	0	261	277	273	66	94	87
607	26	33	31	203	214	221	70	83	73
608	3	1	1	293	310	295	57	71	54
609	1	0	0	314	307	344	87	75	95
610	2	6	4	179	193	169	33	34	34
611	7	2	4	830	793	787	389	418	439
612	14	2	1	187	210	183	69	66	53
613	1	1	3	31	29	31	19	19	18
614	0	0	3	168	160	166	54	57	58
615	0	0	0	48	32	49	15	13	11
616	0	0	0	96	91	79	17	19	13
617	0	0	0	183	178	136	47	69	40
618	1	1	1	138	135	135	6	11	11
619	8	9	8	411	471	394	242	259	234
620	17	18	19	359	403	274	231	225	253
621	6	4	6	255	275	276	119	99	129
622	0	0	0	313	354	293	57	60	48
623	0	2	3	376	383	376	79	79	86
624	2	1	2	556	516	576	252	245	207
625	36	4	0	476	361	428	251	227	251
626	0	1	0	115	110	111	36	37	39
627	0	3	0	282	244	319	66	93	61
628	6	3	4	274	288	283	74	79	66
629	7	8	7	406	380	382	178	189	200
631	1	3	2	149	119	148	53	38	57
632	0	0	0	519	510	472	152	178	173
633	11	8	5	113	133	129	46	39	43
634	0	0	1	368	406	387	187	139	156
635	48	31	32	458	482	491	153	153	147
636	5	4	9	397	401	347	125	121	122
637	1	0	2	173	195	164	51	51	48
638	0	1	0	250	333	229	40	138	ND
639	4	6	2	415	438	407	142	170	155

#### APPENDIX 4: PRETEST OF PBMC BATCHES

##### PBMC 13089 and 13110 / CMV pool / CEF pool / Negative Control

Each pretest lab performed Elispot assay according to "Instruction for the Elispot assay" on a total of 6 vials, using both the CMV peptide pool, the CEF peptide pool and the Negative control with no peptides. Results are shown below.

PBMC batch	Reagent	Pretest Lab 1 #spots/200.000 cells Mean value of 3 vials	Pretest Lab 2 #spots/200.000 cells Mean value of 3 vials	Pretest Lab 1+2 for all vials CV%
13089	CMV peptide pool	41	79	48%
	CEF peptide pool	72	126	39%
	Negative control	2	2	ND
13110	CMV peptide pool	375	231	36%
	CEF peptide pool	45	23	49%
	Negative control	2	2	ND
Viability of all 12 PBMC samples used for pretest were in the range of 88-96%				