T-CELL ELISPOT PROFICIENCY PANEL 2019

June 2019

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T-CELL ELISPOT PROFICIENCY PANEL 2019

This report presents results of the T-cell Elispot Proficiency Panel 2019, where 43 laboratories from 13 countries participated.

Individual test results for each participating laboratory is provided along with an overview of the overall performance.

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 geographic location or field of interest.

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 researchers, clinical developers and clinicians that perform immune monitoring assays. It is the hope and expectation that better immune monitoring assays will lead to better and more efficient immunotherapies.

T-cell Elispot Proficiency Panel is an annual event, and Immudex plan to run the next panel in spring 2020.

Contents

ELISPOT PROFICIENCY PANEL 2019

- 43 laboratories from 13 countries participated.
- Each participant was assigned a confidential Identification Number (Lab Id).
- Each participant received two vials of PBMCs (batch 2010113367 and 2010113384) and three vials of reagents:
  Reagent 1 (PepMixTM HCMVA (pp65) >90%; JPT Product Code: PM-PP65-2)
  Reagent 2 (CEFX Ultra SuperStim Pool 90%; JPT Product Code: PM-CEFX-2)
  Reagent 3 (Negative control PBS/DMSO, no peptides)
- All vials were shipped in liquid nitrogen with a temperature logger to verify that the vials were kept cold during shipment.
- Each participant performed the Elispot assay according to their own protocols.
- All participants were provided with instructions (Appendix 1) and Harmonization Guidelines (Appendix 2).
- Before the Proficiency Panel was initiated, the PBMC batches (2010113367 and 2010113384) were pretested at an external laboratory according to the instructions (Appendix 1). Results from this pretest verified the quality of the PBMC batches and peptide pools (Appendix 5).
- 39 laboratories reported their results back.
- In this report results are shown using the European numeration.

ANALYSES

Each participant received detailed instructions on how to perform the T-cell Elispot proficiency test (Appendix 1).

The participants were asked to determine the number of antigen-specific T cells for each of the two PBMC samples (2010113367 and 2010113384) when analyzed using Reagent 1, Reagent 2 or Reagent 3.

The Elispot assay is carried out in 96-well plate and analyzed by reading the number of spots per well for the PBMC batch/reagent combinations listed in Table 1.

All measurements were done in triplicates.

Table 1 Overview of PBMC batch/Reagent combinations.

1st PBMC batch Reagent 1	1st PBMC batch Reagent 2	1st PBMC batch Reagent 3	1st PBMC batch Medium (no cells)
2nd PBMC batch Reagent 1	2nd PBMC batch Reagent 2	2nd PBMC batch Reagent 3	2nd PBMC batch Medium (no cells)

RESULTS

39 participants in this year T-Cell Elispot Proficiency Panel reported their data. The data is shown in Figures 1-9 on the following pages, and the raw data is presented in Appendices 3-4.

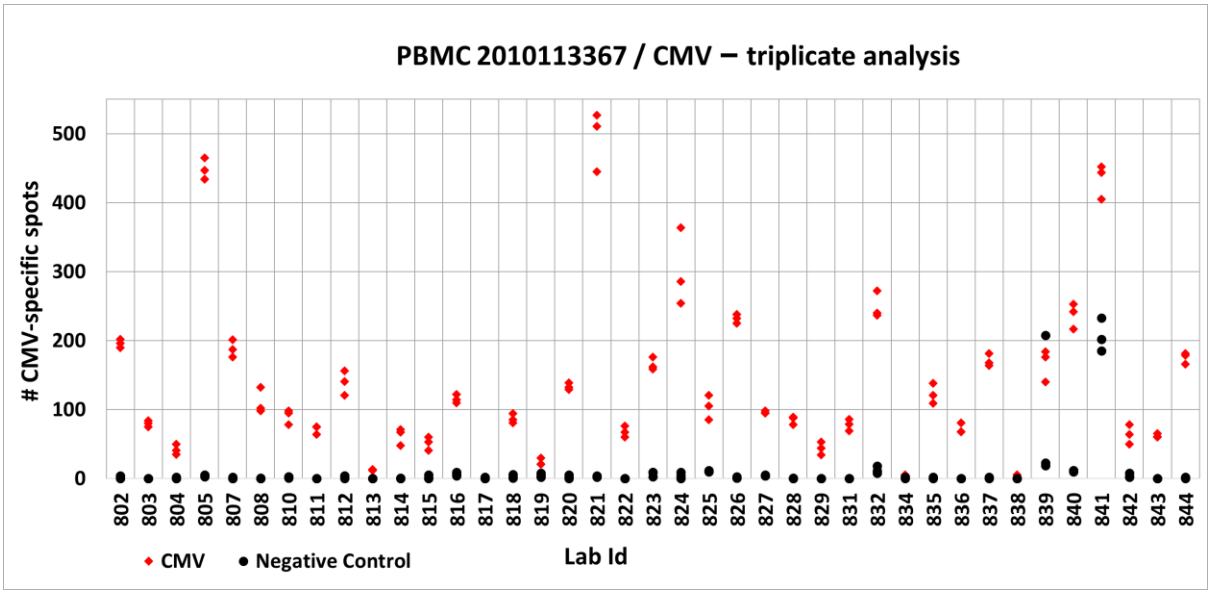

Figure 1A. Results from analysis of sample PBMC 2010113367 with Reagent 1 and Reagent 3. CMV-specific spots (red diamonds) and background spots (black dots) per 200.000 PBMCs (n=3).

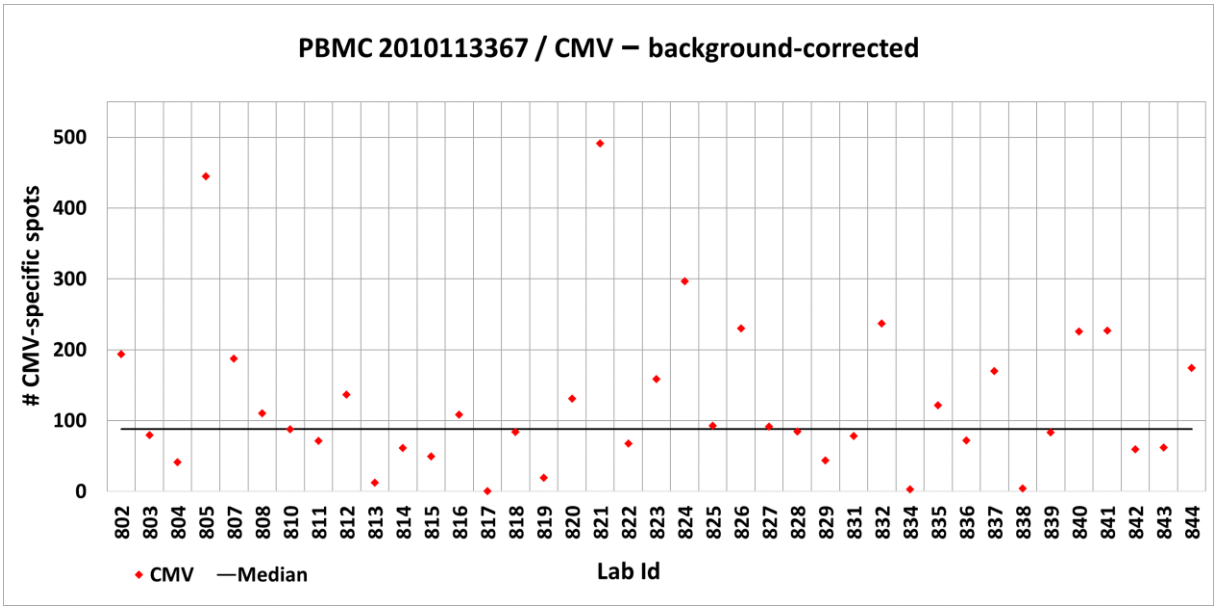

Figure 1B. Results from analysis of sample PBMC 2010113367 with Reagent 1 and Reagent 3. The mean of CMV-specific spots subtracted the mean of background spots is shown (red diamonds). The median of all results was 88 spots and indicated by the black line.

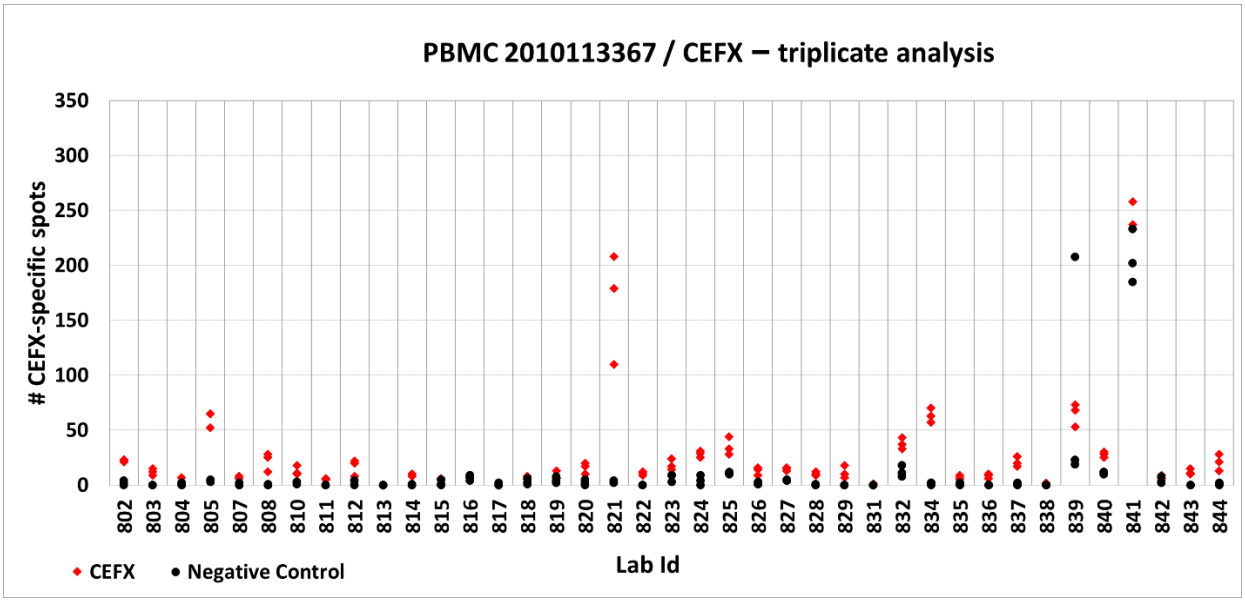

Figure 2A. Results from analysis of sample PBMC 2010113367 with Reagent 2 and Reagent 3. CEFX-specific spots (red diamonds) and background spots (black dots) per 200.000 PBMCs (n=3).

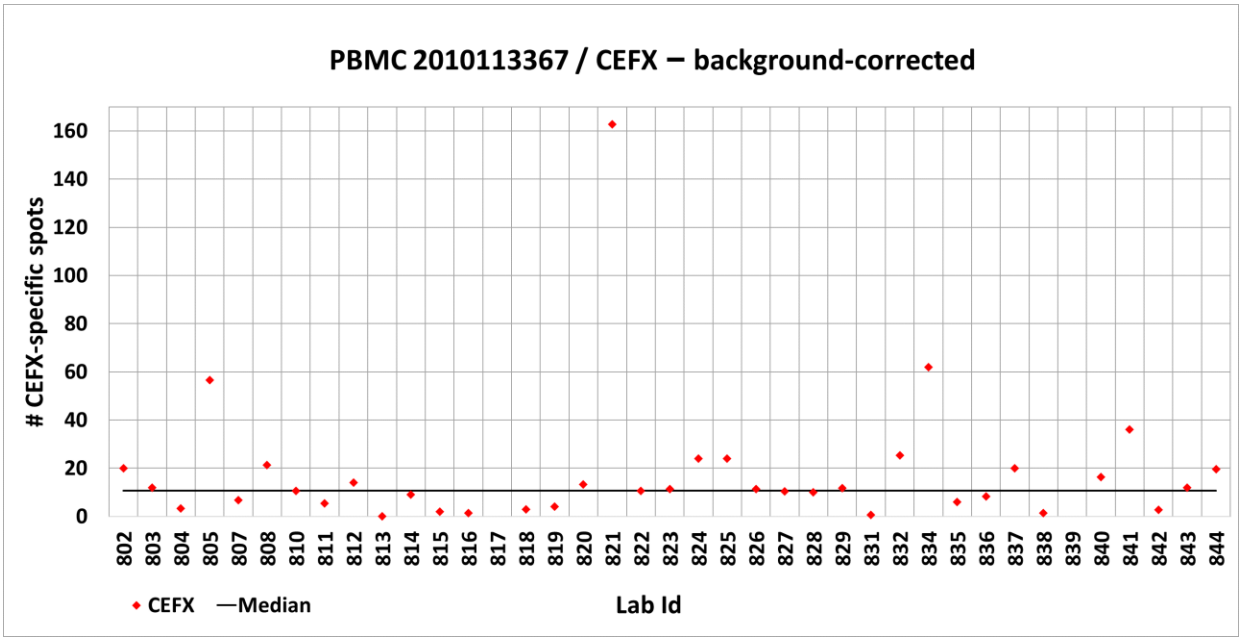

Figure 2B. Results from analysis of sample PBMC 2010113367 with Reagent 2 and Reagent 3. The mean of CEFX-specific spots subtracted the mean of background spots is shown (red diamonds). The median of all results was 11 spots and indicated by the black line.

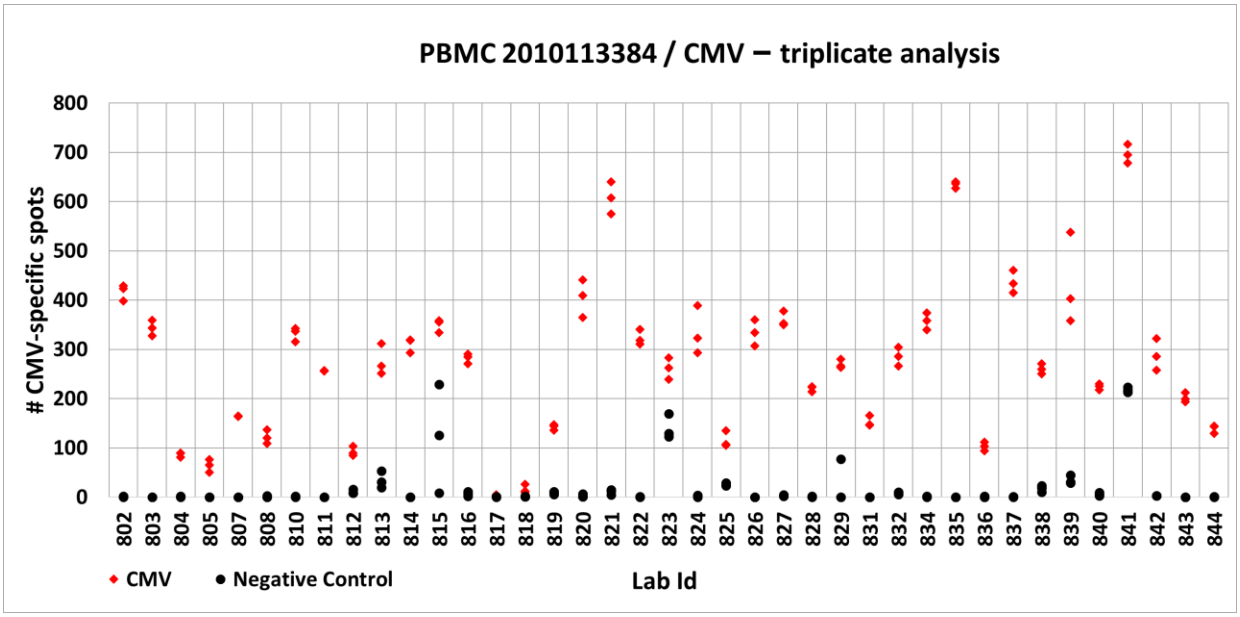

Figure 3A. Results from analysis of sample PBMC 2010113384 with Reagent 1 and Reagent 3. CMV-specific spots (red diamonds) and background spots (black dots) per 200.000 PBMCs (n=3).

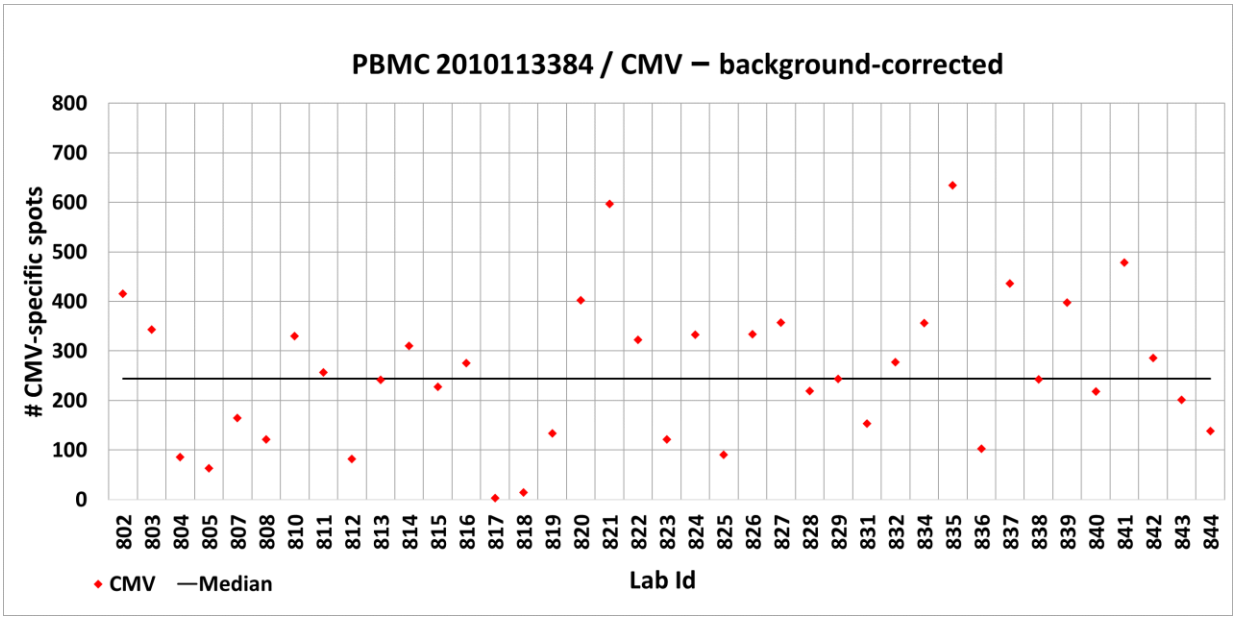

Figure 3B. Results from analysis of sample PBMC 2010113384 with Reagent 1 and Reagent 3. The mean of CMV-specific spots subtracted the mean of background spots is shown (red diamonds). The median of all results was 244 spots and indicated by the black line.

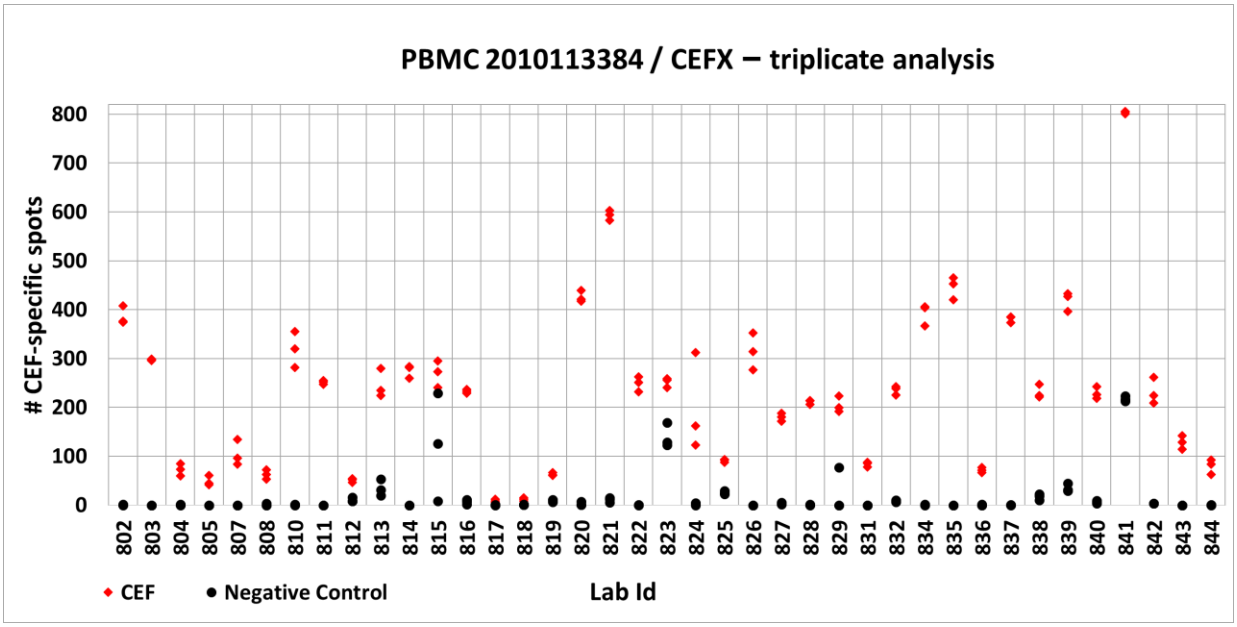

Figure 4A. Results from analysis of sample PBMC 2010113384 with Reagent 2 and Reagent 3. CEFX-specific spots (red diamonds) and background spots (black dots) per 200.000 PBMCs (n=3). Lab Id 841 CEFX-specific spots were 801, 805, 806.

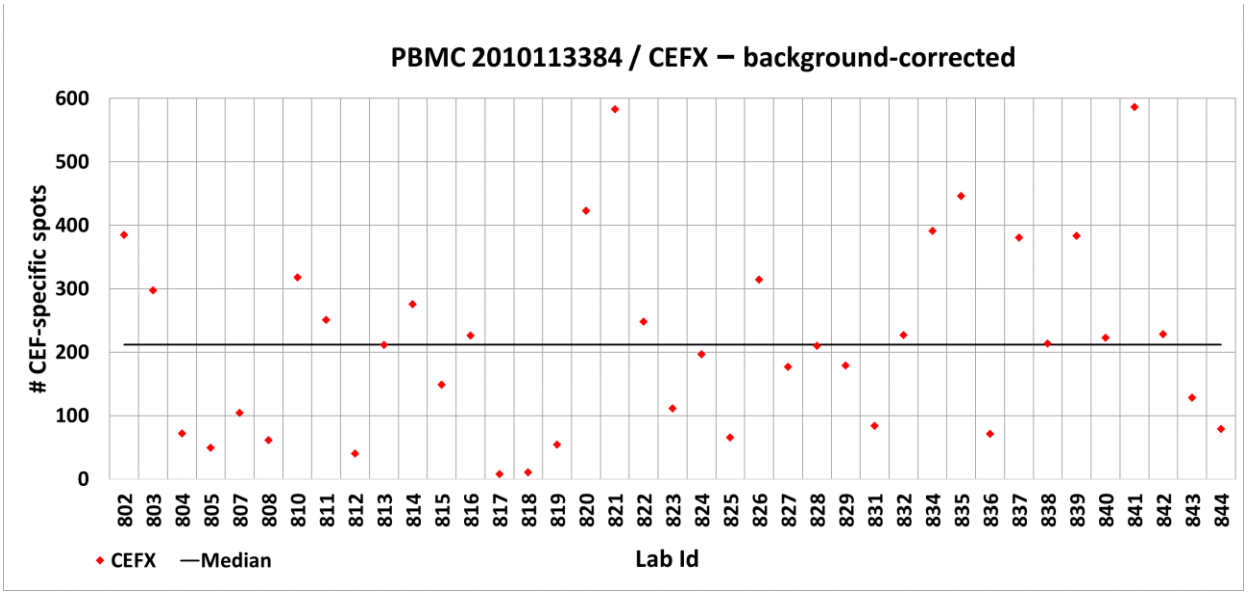

Figure 4B. Results from analysis of sample PBMC 2010113384 with Reagent 2 and Reagent 3. The mean of CEFX-specific spots subtracted the mean of background spots is shown (red diamonds). The median of all results was 212 spots and indicated by the black line.

PROFICIENCY PANEL TESTING RESULTS

In order to compare all the participants results a Relative Accuracy was calculated.

The Relative Accuracy was calculated by dividing the participant result with the median of all participant's results. The median represents the "average value" for that specific PBMC/peptide pool combination.

Any result from 1,5 time lower to 1,5 time higher than the median corresponds to a relative accuracy of 0,66 – 1,5 which is considered "in average range". Any results from 0,50 to 0,65 times lower than the median corresponds to a relative accuracy of 0,50 – 0,65, and any result from 1,6 to 2,0 times higher than the median corresponds to a relative accuracy of 1,6 – 2,0, and both intervals are considered "near the average range". Any result below or above 2,0 times the median corresponds to a relative accuracy below 0,50 and above 2,0 and is considered "far from average".

The Relative accuracy for each of the participants from the four different PBMC/peptide pool combinations is presented in Figure 5, 6, 7, and 8 on the following pages. The Lab Id is presented with increasing relative accuracy from left to right.

Table 2 Summary of the relative accuracies for measuring the overall performance.

Relative accuracy definition	Relative accuracies values	Corresponds to	Presented in the following plots as
1,5 times lower than median - 1,5 times higher than median	0,66 – 1,50	"the average range"	Black filled columns
0,50 – 0,65 times lower than median 1,6 – 2,0 times higher than median	0,50 – 0,65 1,60 – 2,00	"near the average"	Hatched columns
2,0 times higher the median 2,0 times lower the median	Below 0,50 Above 2,00	"far from the average"	Open columns

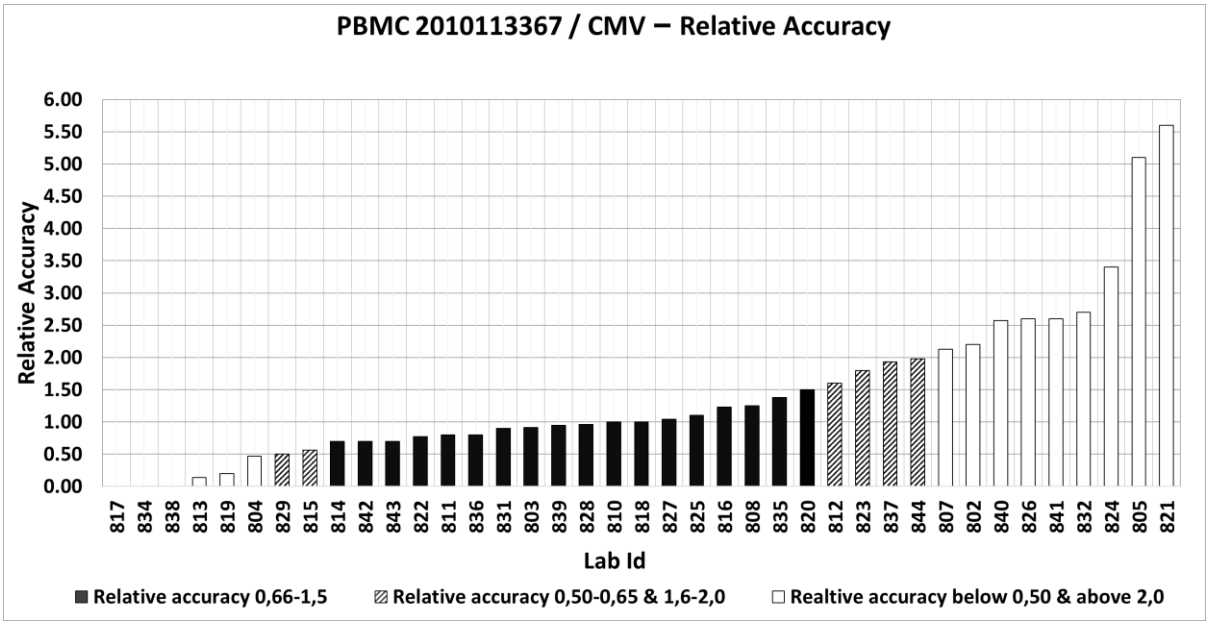

Figure 5. Relative Accuracy for analysis of PBMC 2010113367 with Reagent 1 (CMV). 18 of the 39 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range" (Black filled columns).

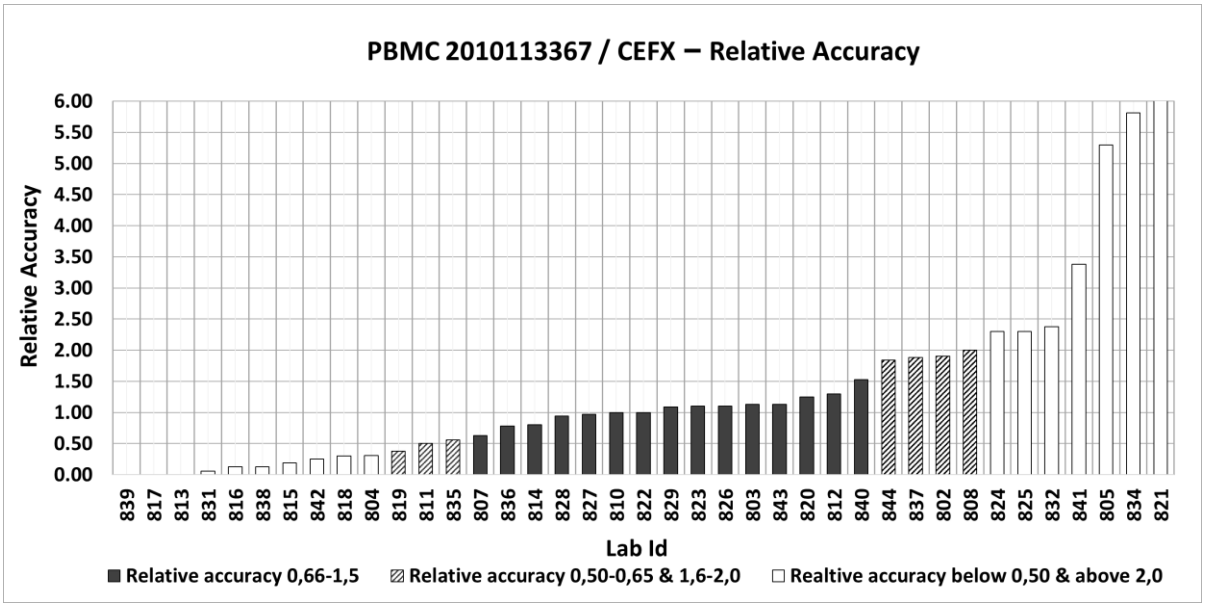

Figure 6. Relative Accuracy for analysis of PBMC 2010113367 with Reagent 2 (CEFX). 15 of the 39 participants had a Relative Accuracy that are considered "in the average range" ((Black filled columns). Lab Id 821 relative accuracy was 15.30.

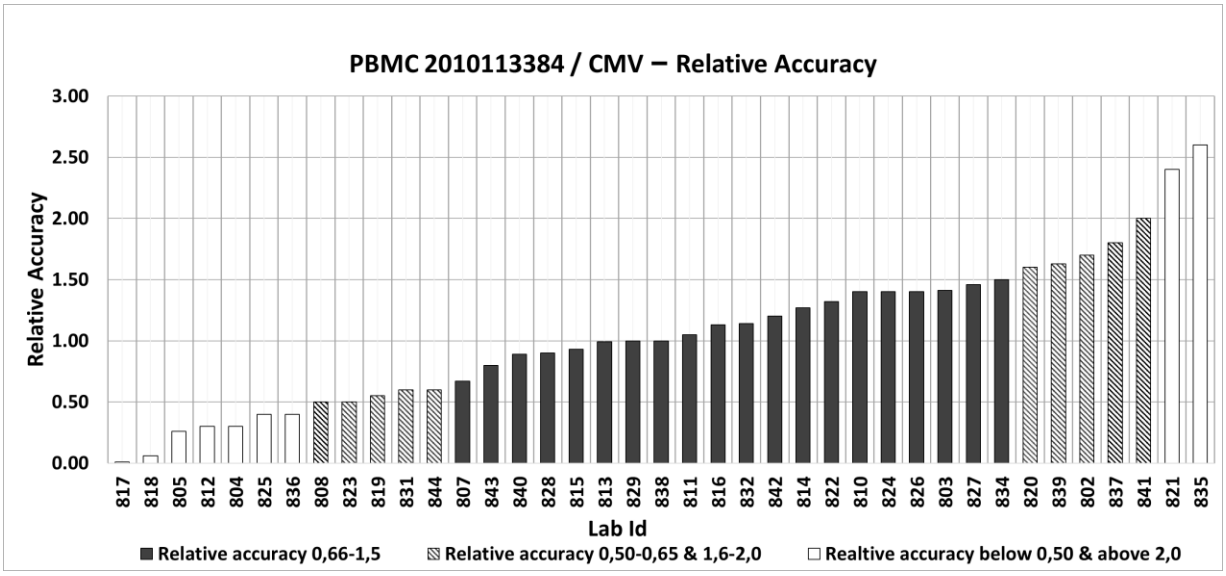

Figure 7. Relative Accuracy for analysis of PBMC 2010113384 with Reagent 1 (CMV). 20 of the 39 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range" (Black filled columns).

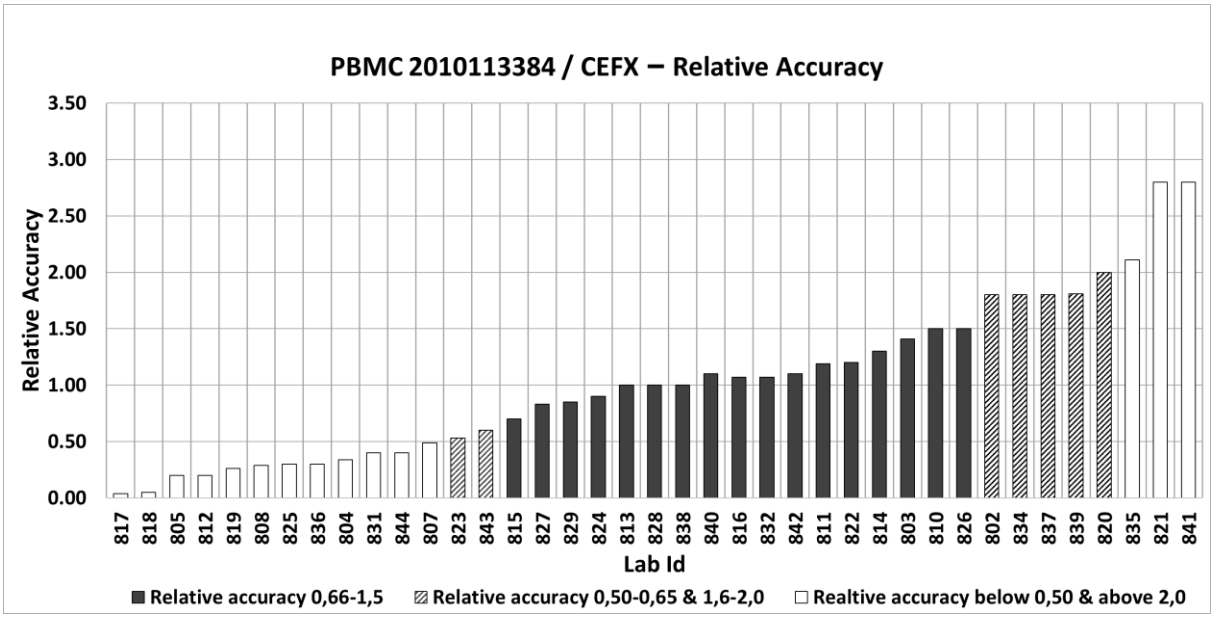

Figure 8. Relative Accuracy for analysis of PBMC 2010113384 with Reagent 2 (CEFX). 17 of the 39 participants had a Relative Accuracy between 0,66-1,5 and are therefore considered "in the average range" (Black filled columns).

OVERALL PROFICIENCY PERFORMANCE

To describe the Overall Proficiency of each of the participants in determining the number of antigen-specific T cells a score was assigned to each laboratory for each of the measurements performed.

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

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

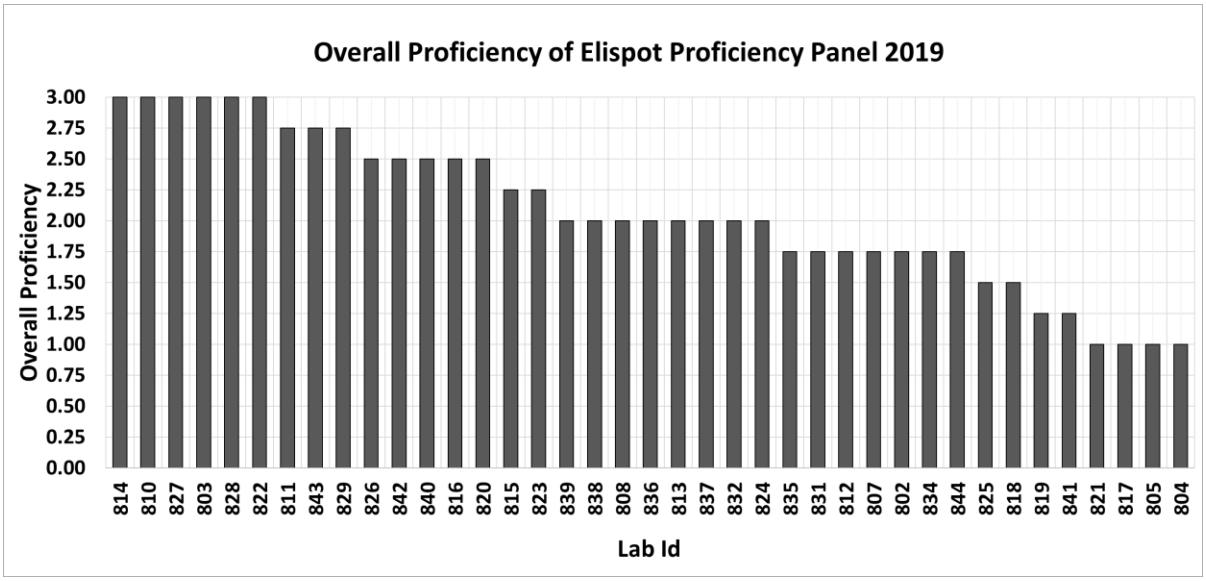

Figure 9. Overall Proficiency in Elispot Proficiency Panel 2019.

ACKNOWLEDGEMENTS

We thank Mabtech 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 for providing helpful advice.

ABOUT IMMUDEX

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex provides MHC Dextramer® for the monitoring of antigen-specific T cells. Under an agreement with the USA 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, *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, autoimmunity, transplantation and infectious diseases.

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

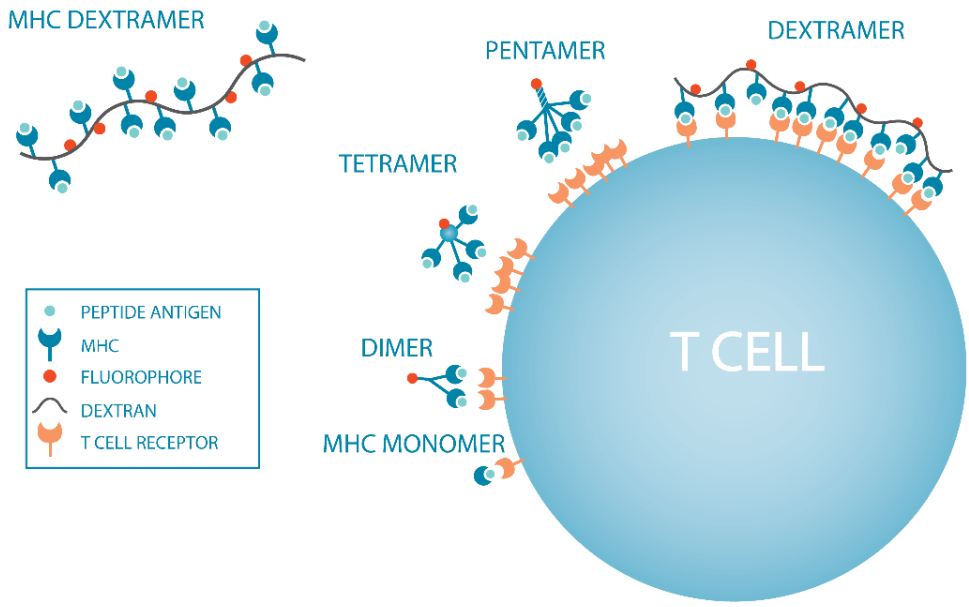

Figure 10 Schematic drawing of MHC Dextramer® and conventional MHC multimers binding to T-cell receptors (TCRs) on the surface of a T cell.
MHC Dextramer® reagents are fluorescent labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a strong and stable interaction between the MHC Dextramer® reagents and the T cell, enabling detection of antigen-specific T cells with even low affinity for the MHC-peptide complex.

Appendix 1: Instructions

All participants will receive two pre-tested Human Peripheral Blood Mononuclear Cell samples and three vials of reagents. All participants must use the Elispot assay to determine the spot count per well as a result of stimulation with PepMixTM HCMVA (pp65) >90%, CEFX Ultra SuperStim Pool 90% and negative control reagent for both PBMC samples.

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 comprising a different PBMC sample; lot 2010113367 and lot 2010113384, respectively. Each vial contains app.10 million cells in 1,5mL.

Reagents:
Each participant will receive three vials; Reagent-1 (PepMixTM HCMVA (pp65) >90%; JPT Product Code: PM-PP65-2), Reagent-2 (CEFX Ultra SuperStim Pool 90%; JPT Product Code: PM-CEFX-2), 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 is 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 IFNγ Elispot Assay, including antibodies, plates, enzyme, substrate, equipment, medium and other miscellaneous chemicals and tools to perform the assay.

Please follow the general 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 3x8 = 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 on next page as the data will be reported and analyzed in this format. Please use columns 3-5 for the assay.

5. Row B3-5, C3-5, D3-5, E3-5, F3-5, G3-5

- 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.

Row A3-5 and H3-5

- Add 100µl/well medium only (no cells or Reagent). This will enable assessment of false positive spots.

6. Perform the assay according to your own SOP.

Plate overview

	1-2	3	4	5	6-12
A		**No cells Medium**	**No cells Medium**	**No cells Medium**	
B		PBMC lot 2010113367 Reagent-1	PBMC lot 2010113367 Reagent-1	PBMC lot 2010113367 Reagent-1	
C		PBMC lot 2010113367 Reagent-2	PBMC lot 2010113367 Reagent-2	PBMC lot 2010113367 Reagent-2	
D		PBMC lot 2010113367 Reagent-3	PBMC lot 2010113367 Reagent-3	PBMC lot 2010113367 Reagent-3	
E		PBMC lot 2010113384 Reagent-1	PBMC lot 2010113384 Reagent-1	PBMC lot 2010113384 Reagent-1	
F		PBMC lot 2010113384 Reagent-2	PBMC lot 2010113384 Reagent-2	PBMC lot 2010113384 Reagent-2	
G		PBMC lot 2010113384 Reagent-3	PBMC lot 2010113384 Reagent-3	PBMC lot 2010113384 Reagent-3	
H		**No cells Medium**	**No cells Medium**	**No cells Medium**	

Reporting of data

Use this link » to record experimental details and data.

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

If you experience any problems or have questions, please contact the organizer:

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Coordinator of Proficiency Panels
mail: ProficiencyPanel@immudex.com
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Appendix 2: 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 3: Results from analysis of PBMC 2010113367

This table shows the triplicate values that the participants reported for analysis with the three reagents. The values represent the number of spots that was read for each sample.

Reagent 1 (CMV) / Reagent 2 (CEFX)/ Reagent 3 (Negative Control)

Lab Id	Well B3-5 CMV			Well C3-5 CEFX			Well D3-5 Negative Control		
802	202	190	196	23	21	22	2	0	4
803	84	75	80	12	9	15	0	0	0
804	41	50	35	7	3	2	2	0	0
805	465	434	447	65	65	52	4	5	3
807	187	176	201	8	8	6	2	0	0
808	102	98	132	25	12	28	0	1	0
810	98	78	95	10	11	18	1	3	3
811	75	75	64	5	5	6	0	0	0
812	121	156	141	22	8	20	4	4	0
813	13	13	12	0	0	0	0	0	0
814	71	48	67	8	10	10	0	1	0
815	41	53	60	5	1	6	1	5	0
816	122	114	110	8	9	7	9	4	7
817	0	1	2	0	0	0	0	0	2
818	94	85	81	8	5	5	1	2	6
819	30	21	21	6	7	13	2	8	4
820	139	129	132	20	10	17	5	0	2
821	511	527	445	179	208	110	4	3	2
822	60	76	67	11	12	9	0	0	0
823	159	162	176	14	24	17	9	3	9
824	286	364	254	31	29	25	4	9	0
825	85	121	105	33	44	28	10	11	12
826	238	232	225	9	16	14	3	1	1
827	95	95	98	16	13	15	5	4	4
828	78	89	88	9	12	10	0	0	1
829	34	44	53	10	18	7	0	0	0
831	69	79	86	0	1	1	0	0	0
832	272	240	237	43	33	37	18	11	8
834	5	3	5	63	57	70	2	2	0
835	121	138	109	5	9	7	1	2	0
836	68	81	68	10	6	9	0	0	0
837	164	168	181	17	20	26	1	2	0
838	5	5	2	2	1	1	0	0	0
839	184	176	140	73	53	68	23	19	208
840	217	253	242	30	25	28	12	12	10
841	444	405	452	237	258	233	185	202	233
842	50	64	78	9	6	6	2	3	8
843	60	65	61	11	15	10	0	0	0
844	179	166	181	28	13	21	0	2	1

Appendix 4: Results from analysis of PBMC 2010113384

This table shows the triplicate values that the participants reported for analysis with the three reagents. The values represent the number of spots that was read for each sample.

Reagent 1 (CMV) / Reagent 2 (CEFX)/ Reagent 3 (Negative Control)

Lab Id	Well E3-5 CMV			Well F3-5 CEFX			Well G3-5 Negative control		
802	429	423	398	376	408	374	0	1	2
803	343	328	359	298	299	296	0	0	0
804	81	89	89	60	73	85	0	2	1
805	50	76	65	42	45	61	0	0	0
807	165	164	164	84	96	134	0	0	0
808	109	120	137	72	53	63	0	0	3
810	342	337	315	282	355	320	1	2	0
811	256	257	257	255	252	247	0	0	0
812	89	103	85	52	54	46	8	16	8
813	312	266	251	224	280	235	31	53	20
814	318	293	319	284	282	260	0	0	0
815	334	355	358	273	241	295	8	126	229
816	285	271	290	233	237	229	11	7	2
817	0	5	5	12	5	8	0	1	1
818	26	12	9	12	15	8	1	1	2
819	147	136	144	67	62	61	11	10	6
820	441	409	365	417	421	439	1	1	7
821	640	575	608	603	594	583	12	5	15
822	318	311	341	251	232	263	1	0	1
823	283	262	239	256	259	241	169	129	123
824	293	389	323	162	312	123	4	0	3
825	105	135	107	93	92	88	29	23	24
826	307	360	334	277	352	314	0	0	0
827	350	378	353	188	180	172	5	2	3
828	214	224	223	214	206	214	1	2	0
829	263	266	280	199	192	223	77	0	0
831	146	147	166	78	88	86	0	0	0
832	304	286	266	242	239	225	8	10	6
834	374	340	358	367	404	406	1	2	0
835	636	627	640	453	420	465	0	0	0
836	94	103	112	67	71	77	2	0	0
837	415	461	434	373	385	385	1	1	0
838	260	271	250	224	247	221	23	19	10
839	538	403	358	433	427	396	31	45	29
840	225	230	218	226	219	242	9	3	6
841	678	695	716	801	805	806	223	213	217
842	258	286	322	224	209	262	3	3	3
843	194	199	212	114	129	142	0	0	0
844	144	129	143	92	84	63	0	1	1

Appendix 5: Pretest of PBMC batches

Elispot assay was preformed according to "Instruction for the Elipspot assay" using PBMC 2010113367 and PBMC 201011384. A total of 6 vials (3 vials from each PBMC batch) were pretested with all three reagents:

Reagent 1 (CMV)
Reagent 2 (CEFX)
Reagent 3 (Negative control)

Viability of all 6 PBMC samples used for pretest were in the range of 97-98%.

Results from this pretest are shown in Table 3, where the values represent the number of spots that was read for each sample.

The mean value of each PBMC batch test/Reagent was calculated and subtracted the mean of background spots (Reagent 3) and listed in Table 4.

To sum up, Table 5 shows the mean of the values listed in Table 4.

Table 3 Results from the pretest.

PBMC batch	Reagent 1			Reagent 2			Reagent 3		
2010113367 (1)	165	185	174	20	19	21	4	3	4
2010113367 (2)	95	120	126	16	ND	29	4	4	7
2010113367 (3)	132	112	110	10	20	6	2	3	2
2010113384 (1)	255	245	285	108	120	122	2	1	3
2010113384 (2)	251	231	206	98	72	105	5	7	8
2010113384 (3)	379	375	322	234	188	206	20	20	13

Table 4 Mean values of the pretest results from Table 3.

PBMC Batch	Reagent 1	Reagent 2
2010113367 (1)	171	16
2010113367 (2)	109	18
2010113367 (3)	116	10
2010113384 (1)	260	115
2010113384 (2)	223	85
2010113384 (3)	341	192

Table 5 Men values of the results from Table 4.

PBMC Batch	Reagent 1	Reagent 2
2010113367	132	15
2010113384	274	130