Immudex

T-CELL ELISPOT PROFICIENCY TESTING 2023 (Group 2)

October 2023

Version 3 of the report

immudex
PRECISION IMMUNE MONITORING

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1. INTRODUCTION TO PROFICIENCY PANELS

Originally developed at the initiative of CIC (the US Cancer Immuno-therapy Consortium of the CRI) and CIMT (the European Association for Cancer Immunotherapy), Immudex has since 2013 offered Proficiency Testing as a service to help researchers and clinicians worldwide evaluate and benchmark their immune monitoring performance with MHC Multimers and T-cell ELISpot assays. The proficiency Testing is open to any laboratory, independent of geographic location or field of interest. Read more about Proficiency Testing here.

Immudex Proficiency Testing is conducted yearly, and the next will take place in 2024.

1.1. T-CELL ELISPOT PROFICIENCY TESTING 2023

In the T-cell ELISpot Proficiency Testing 2023, participants tested their proficiency in detecting the number of IFN-γ secreting antigen-specific cells in two different PBMC samples in response to exposure to defined commercial peptide pools using ELISpot assays.

Each participant received two pre-tested PBMC samples and tested them according to the instructions but with their own protocol for direct human IFN-γ ELISpot Assay. The participants included their own choice of antibodies, plates, enzyme, substrate, equipment, medium, etc. The PBMC samples and reagents were pre-tested at Immudex according to instructions to ensure consistent results between vials and to check the viability of the cells. The viability of the tested PBMC samples was in the range of 64-75% after thawing and after one hour of rest.

This report shows the participants' test results and overall performances without revealing their names and affiliation.

In this Proficiency Test:

- 35 laboratories from 13 countries participated.
- 28 participants were from Academia, and 7 participants were from industry.

The participants were divided into two groups. They received the following PBMC samples for analysis:

Group 1: Lot 2010113384 & Lot 2010113367

Group 2: Lot 2010113384 & Lot 2010113745

2. ANALYSES

Each participant:

- Was assigned a confidential Laboratory Identification Number (Lab ID).
- Received instructions on how to perform the T-cell ELISpot proficiency test (Appendix 1).
- Received two pre-tested vials of PBMC samples (as described above).
- Received three vials of reagents:
    - Reagent 1 (JPT's PepMixTM HCMVA (pp65) >90%; PM-PP65-2.) Pool of 138 peptides derived from a peptide scan (15mers with 11 aa overlap) through 65 kDa phosphoprotein (pp65) (Swiss-Prot ID: P06725) of Human Cytomegalovirus (HCMV) - strain AD169
    - Reagent 2 (JPT's CEFX Ultra SuperStim Pool >90%;PM CEFX-2). Positive Control Pool of 176 known peptide epitopes for a broad range of HLA sub-types and different infectious agents: Clostridium tetani, Coxsackievirus B4, Haemophilus influenza, Helicobacter pylori, Human adenovirus 5, Human herpesvirus 1, Human herpesvirus 2, Human herpesvirus 3, Human herpesvirus 4, Human herpesvirus 5, Human herpesvirus 6, Human papillomavirus, Influenza A, JC polyomavirus, Measles virus, Rubella virus, Toxoplasma gondii, Vaccinia virus
    - Reagent 3 (Negative control: PBS/DMSO)
- Stimulated the two PBMC samples with Reagent 1, 2 and 3.
- Was encouraged to analyze samples with their own standard protocol to reflect routine sample analysis conducted in their laboratory.
- Was recommended to look at the "Assay Harmonization Guidelines" (Appendix 2).
- Reported their results back to Immudex after their analysis (Appendix 4 and Appendix 5).

The reported participant data was analyzed by Immudex. Raw data and calculated values from the data analysis are found in Appendix 2,3.

3. RESULTS

In this year's T-cell ELISpot Proficiency Testing, 35 participants reported their data.

The reported results from the participants are shown in Figures 1-2 and 5-6 on the following pages. All measurements were done in triplicates. Data analysis for Reagents 1 or 2 were corrected for background (Reagent 3) for each PBMC sample.

3.1. Results from Analysis of PBMC 2010113745

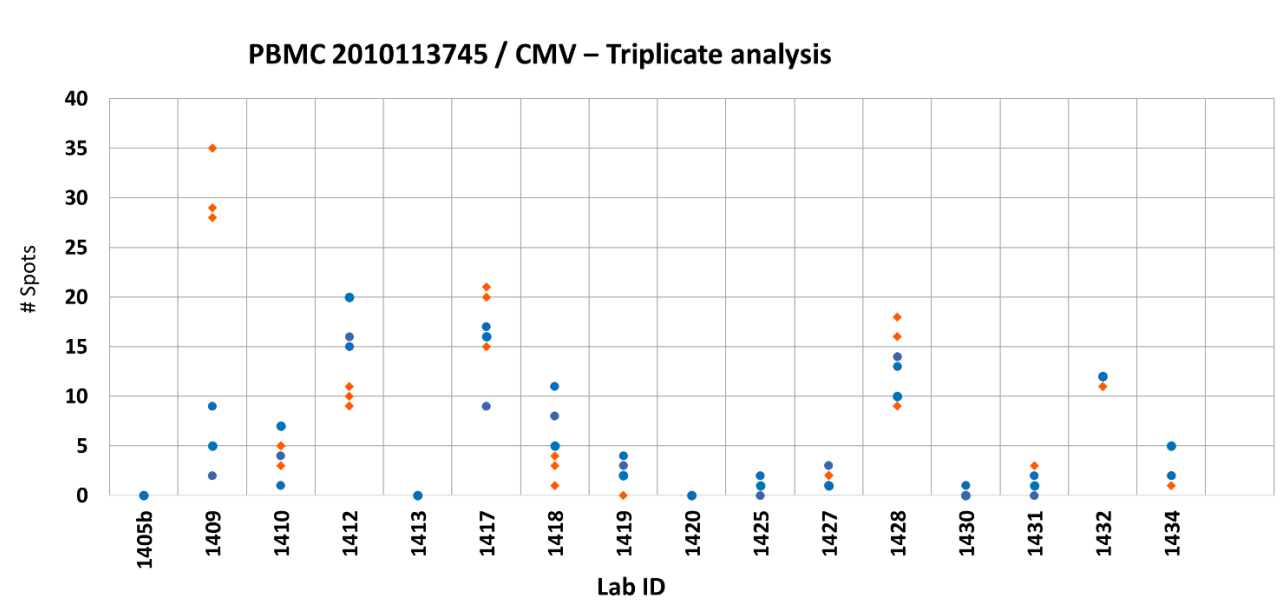

Figure 1A. Results from analysis of sample PBMC 2010113745 with Reagent 1 (CMV) and Reagent 3 (Negative control) (Analysis 1). Triplicate test values for CMV-specific spots (orange diamonds) and background spots (blue dots) per 200.000 PBMCs/well are shown.

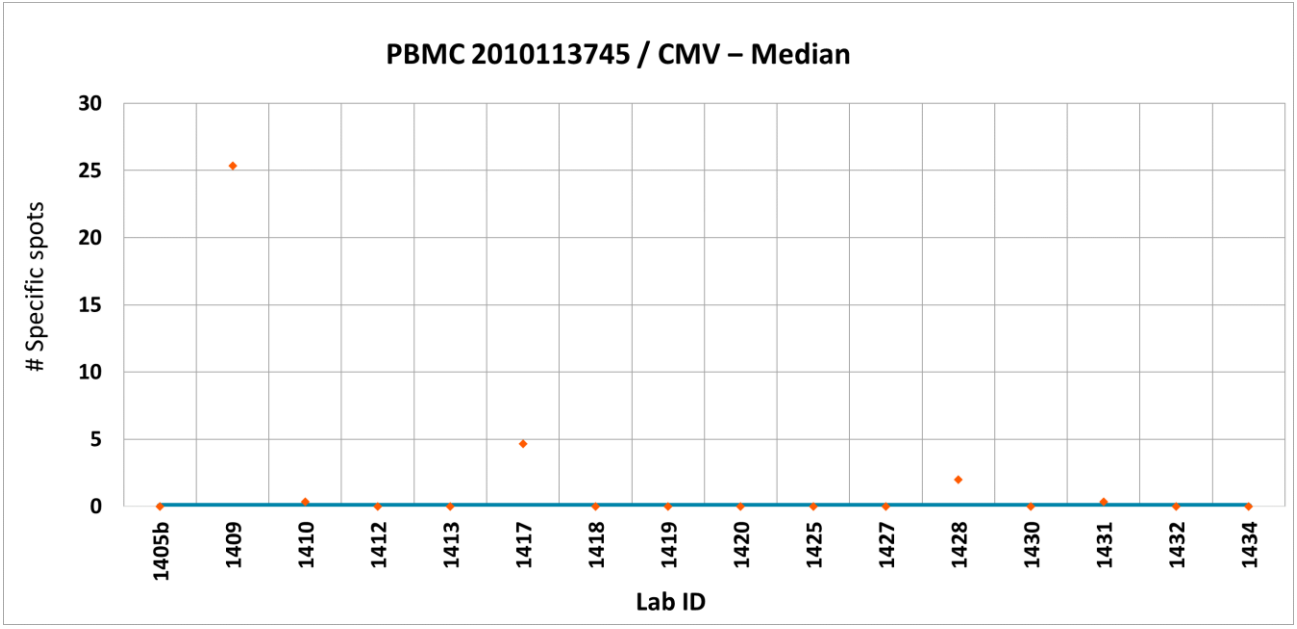

Figure 1B. Background corrected results from analysis of sample PBMC 2010113745 with Reagent 1 (CMV) and Reagent 3 (Negative control) (Analysis 1). The mean of CMV-specific spots subtracted the mean of background spots is shown (orange diamonds). Negative background subtracted values are set to 0. The median of all results is 0 spots/well indicated by the blue line.

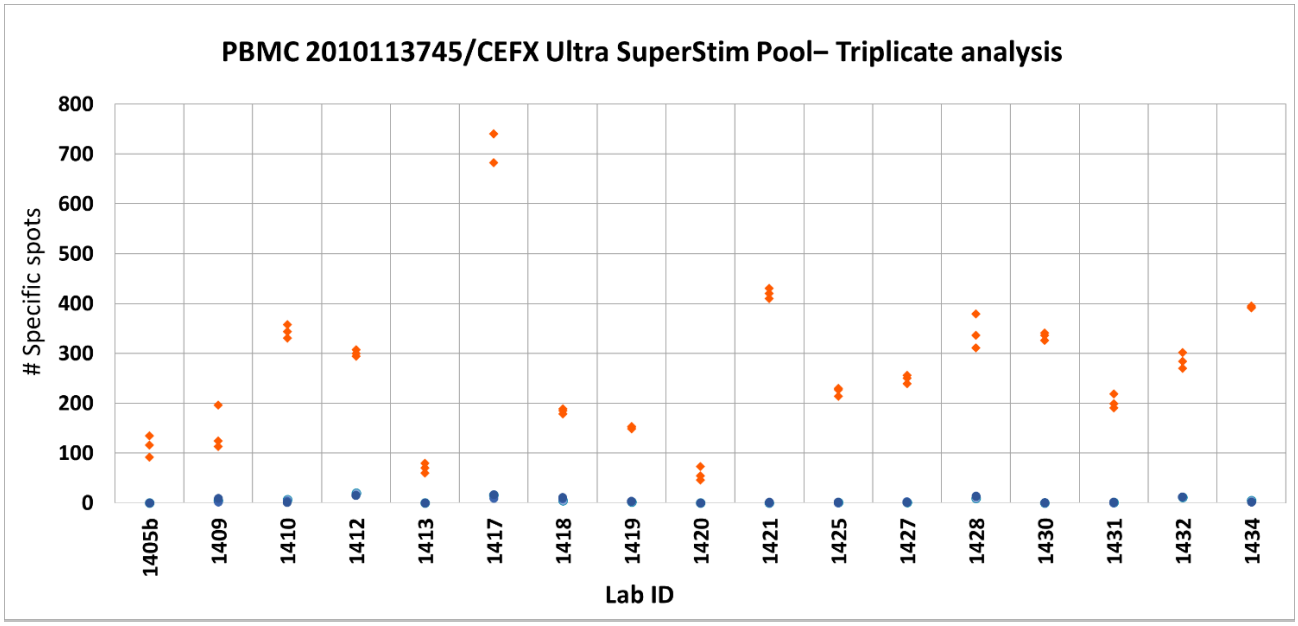

Figure 2A. Results from analysis of sample PBMC 2010113745 with Reagent 2 (CEFX) and Reagent 3 (Negative control) (Analysis 2). Triplicate test values for CEFX-specific spots (orange diamonds) and background spots (blue dots) per 200.000 PBMCs/well are shown.

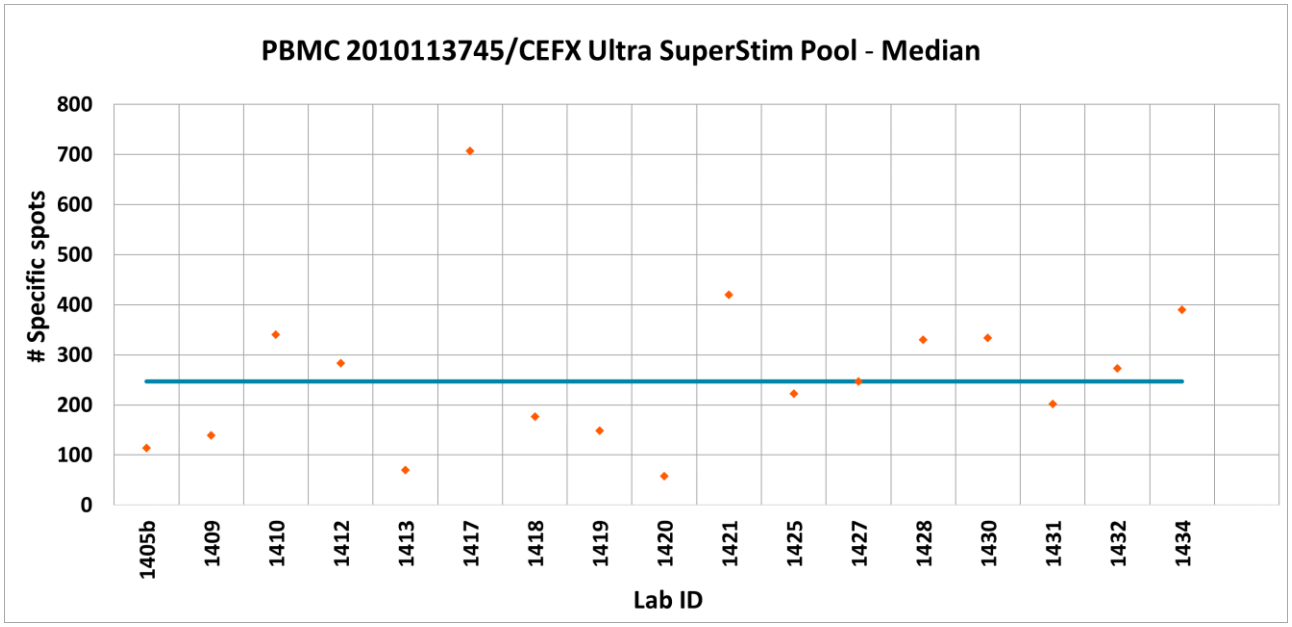

Figure 2B. Results from analysis of sample PBMC 2010113745 with Reagent 2 (CEFX) and Reagent 3 (Negative Control) (Analysis 2). The mean of CEFX-specific spots subtracted the mean of background spots is shown (orange diamonds). The median of all background-corrected test results is 247 spots/well and indicated by the blue line.

3.1.1. Evaluation of Test Results for PBMC 2010113745

3.1.1.1. PBMC 2010113745 stimulated with CMV

This data set is characterized by very few counted spots with a median of zero, but with a few significant outliers. To compare the performance of the participating laboratories we have chosen to base the evaluation on the mean average deviation (MAD) from the median, see Figure 3 & 4, and table 1 below and appendix 4 (analysis 1) to find calculations.

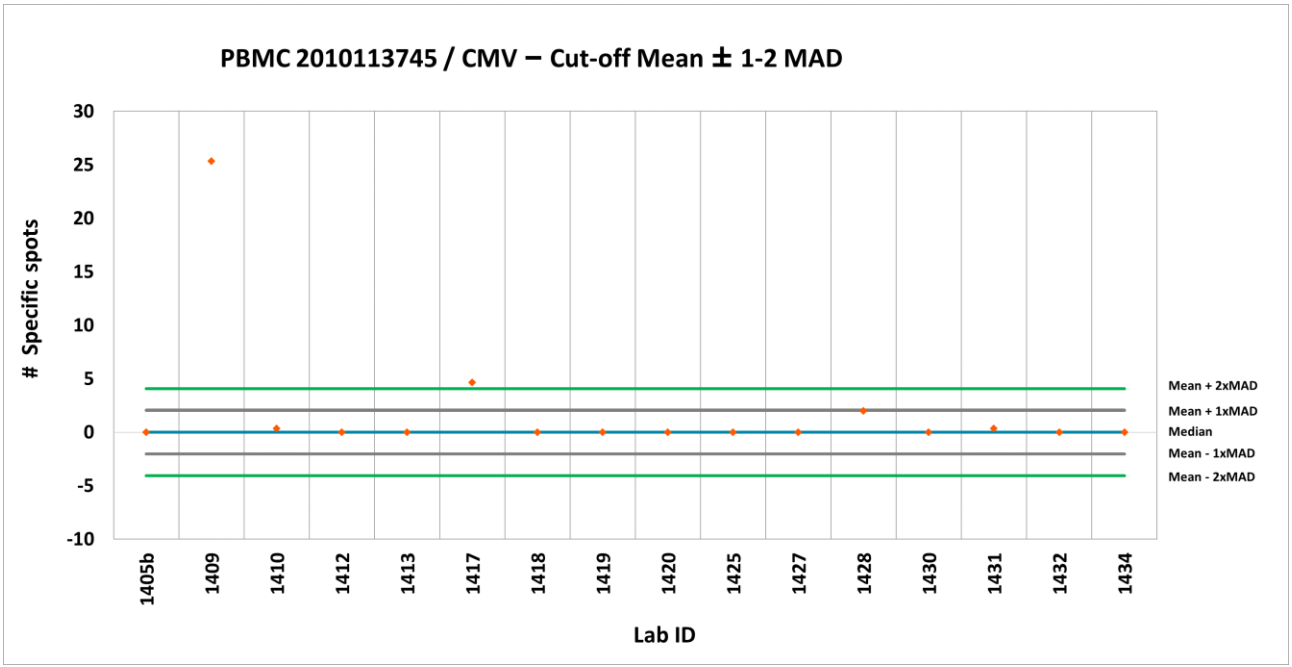

Figure 3. Analysis of PBMC 2010113745 with Reagent 1 (CMV) using median ± 1-2 MAD as a cut-off. The orange diamonds show the mean of CMV-specific spots subtracted the mean of background spots. The blue line shows the median of all results (0 spots). The grey lines are median ± 1MAD, and the green lines are median ± 2MAD.

Table 1. Definition of the test results.

Test Result	Corresponds to	Presented in the figures as	Proficiency Score
[Deviation from Median]<1MAD	Within the average range	Grey lines	3
1MAD≤[Deviation from Median]≤2MAD	Near the average range	Green lines	2
[Deviation from Median]>2MAD	Far from the average range	Above/below green lines	1

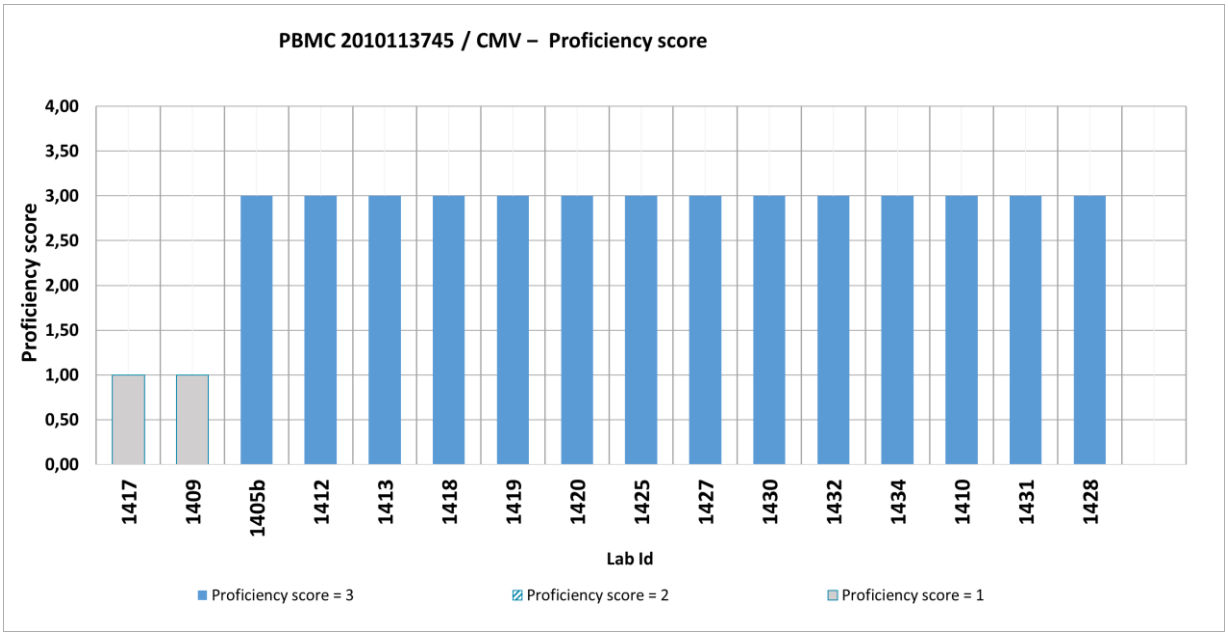

Figure 4. Lab proficiency score of analysis of PMBC 2010113745 with reagent 1 (CMV). See appendix 4 (Analysis 1).

3.1.1.2. PBMC 2010113745 stimulated with CEFX

Relative accuracy was calculated to evaluate the accuracy of each participants' measurements for PBMC 2010113745 stimulated with CEFX. The relative accuracy is defined as the background-corrected test result for each participant divided by the median value of the background-corrected test results for all participants. The relative accuracy is a measure of how close each participant is to the median of data reported by all participating labs. Relative accuracy scores for each laboratory are listed in Appendix 4 and an example of how the relative accuracy is calculated is shown in Appendix 5. The relative accuracy of measurements for PBMC 2010113745 stimulated with CEFX are illustrated in figure 5 below. Lab performances are divided into three groups and assigned a proficiency score according to how close their results are to the average of all participating laboratories – see table 2 and appendix 4 (analysis 2).

Table 2. Definition of proficiency score.

Relative Accuracy	Corresponds to	Presented in the figure below as	Proficiency score
0.66≤RA≤1.50	Within the median range	Blue columns	3
0.50≤RA<0.66 **1.50<RA≤2.00**	Near the median range	Striped columns	2
RA<0.50 **RA>2.00**	Far from the median range	Grey columns	1

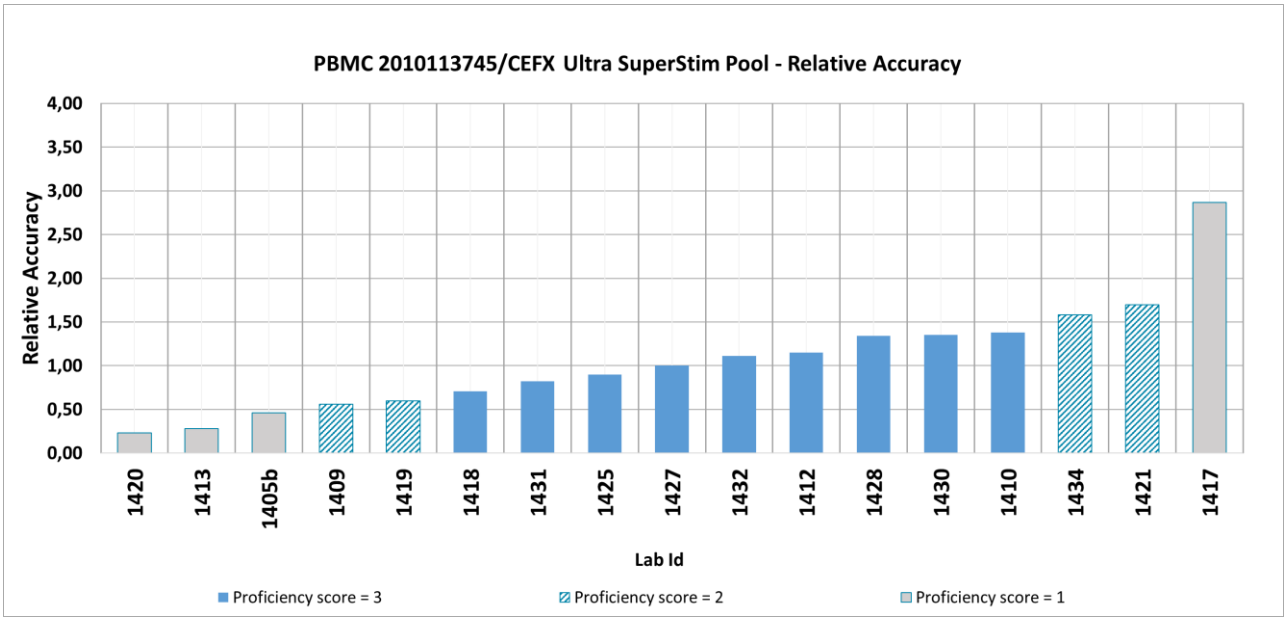

Figure 5. Relative accuracy for analysis of PBMC 2010113345 with Reagent 2 (CEFX). See table 2 and appendix 4 (Analysis 2).

3.2. Results from Analysis of PBMC 2010113384

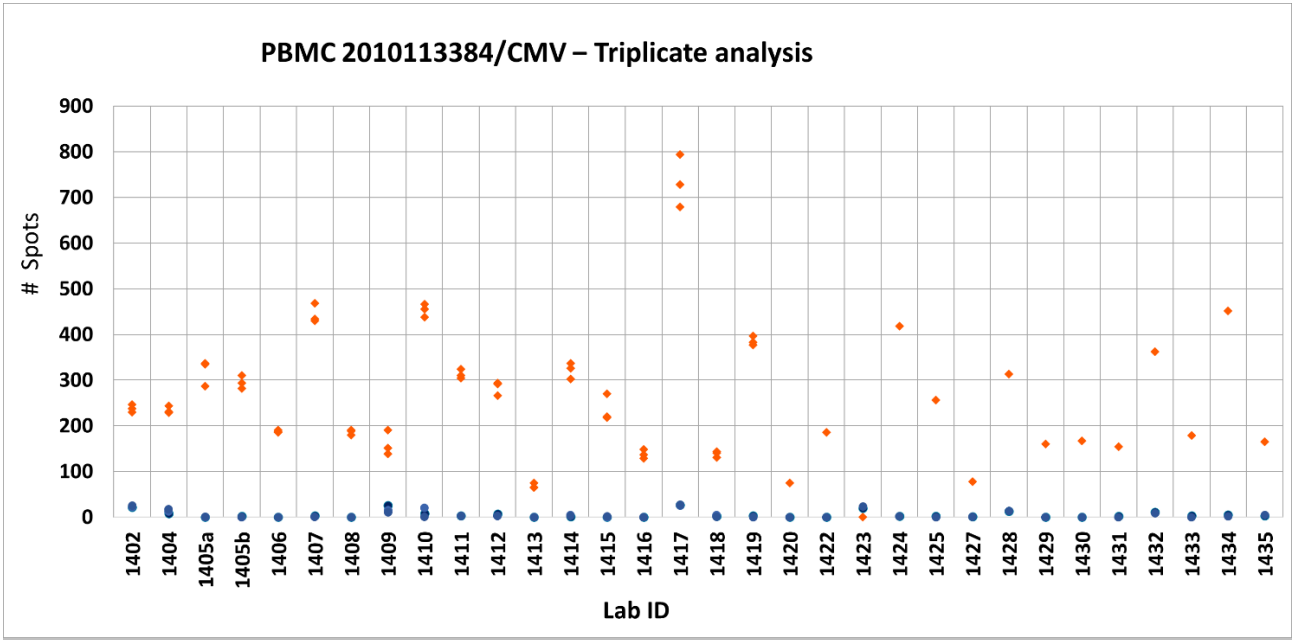

Figure 6A. Results from analysis of sample PBMC 2010113384 with Reagent 1 (CMV) and Reagent 3 (Negative control) (Analysis 3). Triplicate test values for CMV-specific spots (orange diamonds) and background spots (blue dots) per 200.000 PBMCs/well are shown.

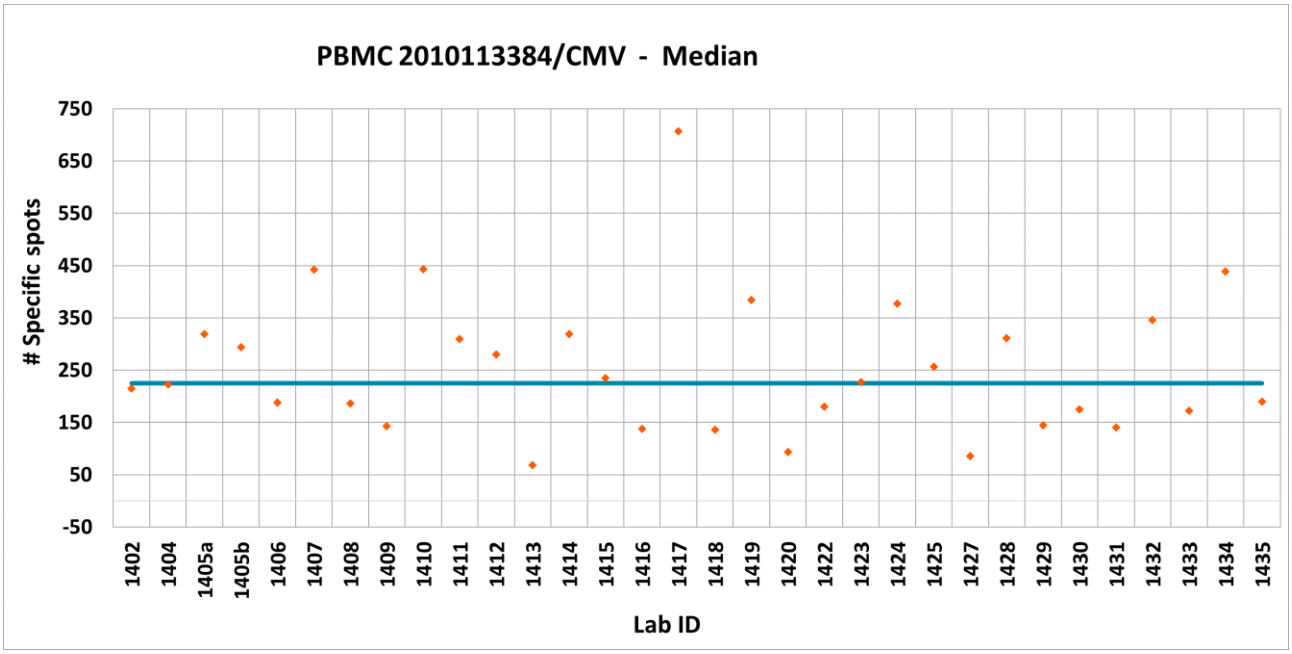

Figure 6B. Results from analysis of sample PBMC 2010113384 with Reagent 1 (CMV) and Reagent 3 (Negative control) (Analysis 3). The mean of CMV-specific spots subtracted the mean of background spots is shown (orange diamonds). The median of all results is 225 spots and indicated by the blue line.

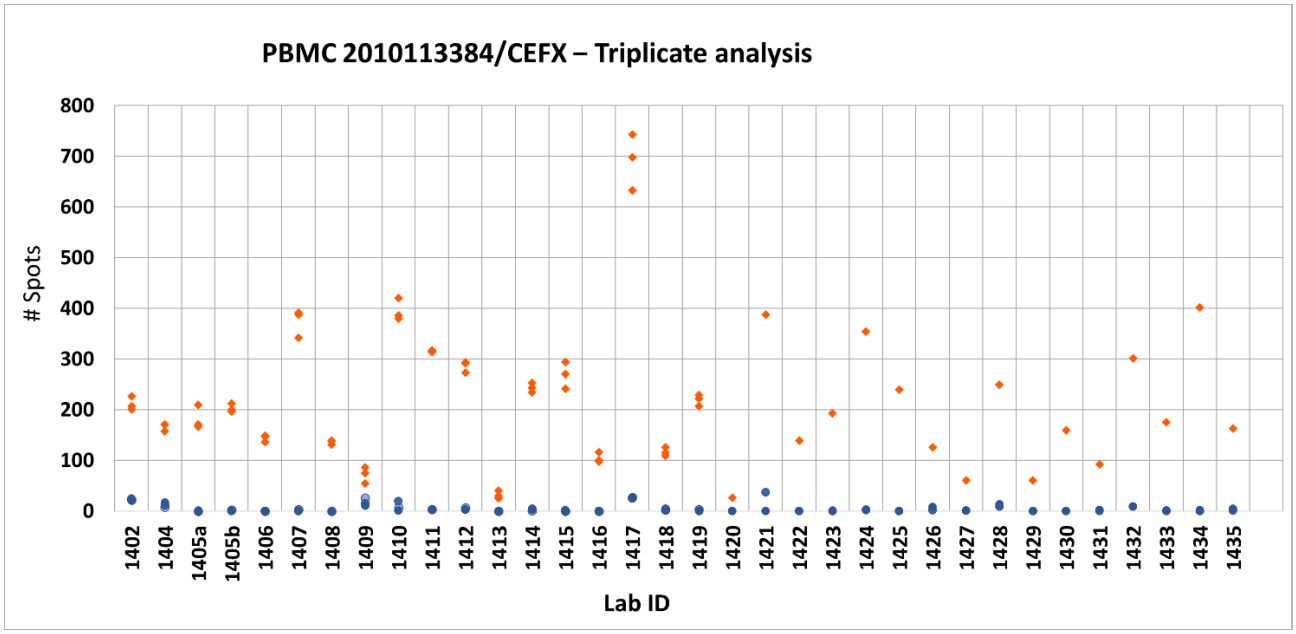

Figure 7A. Results from analysis of sample PBMC 2010113384 with Reagent 2 (CEFX) and Reagent 3 (Negative control) (Analysis 4). Triplicate test values for CEFX-specific spots (orange diamonds) and background spots (blue dots) per 200.000 PBMCs/well are shown.

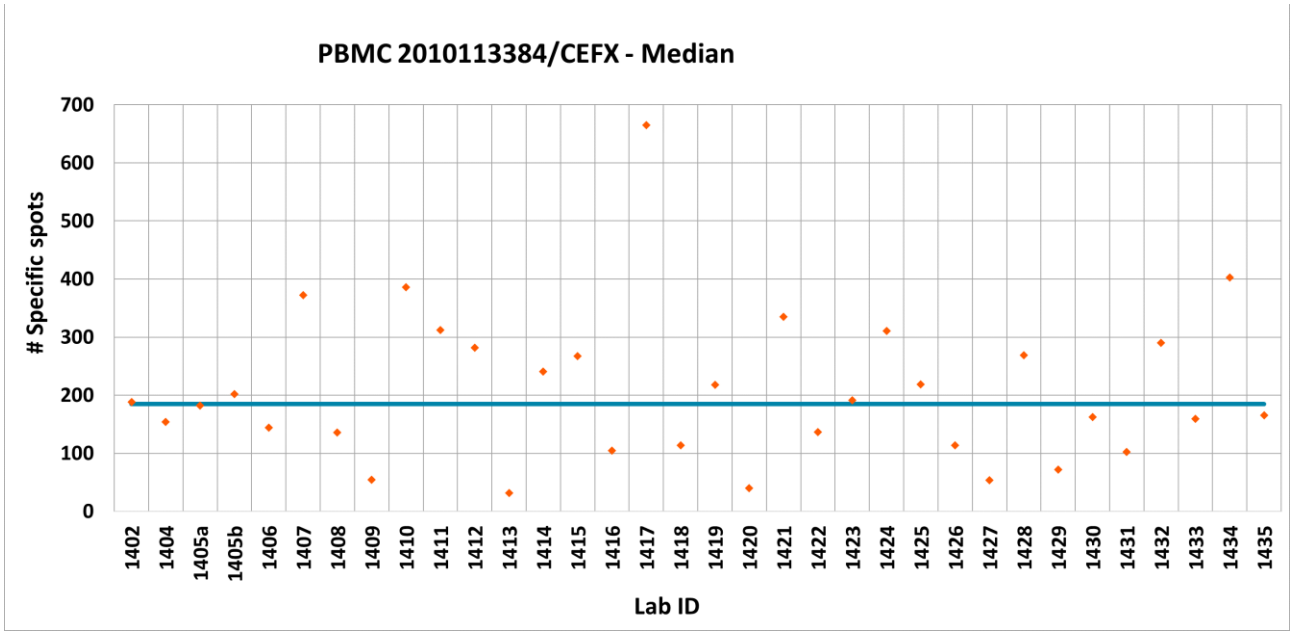

Figure 7B. Results from analysis of sample PBMC 2010113384 with Reagent 2 (CEFX) and Reagent 3 (Negative Control) (Analysis 4). The mean of CEFX-specific spots subtracted the mean of background spots is shown (orange diamonds). The median of all results is 185 spots and indicated by the blue line.

3.2.1. Evaluation of Test Results for PBMC 2010113384

For the data generated with PBMC 2010113384 we have chosen to define the relative accuracy as the background-corrected test result for each participant divided by the median value of the background-corrected test results for all participants. Relative accuracy scores for all laboratories are listed in Appendix 4 and an example of how the relative accuracy is calculated is shown in Appendix 5. The relative accuracy of measurements for PBMC 2010113384 stimulated with CMV and CFEX are illustrated in figures 8 and 9 respectively. Lab performances are divided into three groups and assigned a proficiency score according to how close their results are to the average of all participating laboratories – see table 1 and appendix 4 (analysis 3 & 4).

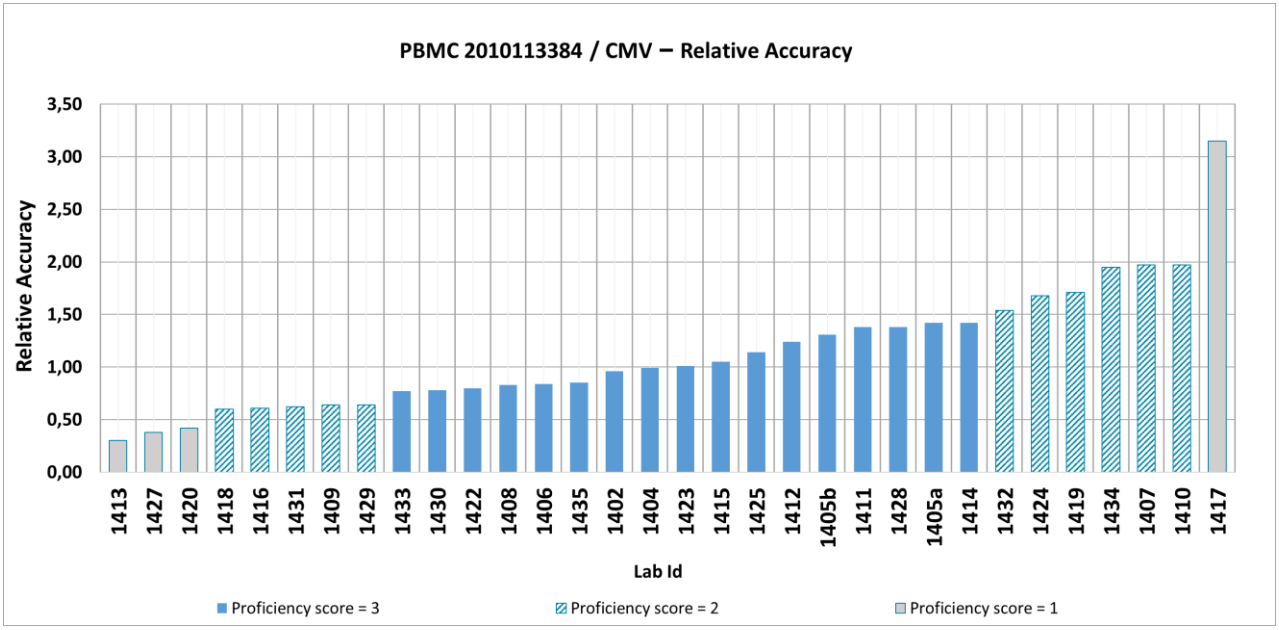

Figure 8. Relative accuracy for analysis of PBMC 2010113384 with Reagent 1 (CMV). See appendix 4 (Analysis 3).

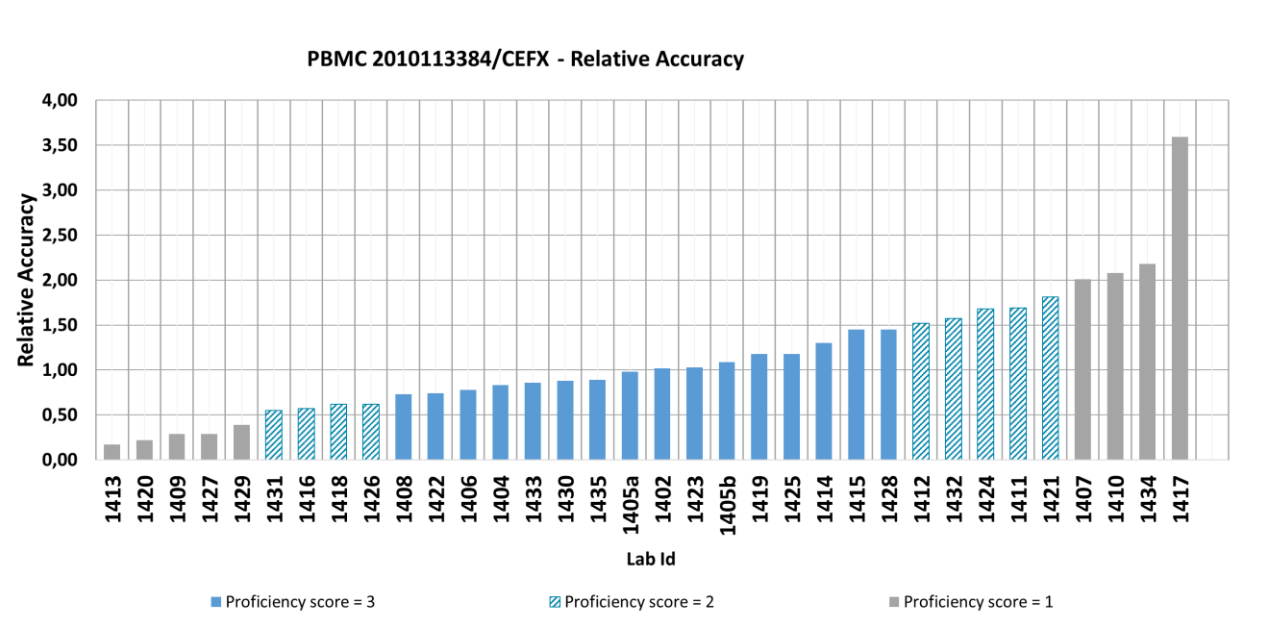

Figure 9. Relative accuracy for analysis of PBMC 2010113384 with Reagent 2 (CEFX). See appendix 4 (Analysis 4).

4. PROFICIENCY PERFORMANCE

The ability of each participant to identify IFN-γ secreting T-cells was described with an overall proficiency score. For each of the four analyses, the laboratories were assigned a proficiency score between 1-3, see figures 4,5,8,9, tables 1,2 and appendix 4. The overall proficiency score was then defined by the average score obtained in the four analyses. Thus, a participant with an overall proficiency score of "3" is in the average range on all four measurements and has the highest possible score. A participant with an average score of "1" is far from average on all four measurements and has the lowest possible score. See calculation of Overall Proficiency Score in Appendix 6

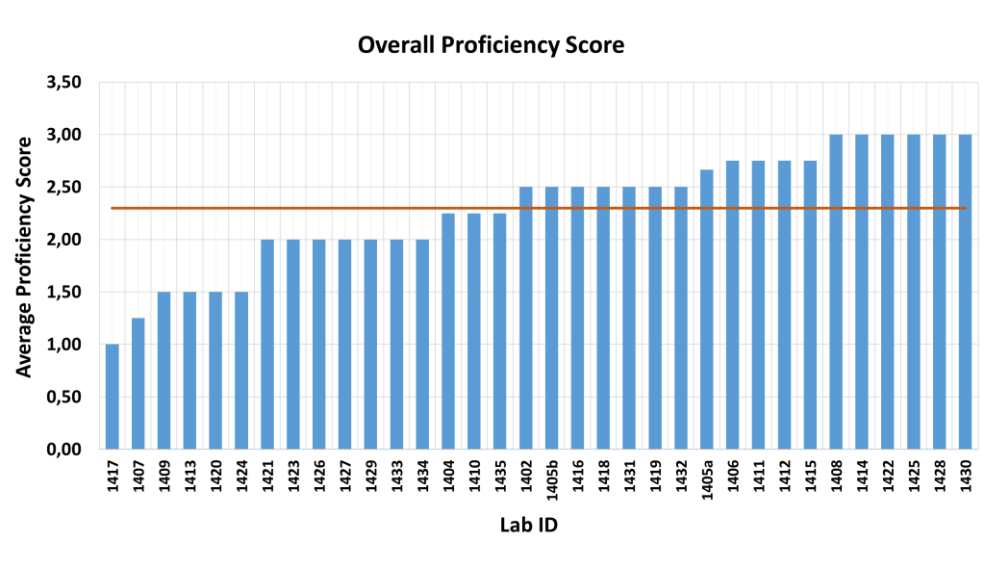

Figure 10. The average overall proficiency score is 2.3 indicated by the red line

5. DISCUSSION

Immudex T-cell ELISpot Proficiency Testing provide an opportunity for laboratories worldwide to assess their proficiency in identifying IFN-γ secreting T-cells with the ELISpot assay. Evaluation of laboratory performance is essential to ensure alignment between laboratories. Harmonized laboratory performance is of high importance in multicenter trials, where clinical results from different sites are compared to evaluate treatment responses.

In this T-cell ELISpot Proficiency Testing, participants used their own laboratory-specific procedure to determine the number of IFN-γ secreting cells after stimulation with two different defined peptide pools (CMV and CEFX). In this report, each participant can see how well their obtained results align with the rest of the participants. This critical knowledge provides each participant with the opportunity to evaluate their assay protocol, to ensure and sustain their ability to identify IFN-γ secreting T-cells accurately, reproducibly, and in alignment with other researchers across sites, or to identify necessary protocol optimizations. To facilitate inter-lab comparisons, we have performed simple statistical data analysis and calculated an overall proficiency score according to criteria chosen by Immudex. However, this is not an exact science and is only meant as a help to get an overview of the results. Different choices of analysis would be equally valid and might have given a slightly different outcome. Visual inspection of the distribution of background corrected results (fig 1b, 2b, 6b and 7b) is also good simple way of assessing overall lab performance.

The variation of spot counts in the triplicate analysis for each lab was low, showing a low intra-lab variation, however, the variation between labs was in general quite high even for assays with intermediate and high numbers of spots with a CV between 52% - 111%. This is probably a reflection of the nature of the ELISpot assay that may be sensitive to small variations in protocols, lab equipment and operator experience. That said the participants in this proficiency test are in general very experienced with 83% reporting that they have conducted >15 ELISpot assays within the last 2 years. In addition, we are unable to identify any trend in the information about the protocol and equipment used that distinguishes the level of lab proficiency, including resting time after thawing. We also investigated whether shipping and the condition of cell samples might have affected lab performance, but we find no trend in viability data either – viability was in general high.

In conclusion, this proficiency test shows that the participating labs have a consistent and reproducible ELISpot assay, however the variation between laboratories is significant and we found no trends in the reported protocols suggesting a general root cause. However, the protocols of the participants do differ on multiple parameters. Establishment of detailed standardized protocols including cell culture conditions and all used equipment is probably crucial to achieve high inter-lab reproducibility of ELISpot assays.

6. ACKNOWLEDGEMENTS

We thank JPT Peptide Technologies (Germany) for providing peptide pools, Mabtech for participating and CureVac for critical review of the report and helpful suggestions that helped shape the content of this report.

7. ABOUT IMMUDEX

Based in Virum, Denmark, with North American operations based in Fairfax, Virginia, Immudex manufactures MHC Dextramer® for the detection 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' 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 to quantify CMV-specific T cells. USA FDA 510(k) clearance for the CMV kit was granted in March 2017.

Our state-of-the-art dCODE Dextramer® reagents enable massive multiplexing of antigen-specific T-cell detection. Detection of over 1000 CD8+ T-cell specificities from a single blood sample has been achieved.

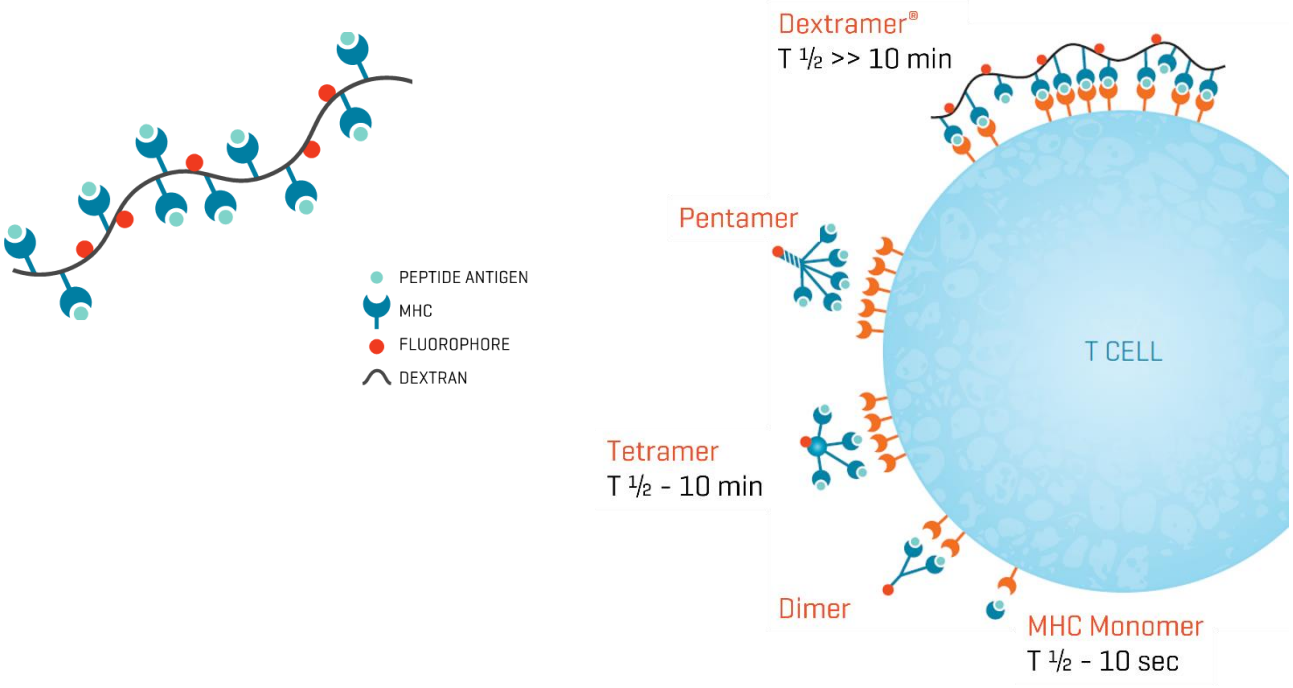

Figure 10 Schematic drawing of MHC Dextramer® and conventional MHC multimers binding to T-cell receptors (TCRs) on the surface of a T cell. MHC Dextramer® reagents are fluorescent-labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a solid and stable 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.

7.1. RESOURCES FROM IMMUDEX

We are committed to building a global community of proficiency in immune monitoring. Reach to us if you have questions or want to know more about the Immudex Proficiency Panels.

Proficiency Panels

Access the Immudex Proficiency Panels site, where you will find information about MHC Multimer and ELISpot Proficiency Panels.

Read more

Contact the Panel Coordinator

We are here to support you through all the process. From the proficiency testing to answering questions regarding deadlines, PBMC samples, data analysis. We want to ensure the process is easy for you.

proficiencypanel@immudex.com

Performance Reports

Curious about previous year's results? Find out more for MHC Multimer and ELISpot Proficiency Panels.

MHC Multimer Proficiency Panel reports

ELISpot Proficiency Panel Reports

Technical Support

Let us know if you experience difficulties or have questions. Immudex will help you get the most out of your Dextramer® products.

customer@immudex.com

8. APPENDIXES

8.1. APPENDIX 1: INSTRUCTIONS

Instructions for T-cell ELISpot Proficiency Testing 2023

Introduction

Originally developed at the initiative of CIC (the US Cancer Immuno-therapy Consortium of the CRI) and CIMT (the European Association for Cancer Immunotherapy), Immudex offers a Proficiency Testing Service to help researchers and clinicians worldwide evaluate and benchmark their immune monitoring performance with T-cell ELISpot assays and MHC multimer reagents and flow cytometry.

In this T-cell ELISpot Proficiency Testing, participants evaluate their ability to accurately detect the number of IFN-γ secreting antigen-specific cells in two different PBMC samples. The participants must determine the spot count per well as a result of stimulation with three different reagents: JPT's PepMixTM HCMVA (pp65), CEFX Ultra SuperStim Pool, and a negative control reagent.

Each participant is asked to test the PBMC samples according to these instructions, but following their own protocol for direct human IFN-γ ELISpot Assays, including own choice of antibodies, plates, enzyme, substrate, equipment, medium, and other miscellaneous chemicals and tools to perform the assay. We encourage participants to analyze samples with their own protocol to reflect routine sample analysis. We also recommend participants to have a look at the "Assay harmonization guidelines" provided by the CIC of CRI and CIMT, see Appendix I.

After analysis, participants report their results to Immudex. Results and performance from all participants are presented in a final report where participants names and affiliations are kept anonymous.

Deadlines and Immudex contact

Data submission:	May 04, 2023
Final report from Immudex:	June, 2023

If you have questions, please contact the proficiency testing coordinator, at proficiencypanel@immudex.com

Samples and Reagents provided

- Two PBMC samples (Lot #2010113745 and Lot #2010113384)
- Reagent-1 (PepMixTM HCMVA (pp65); JPT Product Code: PM-PP65-2)
- Reagent-2 (CEFX Ultra SuperStim Pool; JPT Product Code: PM-CEFX-2)
- Reagent-3 (Negative control PBS/DMSO).

Instructions for how to unload the samples and return the shipper are included in the shipper. We recommend storing the samples at ≤ -140°C until running the ELISpot assay.

Please, remember to return the unloaded shipper within 24 hours after receiving it, instruction is included in the shipper.
NOTE: Failing to return the shipper, we will have to charge you $800, for the shipper.

Experimental setup

ELISpot Step-by-Step

A. Antibody coating
B. Cell incubation
C. Cytokine capture
D. Detection antibodies
E. Streptavidin-enzyme conjugate
F. Addition of substrate
G. Analysis

Please use your own currently established protocol for the IFN-γ ELISpot assay, but follow the general instructions listed here.

General instructions

1. One 96-well plate is required for the assay. Coat columns 3-5 of the plate according to your own IFN-γ ELISpot protocol. Coat 3x8 = 24 wells in total, see plate setup in Table 2 next page.

2. Thaw the two PBMC vials and count the cells using your laboratory's preferred procedure.

For each PBMC vial, record total cell number and the percentage of viable cells. If a resting step is included, please count and record total cell number and the percentage of viable cells after the resting step, see Table 1 below.

Table 1 PBMC status

	Right after thawing		After resting (if you include a resting step)	
PBMC lot	**Total cell number**	**% Viable cells**	**Total cell number**	**% Viable cells**
2010113745				
2010113384				

3. Dilute Reagents:
Reagent-1, Reagent-2, and Reagent-3 contain approximately 100µl and must be diluted 1:10 with the medium used for the assay.

4. Plate PBMC samples and add Reagents exactly as outlined in Table 2 (data are reported in this format).

- Row B3-5, C3-5, D3-5, E3-5, F3-5, G3-5:
Plate 200,000 viable cells/well in 50 µL medium/well. Add Reagents at 50 µL/well. Final volume of cells and Reagent should be 100 µL.

- Row A3-5 and H3-5:
Add 100 µL medium/well (no cells or Reagent), to enable assessment of false positive spots.

5. Perform the assay, following your own established protocol.

Table 2 Plate overview

	1-2	3	4	5	6-12
A		**No cells – Medium**	**No cells – Medium**	**No cells – Medium**	
B		PBMC lot 2010113745 *Reagent-1*	PBMC lot 2010113745 *Reagent-1*	PBMC lot 2010113745 *Reagent-1*	
C		PBMC lot 2010113745 Reagent-2	PBMC lot 2010113745 Reagent-2	PBMC lot 2010113745 Reagent-2	
D		PBMC lot 2010113745 *Reagent-3*	PBMC lot 2010113745 *Reagent-3*	PBMC lot 2010113745 *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**	

Report data

After completing the experiment, please report data and experimental details, using this link https://immudex.wufoo.com/forms/r12a1gqc078ym3v/

Appendix I

Assay harmonization guidelines

Initial ELISpot Harmonization Guidelines to Optimize Assay Performance (based on previously published recommendations from 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. Let only trained personnel (per laboratory SOP) conduct assays.

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

8.2. Appendix 2: Results from Analysis of PBMC 2010113745

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

PBMC 2010113745	Reagent 1			Mean	Reagent 3			Mean	Background corrected data
Reagent	PepMixTM HCMVA (pp65)			B3-B5	Negative control			D3-D5	Mean (B3-B5)-Mean(D3-D5)
Lab ID / Wells	B3	B4	B5		D3	D4	D5		
1405b	0	0	0	0	0	0	0	0	0
1409	28	35	29	31	5	2	9	5	25
1410	5	5	3	4	7	4	1	4	0
1412	11	9	10	10	20	16	15	17	-7
1413	0	0	0	0	0	0	0	0	0
1417	21	20	15	19	16	9	17	14	5
1418	4	3	1	3	5	8	11	8	-5
1419	3	0	2	2	2	3	4	3	-1
1420	0	0	0	0	0	0	0	0	0
1421	na	na	na	na	na	na	na	na	na
1425	0	0	1	0	1	0	2	1	-1
1427	2	2	1	2	1	3	1	2	0
1428	9	16	18	14	10	14	13	12	2
1430	0	0	0	0	0	0	1	0	0
1431	0	1	3	1	1	0	2	1	0
1432	11	12	11	11	12	12	12	12	-1
1434	2	2	1	2	5	2	2	3	-1

PBMC 2010113745	Reagent 2			Mean	Reagent 3			Mean	Background corrected data
Reagent	CEFX Ultra SuperStim Pool			C3-C5	Negative control			D3-D5	Mean (C3-C5)-Mean(D3-D5)
Lab ID / Wells	C3	C4	C5		D3	D4	D5		
1405b	92	116	135	114	0	0	0	0	114
1409	113	196	124	144	5	2	9	5	139
1410	344	358	331	344	7	4	1	4	340
1412	307	300	294	300	20	16	15	17	283
1413	80	60	70	70	0	0	0	0	70
1417	740	740	683	721	16	9	17	14	707
1418	189	179	185	184	5	8	11	8	176
1419	153	152	149	151	2	3	4	3	148
1420	54	46	73	58	0	0	0	0	58
1421	410	420	431	420	0	0	2	1	420
1425	230	227	214	224	1	0	2	1	223
1427	256	239	250	248	1	3	1	2	247
1428	311	336	379	342	10	14	13	12	330
1430	341	326	336	334	0	0	1	0	334
1431	199	191	219	203	1	0	2	1	202
1432	284	302	270	285	12	12	12	12	273
1434	392	391	395	393	5	2	2	3	390

8.3. Appendix 3: 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 read for each sample.

PBMC 2010113384	Reagent 1			Mean	Reagent 3			Mean	Background corrected data
	PepMixTM HCMVA (pp65)			E3-E5	Negative control			G3-G5	Mean (E3-E5)-Mean(G3-G5)
Lab ID / Wells	E3	E4	E5		G3	G4	G5		
1402	246	238	230	238	22	22	25	23	215
1404	244	231	229	235	8	12	17	12	222
1405a	337	287	335	320	0	0	1	0	319
1405b	294	310	282	295	2	0	1	1	294
1406	186	191	188	188	0	0	0	0	188
1407	434	430	469	444	3	1	1	2	443
1408	191	189	180	187	0	0	0	0	187
1409	139	151	191	160	26	15	11	17	143
1410	455	466	438	453	8	20	1	10	443
1411	324	304	310	313	3	3	3	3	310
1412	292	294	266	284	7	3	3	4	280
1413	65	75	65	68	0	0	0	0	68
1414	302	326	337	322	1	5	3	3	319
1415	220	270	218	236	0	0	2	1	235
1416	129	148	137	138	0	0	0	0	138
1417	794	679	728	734	27	25	27	26	707
1418	131	143	141	138	2	5	1	3	136
1419	397	383	377	386	3	0	1	1	384
1420	75	96	109	93	0	0	0	0	93
1421	na	na	na	na	na	na	na	na	na
1422	186	176	180	181	0	0	0	0	181
1423	na	287	211	249	20	22	23	22	227
1424	418	413	307	379	2	3	2	2	377
1425	256	258	257	257	2	0	0	1	256
1426	na	na	na	na	na	na	na	na	na
1427	78	100	83	87	1	1	1	1	86
1428	313	308	347	323	13	9	13	12	311
1429	160	150	125	145	0	0	0	0	145
1430	167	184	173	175	0	0	0	0	175
1431	154	148	123	142	2	2	0	1	140
1432	362	340	366	356	11	9	9	10	346
1433	179	179	163	174	3	1	0	1	172
1434	452	424	447	441	5	0	2	2	439
1435	165	195	219	193	3	1	5	3	190

PBMC 2010113384	Reagent 2			Mean	Reagent 3			Mean	Background corrected data
	CEFX Ultra SuperStim Pool			F3-F5	Negative control			G3-G5	Mean (F3-F5)-Mean(G3-G5)
Lab ID / Wells	F3	F4	F5		G3	G4	G5		
1402	207	226	201	211	22	22	25	23	188
1404	171	158	171	167	8	12	17	12	154
1405a	166	171	210	182	0	0	1	0	182
1405b	212	196	200	203	2	0	1	1	202
1406	149	147	136	144	0	0	0	0	144
1407	388	342	392	374	3	1	1	2	372
1408	138	139	131	136	0	0	0	0	136
1409	54	86	75	72	26	15	11	17	54
1410	386	420	380	395	8	20	1	10	386
1411	317	315	314	315	3	3	3	3	312
1412	292	293	273	286	7	3	3	4	282
1413	30	40	25	32	0	0	0	0	32
1414	234	243	253	243	1	5	3	3	240
1415	294	270	241	268	0	0	2	1	268
1416	100	98	116	105	0	0	0	0	105
1417	698	633	743	691	27	25	27	26	665
1418	115	109	126	117	2	5	1	3	114
1419	229	207	222	219	3	0	1	1	218
1420	26	37	58	40	0	0	0	0	40
1421	388	366	372	375	43	37	na	40	335
1422	139	161	109	136	0	0	0	0	136
1423	193	184	198	192	0	0	1	0	191
1424	354	300	286	313	2	3	2	2	311
1425	240	216	203	220	2	0	0	1	219
1426	126	93	137	119	4	2	8	5	114
1427	61	68	36	55	1	1	1	1	54
1428	249	280	313	281	13	9	13	12	269
1429	61	70	84	72	0	0	0	0	72
1430	159	164	164	162	0	0	0	0	162
1431	92	104	116	104	2	2	0	1	103
1432	301	300	299	300	11	9	9	10	290
1433	175	178	130	161	3	1	0	1	160
1434	402	410	404	405	5	0	2	2	403
1435	163	166	176	168	3	1	5	3	165

8.4. Appendix 4: Calculations of Proficiency Scores

Analysis 1

PBMC 2010113745	PepMixTM HCMVA (pp65)					
Lab ID	Background corrected data	Absolute deviation from median [Data-Median]	Absolute deviation from median X<1MAD (= 2,04)	Absolute deviation from mean 1MAD (= 2,04)≤x≤2MAD (= 4,08)	Absolute deviation from mean X>2MAD (= 4)	Accuracy score
1405b	0,00	0,00	0,00			3
1409	25,33	25,33			25,33	1
1410	0,33	0,33	0,33			3
1412	0,00	0,00	0,00			3
1413	0,00	0,00	0,00			3
1417	4,67	4,67			4,67	1
1418	0,00	0,00	0,00			3
1419	0,00	0,00	0,00			3
1420	0,00	0,00	0,00			3
1421	na	na	na			na
1425	0,00	0,00	0,00			3
1427	0,00	0,00	0,00			3
1428	2,00	2,00	2,00			3
1430	0,00	0,00	0,00			3
1431	0,33	0,33	0,33			3
1432	0,00	0,00	0,00			3
1434	0,00	0,00	0,00			3
	Median = 0	MAD* = 2,04				
Negative background corrected values set to 0		*Mean of Absolute Deviation				
Relative accuracy: Mean X / Mean all						
Absolute deviation: Mean x - Mean all						

Analysis 2

PBMC 2010113745	CEFX Ultra SuperStim Pool				
Lab ID	Background corrected data	0.66≤RA≤1.50	0.50≤RA<0.66 & 1.50<RA≤2.00	RA<0.50 & RA>2.00	Proficiency score
1405b	114			0,46	1
1409	139		0,56		2
1410	340	1,38			3
1412	283	1,15			3
1413	70			0,28	1
1417	707			2,87	1
1418	176	0,71			3
1419	148		0,60		2
1420	58			0,23	1
1421	420		1,70		2
1425	223	0,90			3
1427	247	1,00			3
1428	330	1,34			3
1430	334	1,35			3
1431	202	0,82			3
1432	273	1,11			3
1434	390		1,58		2
	Median= 246,7				
	Relative accuracy (RA)=data/median				

ANALYSIS 3

PBMC 2010113384	PepMixTM HCMVA (pp65)				
Lab ID	Background corrected data	0.66≤RA≤1.50	0.50≤RA<0.66 & 1.50<RA≤2.00	RA<0.50 & RA>2.00	Proficiency score
1402	215	0,96			3
1404	222	0,99			3
1405a	319	1,42			3
1405b	294	1,31			3
1406	188	0,84			3
1407	443		1,97		2
1408	187	0,83			3
1409	143		0,64		2
1410	443		1,97		2
1411	310	1,38			3
1412	280	1,24			3
1413	68			0,30	1
1414	319	1,42			3
1415	235	1,05			3
1416	138		0,61		2
1417	707			3,15	1
1418	136		0,6		2
1419	384		1,71		2
1420	93			0,42	1
1421	na	na	na	na	na
1422	181	0,8			3
1423	227	1,01			3
1424	377		1,68		2
1425	256	1,14			3
1426	na	na	na	na	na
1427	86			0,38	1
1428	311	1,38			3
1429	145		0,64		2
1430	175	0,78			3
1431	140		0,62		2
1432	346		1,54		2
1433	172	0,77			3
1434	439		1,95		2
1435	190	0,85			3
RA (Relative accuracy): Background corrected data/median					
Median = 225					

ANALYSIS 4

PBMC 2010113384	CEFX Ultra SuperStim Pool				
Lab ID	Background corrected data	0.66≤RA≤1.50	0.50≤RA<0.66 & 1.50<RA≤2.00	RA<0.50 & RA>2.00	Proficiency score
1402	188	1,02			3
1404	154	0,83			3
1405a	182	0,98			3
1405b	202	1,09			3
1406	144	0,78			3
1407	372			2,01	1
1408	136	0,73			3
1409	54			0,29	1
1410	386			2,08	1
1411	312		1,69		2
1412	282		1,52		2
1413	32			0,17	1
1414	240	1,30			3
1415	268	1,45			3
1416	105		0,57		2
1417	665			3,59	1
1418	114		0,62		2
1419	218	1,18			3
1420	40			0,22	1
1421	335		1,81		2
1422	136	0,74			3
1423	191	1,03			3
1424	311		1,68		2
1425	219	1,18			3
1426	114		0,62		2
1427	54			0,29	1
1428	269	1,45			3
1429	72			0,39	1
1430	162	0,88			3
1431	103		0,55		2
1432	290		1,57		2
1433	160	0,86			3
1434	403			2,18	1
1435	165	0,89			3
RA (Relative accuracy): Background corrected data/median					
Median = 185					

8.5 APPENDIX 5: CALCULATION OF THE RELATIVE ACCURACY

Example of relative accuracy calculation of results obtained with PBMC 2010113384 stimulated with CEFX peptide pool.

	PBMC 2010113384										
Lab ID	CMV				Negative control						
	E1	E2	E3	Mean	G1	G2	G3	Mean	Background corrected mean	Median of all participants	Relative accuracy
1402	246	238	230	238	22	22	25	23	215	224,8	(215,0/224,8)= 0,96

8.6 Appendix 6: Calculation of Overall Proficiency Score

	Proficiency score				Overall proficiency score
Lab ID no.	Analysis 1	Analysis 2	Analysis 3	Analysis 4	(Mean)
1402	2	2	3	3	2,5
1404	1	2	3	3	2,3
1405a	na	2	3	3	2,7
1405b	3	1	3	3	2,5
1406	3	2	3	3	2,8
1407	1	1	2	1	1,3
1408	3	3	3	3	3,0
1409	1	2	2	1	1,5
1410	3	3	2	1	2,3
1411	3	3	3	2	2,8
1412	3	3	3	2	2,8
1413	3	1	1	1	1,5
1414	3	3	3	3	3,0
1415	2	3	3	3	2,8
1416	3	3	2	2	2,5
1417	1	1	1	1	1,0
1418	3	3	2	2	2,5
1419	3	2	3	3	2,8
1420	3	1	1	1	1,5
1421	na	2	na	2	2,0
1422	3	3	3	3	3,0
1423	1	1	3	3	2,0
1424	1	1	3	2	1,8
1425	3	3	3	3	3,0
1426	na	2	na	2	2,0
1427	3	3	1	1	2,0
1428	3	3	3	3	3,0
1429	2	3	2	1	2,0
1430	3	3	3	3	3,0
1431	3	3	2	2	2,5
1432	3	3	3	2	2,8
1433	1	1	3	3	2,0
1434	3	3	2	1	2,3
1435	1	2	3	3	2,3
1436	na	na	na	na	na

8.7 APPENDIX 7: DEVIATION IN DATA HANDLING

Group 1: Deviation in data handling	
Lab	**Reason for deviations in data handeling**
1405	This lab commented on the the R1 response of PBMC 2010113367, for which they get no significant spots. This lab comment that in all previous participations they have seen many spots with this combination. They have no explanation for the discrepancy. We have considered this a result of experimental error and taken this dataset out of the calculation.
1411	This lab observed a large amount of spots in the negative controls for PBMC 201113384 for which they have no explanation. We have chosen to consider this a result of experimental error. To be able to include the lab in the overall score, the negative control for this dataset is set to the average of the negative controls of all participants. Negaitve control (R3) is set to 3 spots in the calculations.
1423	This lab comment that they reject the data in well E3 on PBMC 2010113384. We have calculated the average of the remaining two wells.
1426	This lab has used the CEFX Ultra SuperStim Pool (R2) in both sets of stimulation. The R1 (PepMixTM HCMVA (pp65)) of both PBMC sampels is taken out of the analysis (na)

Group 2: Deviation in data handling	
Lab	**Reason for deviation in data handeling**
1421	This lap has used another peptide pool for stimulation (R1) on both samples. These data are taken out of the calculations.
1436	This lab is removed due to reported low cell viability, cell clumping, and no significant spot forming units in any of the assays.

8.8 Appendix 8: Pre-testing by flow cytometry at Immudex of donor samples stained with MHC-Dextramer®

PBMC	Reagent	Pre-test result
2010113745	Reagent 1: PepMixTM HCMVA (pp65) and Reagent 3: Negative Control	Negative
2010113745	Reagent 2: CEFX Ultra SuperStim Pool and Reagent 3 (Negative Control)	High response
2010113384	Reagent 1: PepMixTM HCMVA (pp65) and Reagent 3: Negative Control	Medium/high response
2010113384	Reagent 2: CEFX Ultra SuperStim Pool and Reagent 3 (Negative Control)	Medium/high response

8.9 Change Log

For analysis 2, 3 and 4 we have changed the calculation of relative accuracy. In the previous report (version 2) we evaluated lab performance relative to the mean of all measurements. However, since the data are not normal distributed it is better to calculate the accuracy relative to the median of all measurements. We have redone the analysis accordingly which has impacted figures 2B, 5, 6B, 7B, 8, 9 and 10, and appendix 4 (analysis 2, 3, 4), appendix 5, 6.

Table 2 has been changed slightly to make it easier to understand.

Appendix 2 – minor correction for lab 1421 (see appendix 7).

Appendix 3: minor correction for lab 1421 & 1426 (see appendix 7)