MHC Multimer Proficiency Panel 2019

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Contents

INTRODUCTION TO PROFICIENCY PANELS

The ability to compare data generated by different laboratories is a powerful tool to ensure alignment and drive improvements in research and development.

Supported by the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC of CRI) and the European Association for Cancer Immunotherapy (CIMT), Immudex conducts Proficiency Panels yearly, providing a program for laboratories around the globe to assess their proficiency in monitoring antigen-specific T-cell responses. It is a non-profit service offered with the intent of increasing the level of proficiency among researchers and clinicians that perform the immune monitoring assays, the MHC Multimer assays, and T-cell ELISpot assays. Check out the science behind Immudex' Proficiency Panels here.

Immudex encourages participants to analyze samples using their own protocol to reflect routine sample analysis in their laboratory. Furthermore, we recommend participants to have a look at the "Assay Harmonization Guidelines" provided by CIC of CRI and CIMT (Appendix 2). By evaluating the accuracy to enumerate T cells using laboratory-specific procedures, participants get information on the degree of concordance of results obtained by the other laboratories in the panel, and thereby access to evaluate their competency and laboratory protocol.

MHC MULTIMER PROFICIENCY PANEL 2019

In the MHC Multimer Proficiency Panel 2019 participants evaluated the accuracy of enumerating antigen specific CD8+ T cells in PBMC samples using MHC multimer assay and flow cytometry.

Each participant received two pretested PBMC samples with low, medium, or high responses of antigen-specific T cells specific for predefined EBV-, CMV1-, FLU- or CMV2-epitopes. Each participant measured the percentages of antigen-specific CD8+ T cells in the PBMC samples according to the instructions, but with their own choice of materials (multimer, antibodies, viability marker etc.), and following their own assay protocol (staining tubes/plates, washing buffer etc.).

Results and performance from all participants are presented in this report. The participants' names and affiliations are kept anonymous.

- 19 laboratories from 9 different countries participated in this Proficiency Panel
- 18 participants used MHC Dextramer® reagents and 1 participant used their own MHC multimer reagents
- 10 participants were from Academia and 9 participants were from industry
- 84% of the participating laboratories got a proficiency score of ≥ 2.5

ANALYSES

Table 1 MHC Multimer reagents used in the MHC Multimer Proficiency Panel 2019:

MHC Multimer reagent	MHC Multimer specificity
HLA-B*3501/HPVGEADYFEY	EBV-specific
HLA-B*3501/IPSINVHHY	CMV-specific
HLA-A*0201/GILGFVFTL	FLU-specific
HLA-A*0201/NLVPMVATV	CMV-specific
HLA-A*0201/negative control	Negative Control

Each participant

- was assigned a confidential laboratory identification number (Lab ID)
- received instructions on how to perform the MHC Multimer proficiency test (Appendix 1)
- received Harmonization Guidelines (Appendix 2)
- received two pretested PBMC samples
- received MHC Dextramer® reagents if requested or used their own MHC Multimer reagents
- ran the proficiency testing according to their own protocol. Each analysis was made in duplicates
- reported the following numbers for each of the 12 analyses:
    - The number of CD8+ cells
    - The number of CD8+ Multimer+ cells
    - The % of CD8+ Multimer+ cells out of the CD8+ cell population

For further details, see Appendix 1 for instructions.

RESULTS

The duplicate measurements of the percentage of the antigen-specific CD8+ T cells (EBV-, CMV-, FLU- and CMV2-specific) and the medians are presented in Figure 1, Figure 2, Figure 3, and Figure 4.

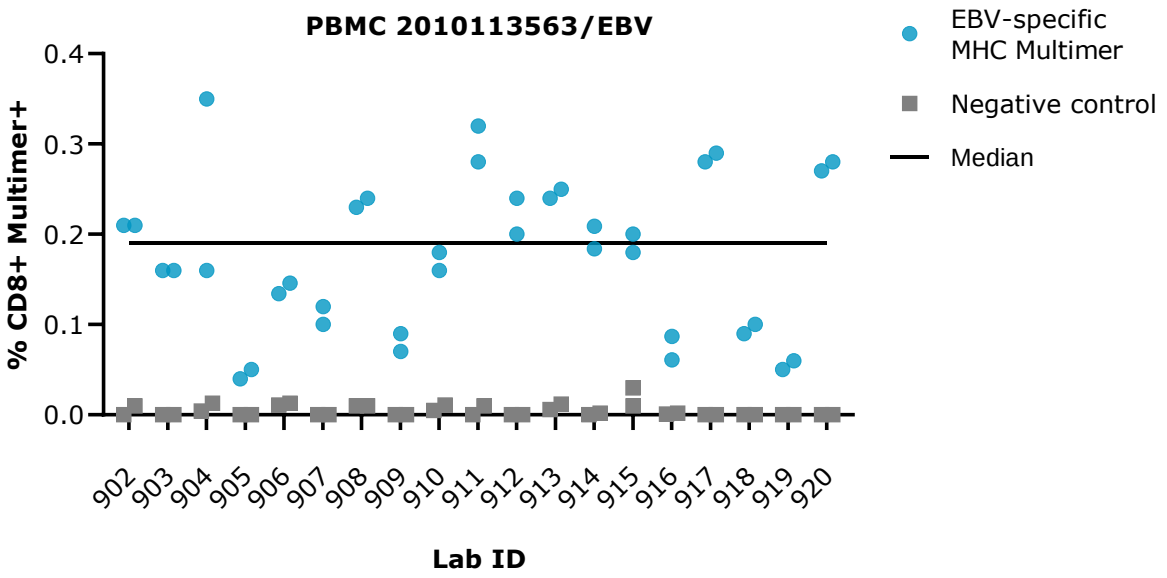

Figure 1 Results from analysis of samples PBMC 2010113563 stained with HLA-B*3501/HPVGEADYFEY MHC Multimer (EBV-specific) and Negative control MHC Multimer. Median for EBV-specific CD8+ T cells is 0.19%.

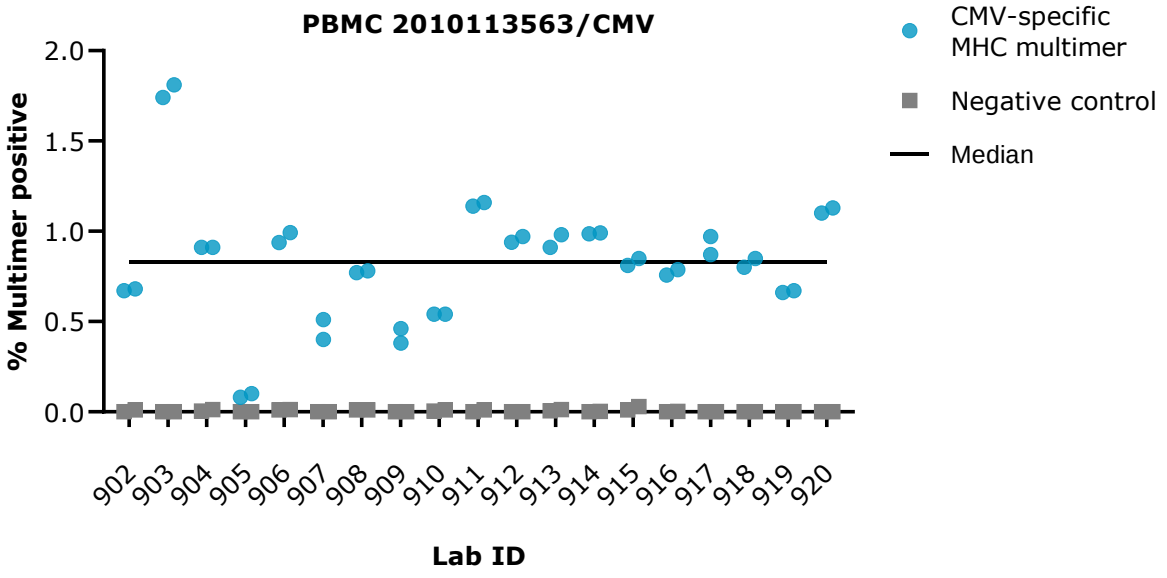

Figure 2 Results from analysis of samples PBMC 2010113563 stained with HLA-B*3501/IPSINVHHY MHC Multimer (CMV-specific) and Negative control MHC Multimer. Median for CMV-specific CD8+ T cells is 0.83%.

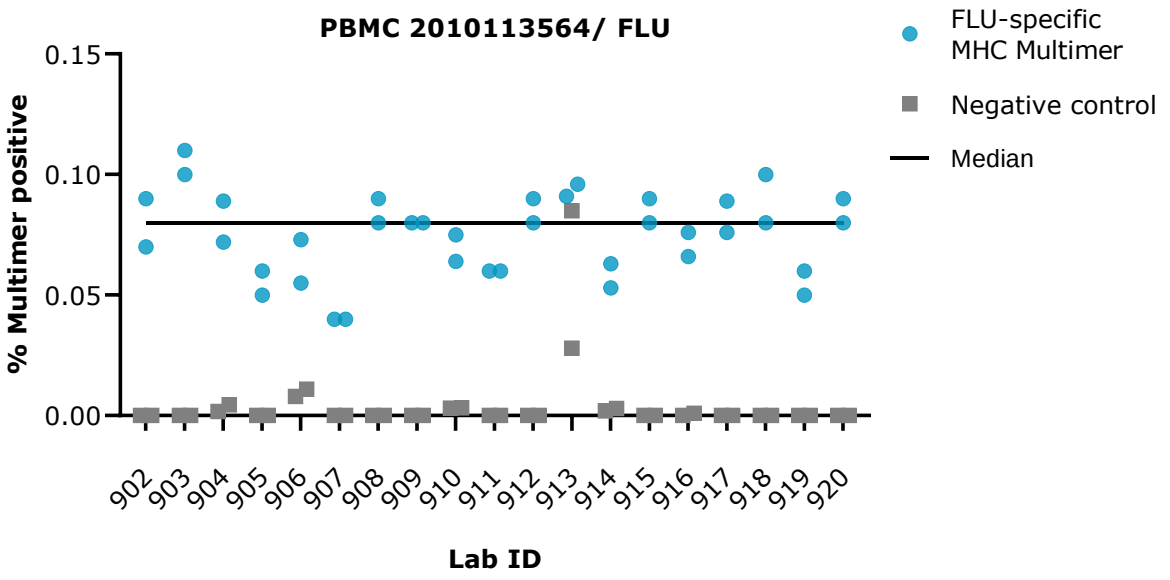

Figure 3 Results from analysis of samples PBMC 2010113564 stained with HLA-A*0201/GILGFVFTL MHC Multimer (FLU-specific) and Negative control MHC Multimer. Median for FLU-specific CD8+ T cells is 0.08%.

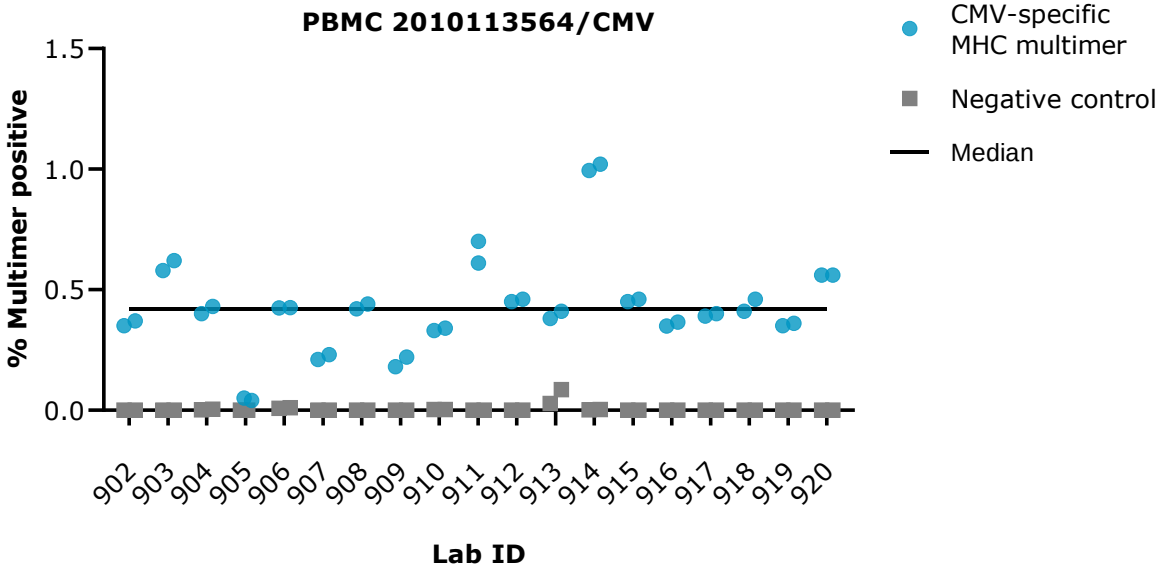

Figure 4 Results from analysis of samples PBMC 2010113564 stained with HLA-A*0201/NLVPMVATV (CMV-specific) and Negative control MHC Multimer. Median for CMV-specific CD8+ T cells is 0.42%.

PERFORMANCE

To evaluate the accuracy of each participants' measurements, we used the relative accuracy. The relative accuracy tells you how close each participant is to the average value reported by all participants. The medians shown in Figure 1-4 were used as the average value to calculate the relative accuracy, see example of calculation in Appendix 3. The individual laboratories' relative accuracies are presented in Figure 5-8 on the following pages, and the definition of what the values correspond to is listed in Table 2. Results of pretesting the two PBMC samples are shown in Appendix 4.

Table 2 Definition of the relative accuracy:

Relative accuracy	Corresponds to	Presented in the figures as
< 0.50 > 2.00	far from the average range	black columns
0.50 – 0.65 1.50 – 2.00	near the average range	grey columns
0.66 – 1.50	within the average range	orange columns

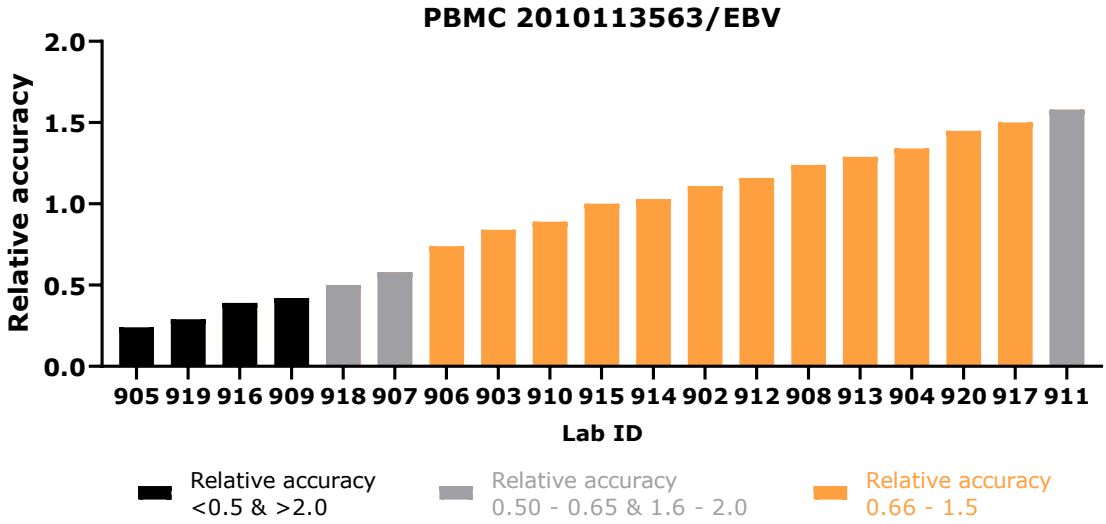

Figure 5 Relative Accuracy for analysis of PBMC 2010113563 stained with the EBV-specific Multimer: HLA-B*3501/HPVGEADYFEY. 12 out of 19 participants are within "the average range" (orange columns).

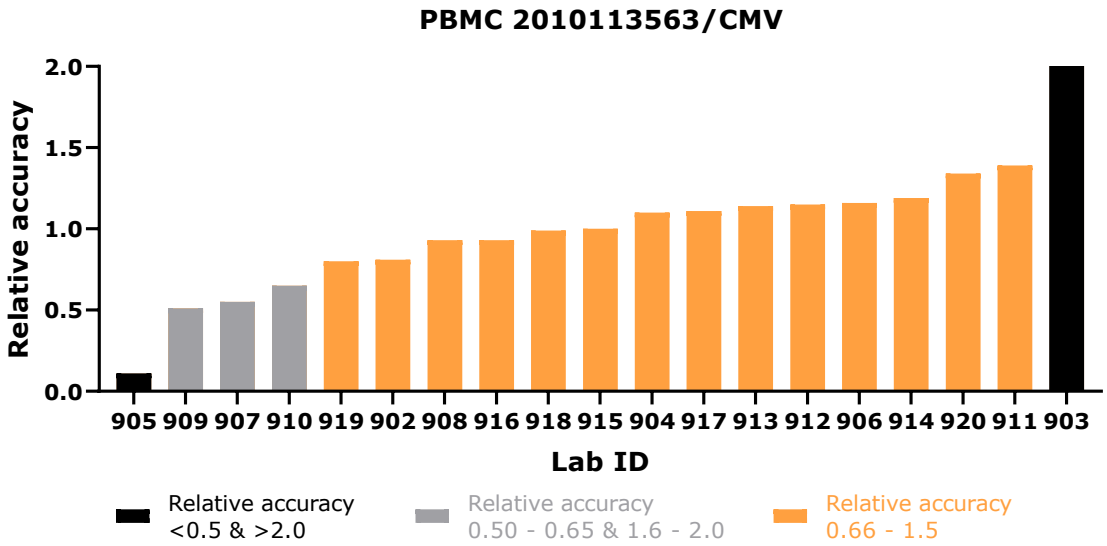

Figure 6 Relative Accuracy for analysis of PBMC 2010113563 stained with the CMV-specific Multimer: HLA-B*3501/IPSINVHHY. 14 out of 19 participants are within "the average range" (orange columns).

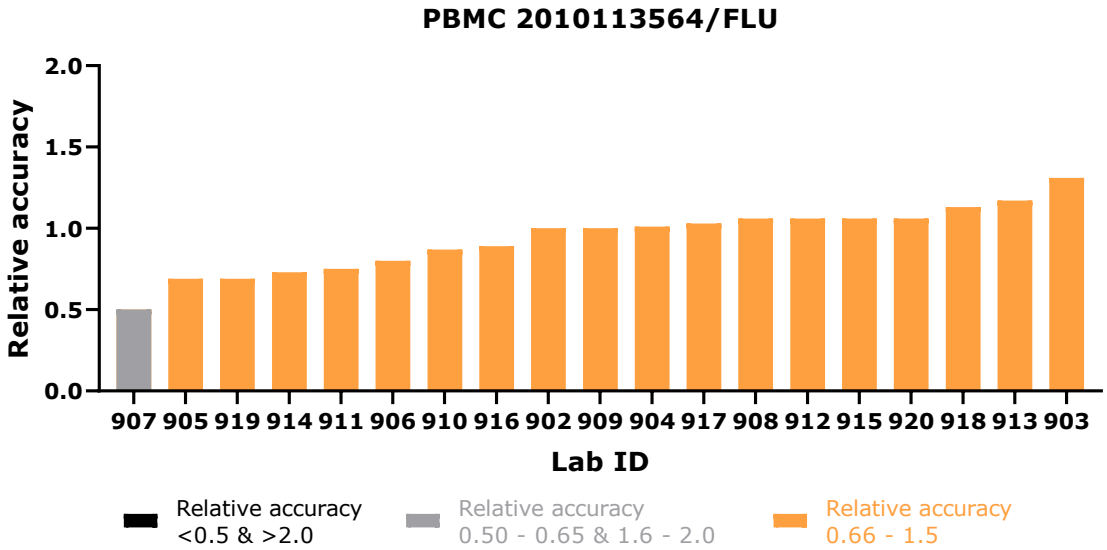

Figure 7 Relative Accuracy for analysis of PBMC 2010113564 stained with the FLU-specific Multimer: HLA-A*0201/GILGFVFTL. 18 out of 19 participants are within "the average range" (orange columns).

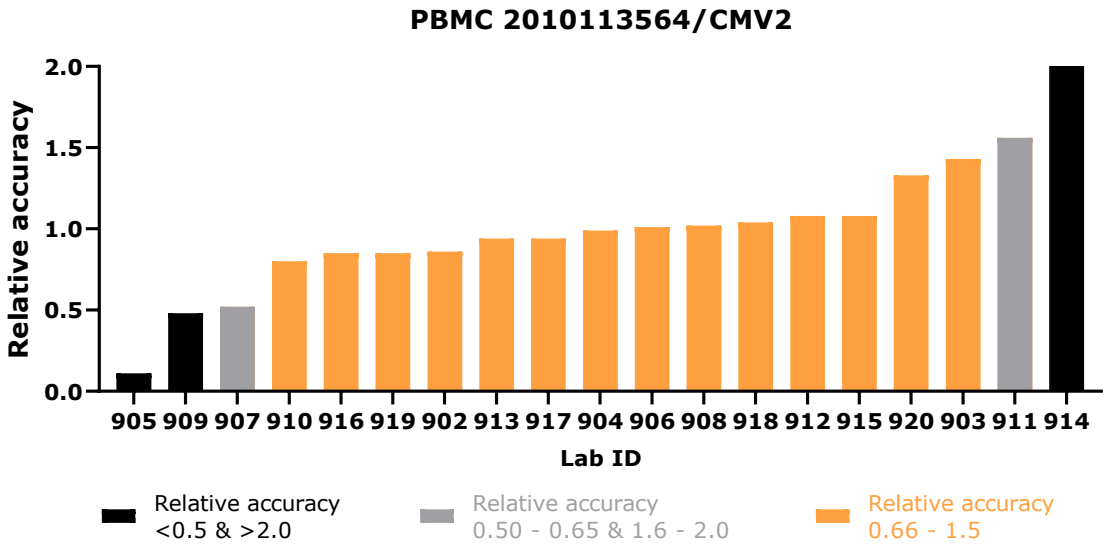

Figure 8 Relative Accuracy for analysis of PBMC 2010113564 stained with the CMV-specific Multimer: HLA-A*0201/NLVPMVATV. 14 out of 19 participants are within "the average range" (orange columns).

OVERALL PROFICIENCY

The overall proficiency of each individual participant was assessed by using a scoring system. Each participant was assigned a proficiency score of 1, 2 or 3 based on the relative accuracies (Table 3), and the overall proficiency of an individual laboratory was then defined by the average score obtained for all four measurements. For example, participants with an overall proficiency of "3" were within the average range on all four measurements and has obtained the highest possible score. Figure 9 shows the overall proficiency of all individual laboratories.

Table 3 Proficiency score to assess the overall performance of each individual laboratory. Each individual laboratory got a proficiency score for all four measurements:

Relative accuracies	Definitions of the different ranges.	Proficiency Score
0.66-1.5	within the average range	3
0.50- 0.65 1.6-2.0	near the average range	2
< 0,50 > 2,0	far from the average range	1

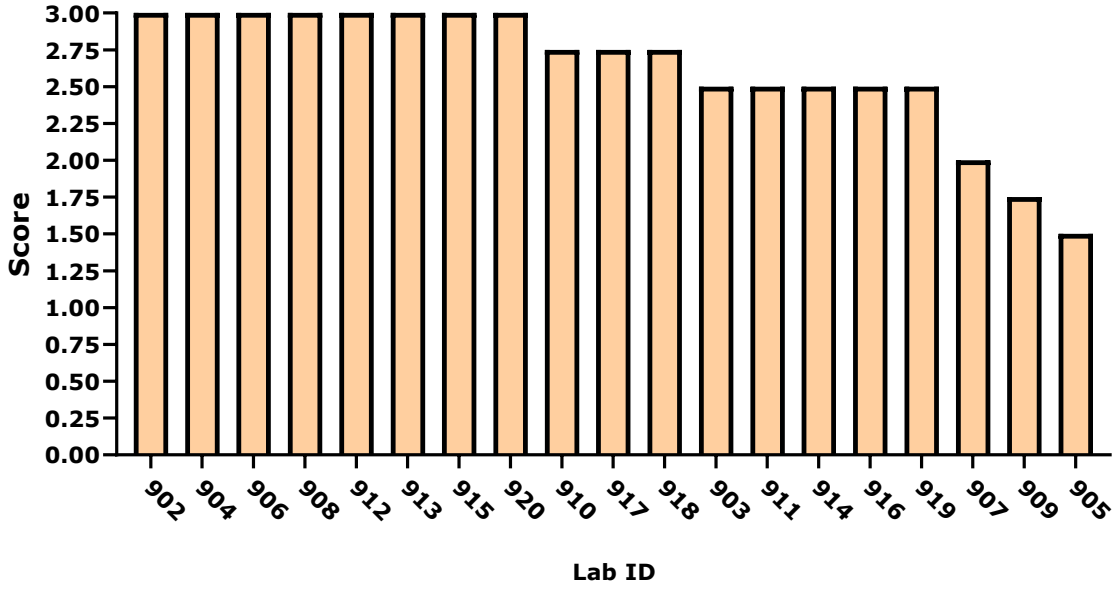

Figure 9. Participants' overall proficiency score in the MHC Multimer Proficiency Panel 2019.

DISCUSSION

The mission of Immudex' Proficiency Panels is to provide a program for laboratories to evaluate their proficiency in monitoring antigen-specific T cell responses. We hope that the Proficiency Panels can contribute to the generation of high-quality data which in turn can drive improvements in immunotherapeutic research and development.

Evaluation of laboratory performance is essential if data generated by different laboratories is going to be compared. With clinical samples being tested at different laboratories in multicenter trials, aligned laboratory proficiency is needed to assess the relative potency of the therapeutic or vaccine candidate being investigated at several locations.

In the MHC Multimer Proficiency Panel participants were able to evaluate their accuracy to enumerate antigen-specific CD8+ T cells using laboratory-specific procedures, informing each laboratory on the degree of concordance of its results with those obtained by the other participating laboratories. Furthermore, this provides each laboratory with the opportunity to check their MHC Multimer assay protocol – to ensure and sustain its ability to accurately identify antigen-specific T-cell responses, or to possibly identify necessary protocol optimization.

76% of all reported measurements were within the average range (defined as 1.5x lower or higher than the median). If the near average range (defined as 1.6 - 2.0 times higher and 0.50 - 0.65 lower than the median) was included - 88% of all reported measurements were covered. Interestingly, identification of the FLU-antigen specific CD8+ T cells, which was a sample with relatively low response (median was 0.08%), 18 out of 19 participants got measurements within the average

range. Usually, measurements for low responses are less aligned. One possible explanation is that the FLU-antigen specific CD8+ T cells population is of high-affinity despite being present in a low frequency, making the positive population easily separable from the negative population and consequently more aligned results among the participants. For the next proficiency panel we aim at finding PBMC donors that have antigen-specific T cells in low responses and additionally are low-affinity T cells in order for the participants to enumerate those antigen-specific T cells that might be more difficult to identify. MHC Multimer Proficiency Panel is an annual event, and Immudex plan to run the next panel in the Fall of 2020.

In the overall proficiency, 16 out of the 19 participating laboratories got a proficiency score of ≥ 2.5. This corresponds to 84% which is in line with the two former MHC Multimer Proficiency Panels (the panels from 2017 and 2018) where 83% and 78% respectively obtained a proficiency score of ≥ 2.5.

This proficiency panel shows that MHC multimer assays in general are well harmonized across different laboratories and thus a useful tool for evaluating treatment response in immunotherapeutic research and development.

ABOUT IMMUDEX

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex manufactures MHC Dextramer® for the detection of antigen-specific T cells.

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, for the quantification of CMV-specific T cells. USA FDA 510(k) clearance for the CMV kit was granted March 2017. GMP Grade reagents are available.

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.

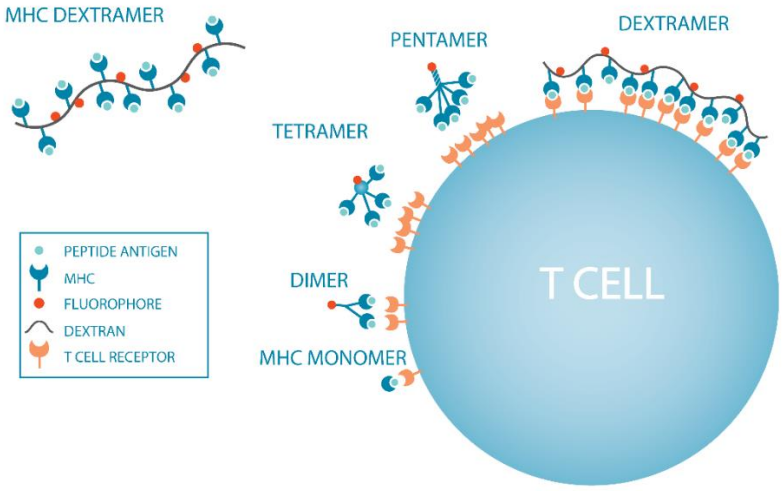

Figure 10 Schematic drawing of MHC Dextramer® and conventional MHC multimer reagents binding to T-cell receptors (TCRs) on the surface of a T cell. MHC Dextramer® reagents are fluorescent labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a strong and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

APPENDIX 1: INSTRUCTIONS FOR PROFICIENCY TESTING

Introduction

This Proficiency Panel evaluates performance of laboratories running MHC multimer assays for determination of antigen-specific T cells in PBMC samples.

In the MHC Multimer Proficiency Panel 2019 participants must determine the percentage of CMV-, FLU- and EBV-specific T cells using predefined MHC Multimer reagents. Analyses are done by flow cytometry.

The purpose of proficiency testing is to evaluate the competency of the staff and the laboratory's processes. The proficiency testing samples should therefore follow the same steps as any routine sample processed by your laboratory, and we encourage participants to analyze samples according to their own protocol.

Identical cell samples (2 different PBMCs) are sent to the participating laboratories. Each participant measures the percentages of antigen-specific T cells in the PBMC samples according to the instructions, but with their own choice of materials (multimer, antibodies, viability marker etc.), and following their own protocol (staining tubes/plates, washing buffer etc.).

After analysis, participants report their results to Immudex. Performance results from all participants are presented in a final report. The participants' names and affiliations are kept anonymous.

Deadlines and Immudex contact

Data submission:	December 20, 2019
Performance report from Immudex:	February 2020

If you have any questions, please contact the organizer:

Rikke Yding Tingleff, PhD at proficiencypanel@immudex.com

Reagents supplied

PBMC samples

Each participant receives two vials of pretested PBMC samples: PBMC 2010113563 and PBMC 2010113564 (each vial contains 10 million cells in 1.5ml). Please store PBMCs at ≤ -150°C.

MHC Multimer reagents

MHC Multimer reagents needed for analysis:

- HLA-B*3501/HPVGEADYFEY MHC Multimer – **EBV-specific**
- HLA-B*3501/IPSINVHHY MHC Multimer – **CMV-specific**
- HLA-A*0201/GILGFVFTL MHC Multimer – **FLU-specific**
- HLA-A*0201/NLVPMVATV MHC Multimer - **CMV-specific**
- Negative Control MHC Multimer

Participants requesting MHC Dextramer® reagents receive these five PE-labeled reagents:

• WK2145-PE	HLA-B*3501/HPVGEADYFEY MHC Dextramer	10 tests
• WK2138-PE	HLA-B*3501/IPSINVHHY MHC Dextramer	10 tests
• WB2161-PE	HLA-A*0201/GILGFVFTL MHC Dextramer	10 tests
• WB2132-PE	HLA-A*0201/NLVPMVATV MHC Dextramer	10 tests
• WB2666-PE	Neg. Control MHC Dextramer	15 tests

MHC Dextramer® reagents must be stored at 2-8°C (protected from light).

Additional reagents needed for analysis

In addition to the MHC Multimer reagents, other relevant reagents for the data analysis must be provided by the participant. This include anti-CD8 antibody and other relevant antibody markers used for exclusion or inclusion of specific cell population(s) (e.g. anti-CD4 antibody, anti-CD3 antibody, viability dye).

You are free to use your own choice of cell markers, staining - and washing buffers, buffer volume, staining tubes/plates, and incubation times.

Experimental setup

Each participant performs a total of 12 analyses, corresponding to 6 analyses on each of the two supplied PBMC samples (Table 4).

Analyze the two supplied donor samples accordingly:

PBMC-2010113563

- Negative control; staining with Negative Control MHC Multimer
- Measurement of EBV-specific CD8+ T cells using EBV (B3501/HPVGEADYFEY) MHC Multimer
- Measurement of CMV-specific CD8+ T cells using CMV (B3501/IPSINVHHY) MHC Multimer

PBMC-2010113564

- Negative control; staining with Negative Control MHC Multimer
- Measurement of FLU-specific CD8+ T cells using FLU (A0201/GILGFVFTL) MHC Multimer
- Measurement of CMV-specific CD8+ T cells using CMV (A0201/NLVPMVATV) MHC Multimer

Please note, that the indicated staining IDs from Table 4 must be used for naming the FCS files.

Table 4 Required analyses for the MHC Multimer Proficiency Panel 2019:

Staining ID	Donor sample	MHC Multimer specificity
R1-563-Neg	PBMC-2010113563	Negative control
R2-563-Neg	PBMC-2010113563	Negative control
R3-563-EBV	PBMC-2010113563	EBV (B3501/HPVGEADYFEY)
R4-563-EBV	PBMC-2010113563	EBV (B3501/HPVGEADYFEY)
R5-563-CMV1	PBMC-2010113563	CMV (B3501/IPSINVHHY)
R6-563-CMV1	PBMC-2010113563	CMV (B3501/IPSINVHHY)
R7-564-Neg	PBMC-2010113564	Negative control
R8-564-Neg	PBMC-2010113564	Negative control
R9-564-FLU	PBMC-2010113564	FLU (A0201/GILGFVFTL)
R10-564-FLU	PBMC-2010113564	FLU (A0201/GILGFVFTL)
R11-564-CMV2	PBMC-2010113564	CMV (A0201/NLVPMVATV)
R12-564-CMV2	PBMC-2010113564	CMV (A0201/NLVPMVATV)

We recommend you to follow guidelines provided by the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC of CRI) and the Association for Cancer Immunotherapy (CIMT).

These guidelines are outlined in Appendix 2: "Multimer Harmonization Guidelines".

Instructions for sample analysis

Cell preparation

Thaw PBMCs and count the cells using your laboratory's preferred procedure.

- Record the total cell number, and the number of viable cells for each of the PBMC samples provided

Staining and gating

Use your own protocol for staining and gating of the MHC Multimer-specific CD8+ T cells.

In addition to the four virus-specific MHC Multimer reagents and the Negative Control MHC Multimer reagent, it is mandatory to:

- Include anti-CD8 antibody staining

Preferably, acquire a minimum of 100,000 CD8+ T cells. To achieve this, we recommend staining at least 1.5 x 10^6 viable cells per staining.

If you use MHC Dextramer® reagents, please read the staining protocol provided with the MHC Dextramer® reagents.

Data recording

For each of the 12 required analyses in this Proficiency Panel, it is mandatory to:

- Record the number of CD8+ T cells (e.g. number of events in gate R6 in Figure 11)
- Record the number of MHC multimer+ CD8+ T cells (e.g. number of events in gate R5 in Figure 11)
- Calculate the percentage of MHC multimer+ CD8+ T cells out of total CD8+ T cells (R5/R6x100 % in Figure 11). All results are recorded with two decimals

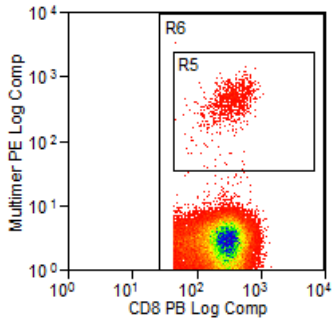

Figure 11 Example of CD8+ MHC Multimer+ cells.
Please see "PowerPoint Dot plot" (provided by email) for gating example.

Reporting data

1. Fill-in the "PowerPoint Dot plot" slide (provided by email) with your gating strategy and dot plots. The dot plots must show the CD8 staining on the x-axis and MHC Multimer staining on the y-axis as illustrated on the first slide of this PowerPoint.

2. Create a Zip file, name it with your Lab ID (provided by email), and include the following files:
    a. The filled-in "PowerPoint Dot plot".
    b. The 12 FCS files, named exactly as described in Table 1.
    c. If acquired, include your single-color compensation files.

3. Data reporting
    a. Go to » Proficiency panel data reporting (If required to, select region).
    b. Upload data Zip file.
    c. Report recorded data and results obtained from sample analysis by filling in the survey.

APPENDIX 2: ASSAY HARMONIZATION GUIDELINES

Multimer Harmonization Guidelines to Optimize Assay Performance

A. **Establish lab SOP for MHC peptide multimer staining:**
   A1. Count at least 100,000 CD8+ T cells per staining. A2. Establish adequate measures to quantify non-specific binding of Multimer to CD8+ cells (e.g. irrelevant Multimer or autofluorescence). A3. Establish adequate measures to reduce the amount of non-specific binding of Multimer in the CD8+ population to allow accurate quantification (e.g. DUMP channel or DEAD cell dyes).

B. **Establish SOP for software analyses of stained samples, including:**
   B1. Gating strategy.
   B2. Rules to set the gates.

C. **Establish a human auditing process of all results:**
   C1. Are all dot plots correctly compensated?
   C2. Have the gates been set correctly?
   C3. Are the reported frequencies of multimer-positive cells plausible?

D. **Lab environment**
   D1. Only let experienced personnel (per lab SOP) conduct assay.

APPENDIX 3: PARTICIPANT DATA

Table 5 Example of relative accuracy calculation on PBMC donor 2010113563 stained with the EBV-specific Multimer: HLA-B*3501/HPVGEADYFEY:

	% of CD8+ Multimer+ T cells						
Lab ID	Neg. control multimer	Neg. control multimer	EBV - specific multimer	EBV - specific multimer	Mean value of Lab ID 902, EBV-specific multimer	Median for all participants, EBV-specific multimer	Relative Accuracy for Lab ID 902 on the EBV-specific Multimer
902	0.00	0.01	0.21	0.21	0.21	0.18	$\frac{0.21}{0.18} = 1.17$

Table 6 shows the doublet values reported by the participants for analysis of PBMC donor lot: 2010113563 stained with the three different MHC multimer reagents. R1, R2: PBMC stained with Negative control multimer (NC), R3, R4: PBMC stained with EBV epitope -specific MHC multimer. R5, R6: PBMC stained with CMV1 epitope -specific MHC multimer:

Lab ID	Cell viability	R1 % CD8+ T cells (NC)	R2 % CD8+ T cells (NC)	R3 % CD8+ EBV-specific T cells	R4 % CD8+ EBV-specific T cells	Relative accuracy EBV-specific T cells	R5 % CD8+ CMV-specific T cells	R6 % CD8+ CMV-specific T cells	Relative accuracy CMV-specific T cells
902	96%	0.00	0.01	0.21	0.21	1.17	0.67	0.68	0.81
903	89.2%	0	0	0.16	0.16	0.89	1.81	1.74	2.14
904	96.1%	0.013	0.00419	0.35	0.16	1.42	0.91	0.91	1.1
905	82%	0.00	0.00	0.04	0.05	0.25	0.10	0.08	0.11
906	93.4%	0.011	0.013	0.134	0.146	0.78	0.992	0.937	1.16
907	100 %	0.0	0.0	0.1	0.12	0.61	0.51	0.40	0.55
908	96.8%	0.01	0.01	0.23	0.24	1.31	0.78	0.77	0.93
909	88%	0	0	0.07	0.09	0.44	0.38	0.46	0.51
910	79%	0.011	0.00467	0.16	0.18	0.94	0.54	0.54	0.65
911	90%	0.00	0.01	0.28	0.32	1.67	1.14	1.16	1.39
912	94%	0.00	0.00	0.24	0.2	1.22	0.94	0.97	1.15
913	64%	0.012	0.006	0.25	0.24	1.36	0.91	0.98	1.14
914	62%	0.000	0.002	0.184	0.209	1.09	0.991	0.985	1.19
915	97.3%	0.01	0.03	0.20	0.18	1.06	0.85	0.81	1
916	95.3%	.002	.001	.087	.061	0.41	.758	.788	0.93
917	96%	0.00	0.00	0.28	0.29	1.58	0.87	0.97	1.11
918	84%	0	0	0.10	0.09	0.53	0.80	0.85	0.99
919	98.5%	0.00	0.00	0.06	0.05	0.31	0.66	0.67	0.8
920	97.5%	0.00	0.00	0.27	0.28	1.45	1.1	1.13	1.34

Table 7 shows the doublet values reported by the participants for analysis of PBMC donor lot: 2010113564 stained with the three different MHC multimer reagents. R7, R8: PBMC stained with Negative control multimer (NC), R9, R10: PBMC stained with FLU epitope -specific MHC multimer. R11, R12: PBMC stained with CMV2 epitope-specific MHC multimer:

Lab ID	Cell viability	R7 % CD8+ T cells (NC)	R8 % CD8+ T cells (NC)	R9 % CD8+ FLU-specific T cells	R10 % CD8+ FLU-specific T cells	Relative accuracy FLU-specific T cells	R11 % CD8+ CMV-specific T cells	R12 % CD8+ CMV-specific T cells	Relative accuracy CMV-specific T cells
902	98%	0.00	0.00	0.09	0.07	1	0.37	0.35	0.88
903	90.6%	0	0	0.11	0.1	1.31	0.62	0.58	1.46
904	95.4%	0.0045	0.00167	0.072	0.089	1.01	0.43	0.4	1.01
905	88%	0.00	0.00	0.06	0.05	0.69	0.05	0.04	0.11
906	95.6%	0.011	0.008	0.073	0.055	0.8	0.424	0.426	1.04
907	100%	0.0	0.0	0.04	0.04	0.5	0.23	0.21	0.54
908	98.3%	0	0	0.09	0.08	1.06	0.44	0.42	1.05
909	91%	0	0	0.08	0.08	1	0.18	0.22	0.49
910	91%	0.0031	0.0032	0.075	0.064	0.87	0.34	0.33	0.82
911	90%	0.00	0.00	0.06	0.06	0.75	0.61	0.70	1.6
912	95%	0	0,00	0.09	0.08	1.06	0.46	0.45	1.11
913	70%	0.028	0.085	0.091	0.096	1.17	0.38	0.41	0.96
914	47.4%	0.003	0.002	0.053	0.063	0.73	1.020	0.994	2.46
915	97.1%	0.00	0.00	0.08	0.09	1.06	0.46	0.45	1.11
916	97.4%	0	.001	.066	.076	0.89	.349	.366	0.87
917	98%	0.00	0.00	0.089	0.076	1.03	0.39	0.40	0.96
918	72%	0	0	0.10	0.08	1.13	0.41	0.46	1.06
919	97.7%	0.00	0.00	0.06	0.05	0.69	0.36	0.35	0.87
920	97.3%	0.00	0.00	0.08	0.09	1.06	0.56	0.56	1.33

APPENDIX 4: PRETEST RESULTS

At Immudex we pretested the PBMCs before sending them to the participants. MHC Multimer staining was performed according to the instructions, but with 3 vials from each PBMC Donor (Lot: 2010113563, and Lot: 2010113564), giving a total of 6 vials stained with Dextramer specificities listed in Table 1. Viability of all 6 PBMC samples were in the range of 97-98%.

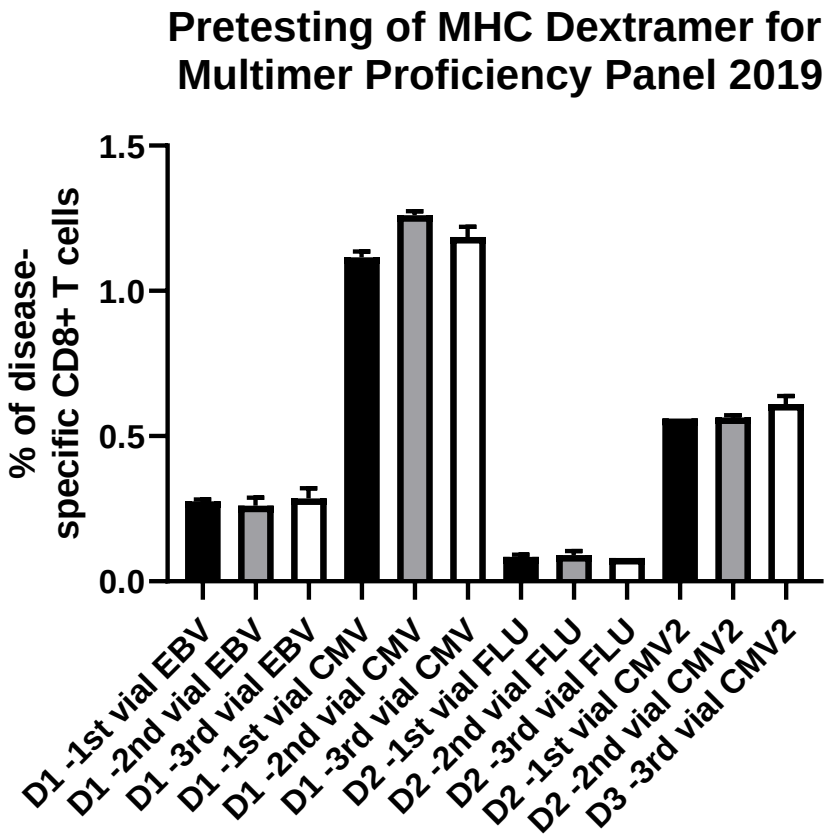

Figure 12 Result of pretesting the PBMCs. D1 represents PBMC lot: 2010113563, and D2 represents 2010113564. 3 vials of each donor were stained with the Dextramer reagents listed in Table 1 (EBV-epitope, CMV-epitope, FLU-epitope, or CMV epitope specific Dextramer). Duplicate measurements were made for each Dextramer/PBMC vial combination.