MHC Multimer Proficiency Panel 2021

May 2022

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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.

Immudex Proficiency Panels are programs that provide laboratories worldwide with the opportunity to assess their proficiency in monitoring antigen-specific T-cell responses. It is a non-profit service offered to increase the proficiency among researchers and clinicians who perform the immune monitoring assays, MHC multimer, and T-cell ELISpot. The Proficiency Panels are open to any laboratory, independent of geographic location or field of interest.

In 2013, Immudex took over the Proficiency Panels from the CIC of CRI (Cancer Immunotherapy Consortium of the Cancer Research Institute, USA) and the CIMT (Association for Cancer Immunotherapy, Europe). We are very honored to be responsible for conducting Proficiency Panels and continue the age-long efforts of CIC and CIMT to improve the level of accuracy, robustness, and reliability of the immune monitoring assays. Read more about the Proficiency Panels here.

Immudex Proficiency Panels are conducted yearly, and the next MHC Multimer Proficiency Panel will take place in the fall of 2022.

MHC MULTIMER PROFICIENCY PANEL 2021

In the MHC Multimer Proficiency Panel 2021, 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 CMV-, and EBV-epitopes. The two PBMC samples were pretested at Immudex to ensure consistent results between vials and to check viability of the cells. Viability of the tested PBMC samples were in the range of 96-98%. 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.).

This report shows the test results and overall performance of the participants without revealing their names and affiliations.

In this Multimer Proficiency Panel:

- 10 laboratories from 6 different countries participated.
- All the participants used MHC Dextramer® reagents
- 5 participants were from Academia, and 5 participants were from industry.
- 90% of the participating laboratories got a proficiency score of ≥ 2.0.

ANALYSES

Table 1 MHC Multimer reagents used in the MHC Multimer Proficiency Panel 2021:

MHC Multimer reagent	***MHC Multimer specificity***
HLA-A*0101/VTEHDTLLY	*CMV-specific*
HLA-A*0201/NLVPMVATV	*CMV-specific*
HLA-A*0201/CLGGLLTMV	*EBV-specific*
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 two pretested PBMC samples (HHU20181002 and HHU20190623)
- Received MHC Dextramer® reagents if requested, or used their own MHC Multimer reagents
- Was recommended to look at the "Assay Harmonization Guidelines" (Appendix 2)
- Was encouraged to analyze samples with their own standard protocol to reflect routine sample analysis conducted in their laboratory.
- 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
- Reported FCS files, compensation files, and "PowerPoint Dot plot" file.

RESULTS

10 participants in this year's MHC Multimer Proficiency Panel reported their data. Figure 1-4 show results of the duplicate measurements made by each participant. Percentage of antigen-specific CD8+ T cells were calculated out of the total number of CD8+ T cells. The raw data is presented in Appendix 3.

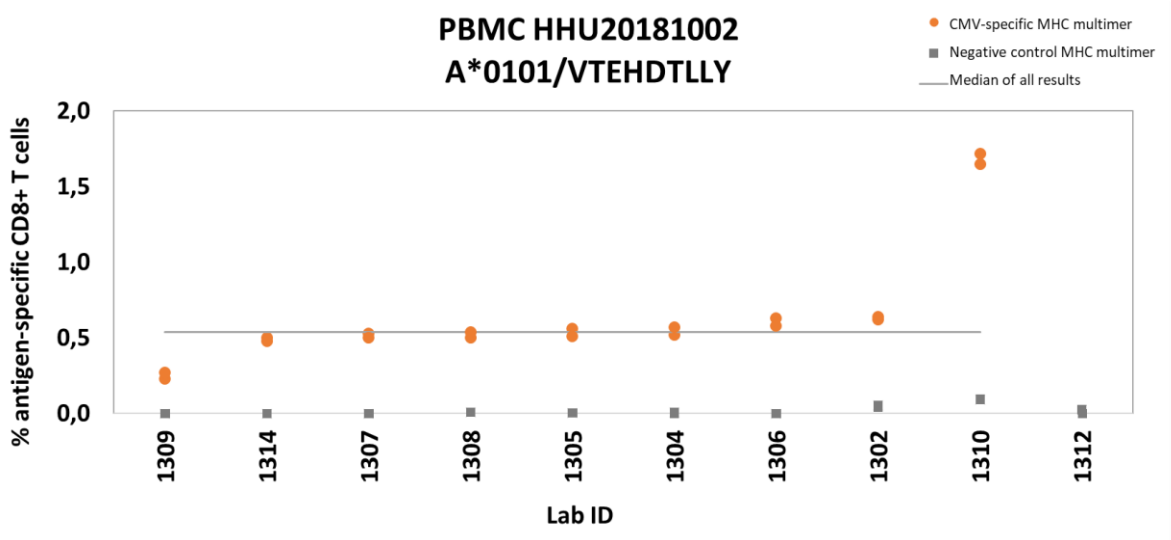

Figure 1 Results from analysis of samples PBMC HHU20181002 stained with HLA-A*0101/VTEHDTLLY MHC Multimer (CMV-specific) and Negative control MHC Multimer. Median for CMV-specific CD8+ T cells is 0,54%. * The result reported for the lab 1312 was omitted because it was far outside the generally observed range for this assay point.

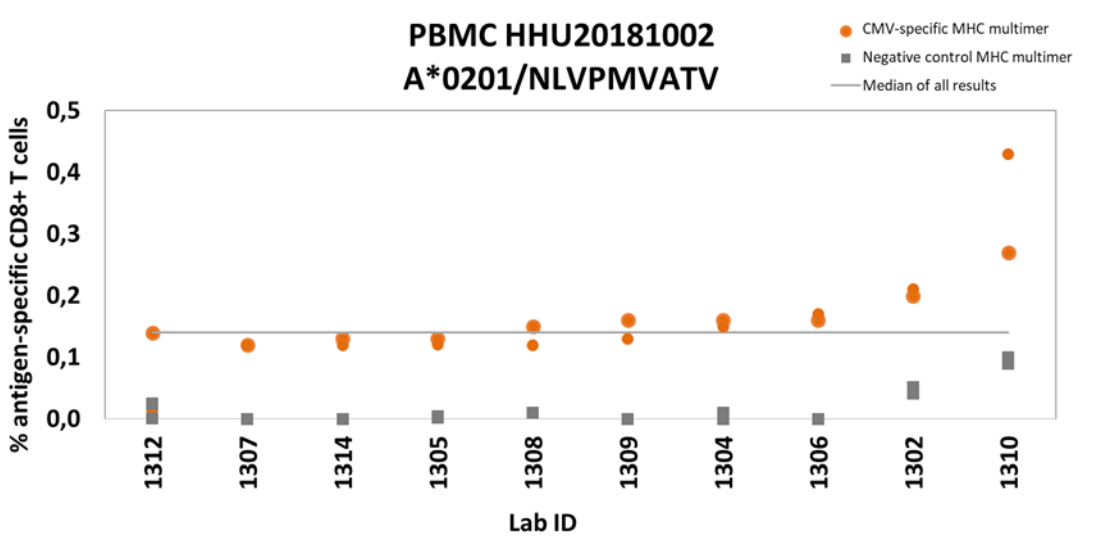

Figure 2 Results from analysis of samples PBMC HHU20181002 stained with HLA-A*0201/NLVPMVATV MHC Multimer (CMV-specific) and Negative control MHC Multimer. Median for CMV-specific CD8+ T cells is 0.14%.

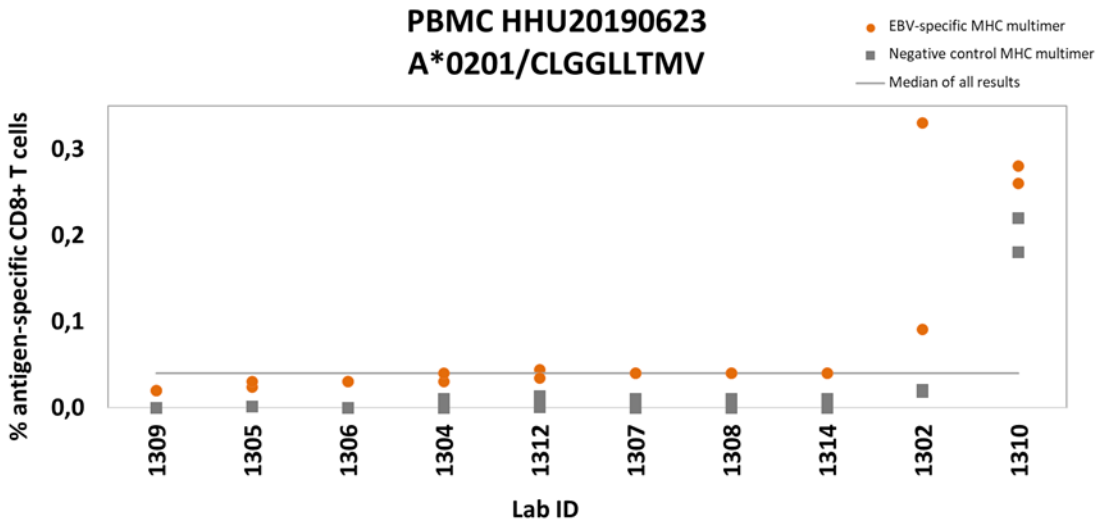

Figure 3 Results from analysis of samples PBMC HHU20190623 stained with HLA-A*0201/CLGGLLTMV MHC Multimer (EBV-specific) and Negative control MHC Multimer. Median for EBV-specific CD8+ T cells is 0,04%.

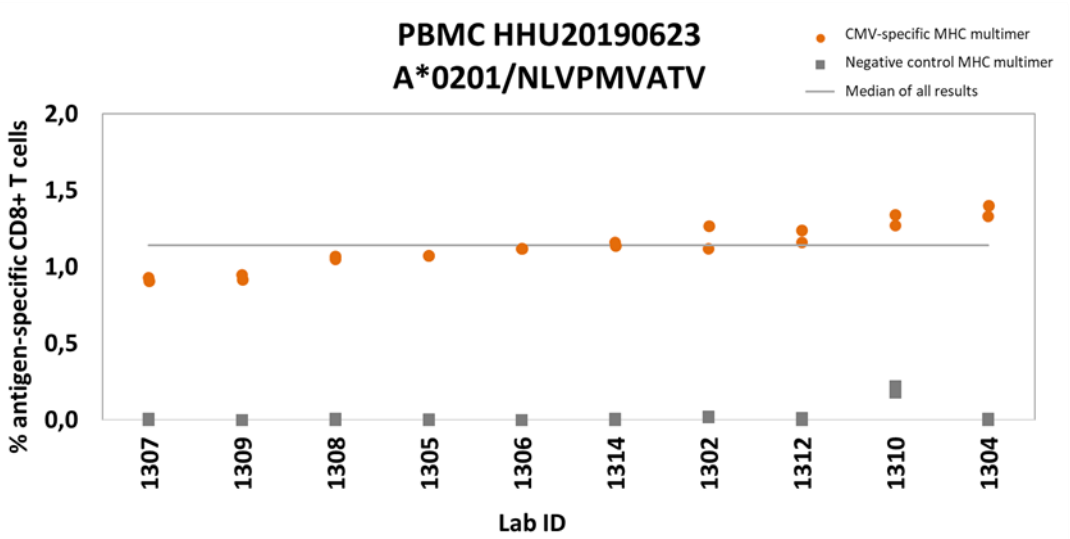

Figure 4 Results from analysis of samples PBMC HHU20190623 stained with HLA-A*0201/NLVPMVATV MHC Multimer (CMV-specific) and Negative control MHC Multimer. Median for CMV-specific CD8+ T cells is 1,14%.

PERFORMANCE

To evaluate the accuracy of each participants' measurements, we calculated the relative accuracy. The relative accuracy is calculated as the mean value of the duplicates for each participant measurement divided by the median for all participants. The relative accuracy tells you how close each participant is to the average value reported by all participants. See appendix 4 for calculation example of relative accuracy. The medians shown in Figure 1-4 were used as the average value to calculate the relative accuracy. The individual laboratories' relative accuracies are presented in Figures 5-8, and the definition of what the values correspond to is listed in Table 2.

Table 2 Definition of the relative accuracy:

Relative accuracy	***Corresponds to***	***Presented in the figures as***
0,66 – 1,5	*within the average range*	*orange columns*
0,50 – 0,65 ***1,6 – 2,0***	*near the average range*	*grey columns*
< 0,50 ***> 2,0***	*far from the average range*	*black columns*

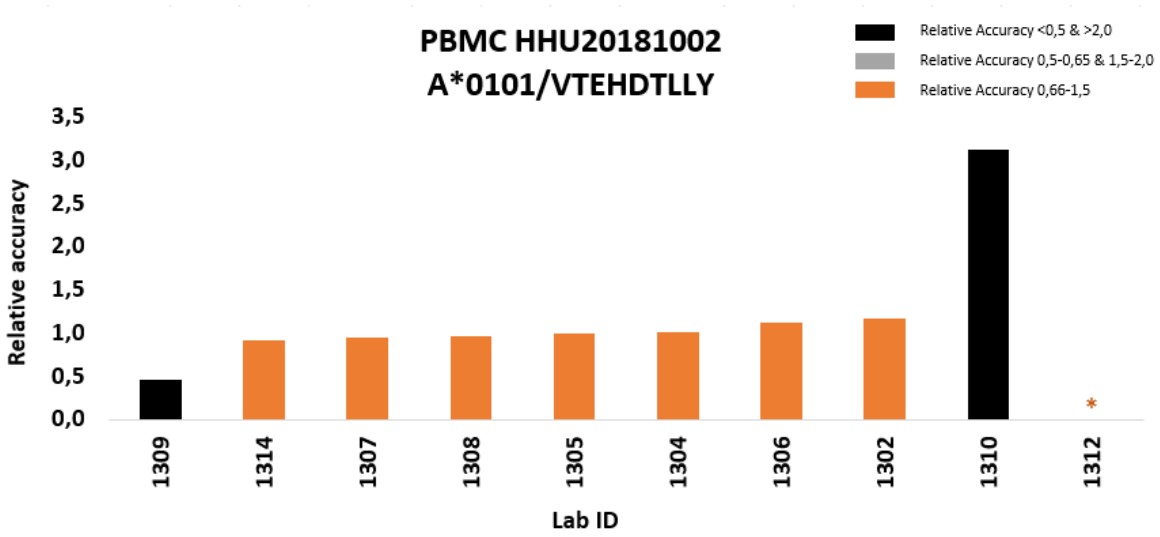

Figure 5 Relative accuracy for analysis of PBMC HHU20181002 stained with the CMV-specific Multimer: HLA-A*0101/VTEHDTLLY. 7 out of 10 participants are within "the average range" (orange columns). *The result reported for the lab 1312 was omitted because it was far outside the generally observed range for this assay point.

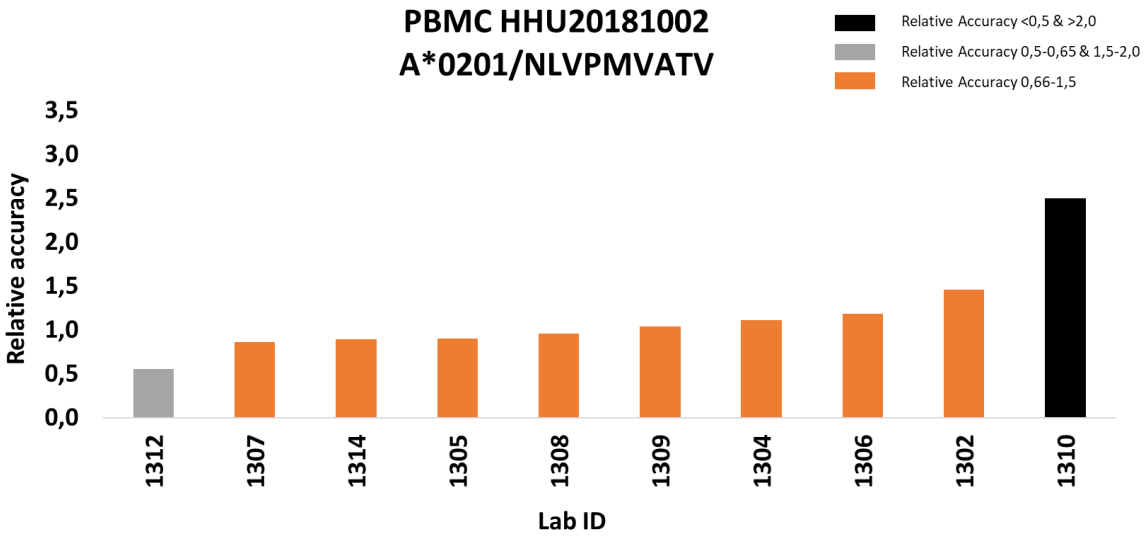

Figure 6 Relative accuracy for analysis of PBMC HHU20181002 stained with the CMV-specific Multimer: HLA- A*0201/NLVPMVATV. 8 out of 10 participants are within "the average range" (orange columns).

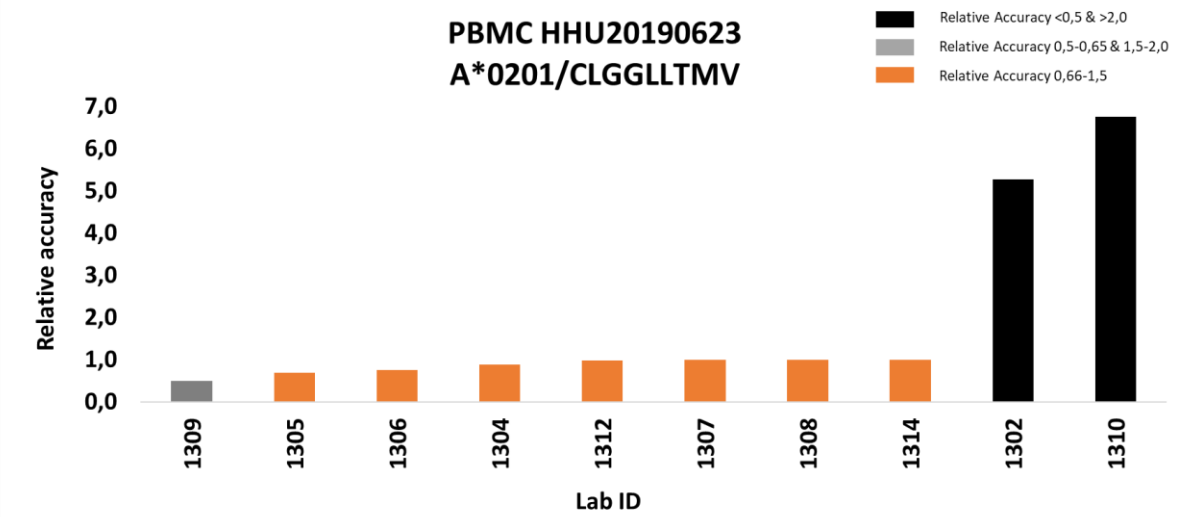

Figure 7 Relative accuracy for analysis of PBMC HHU20190623 stained with the EBV-specific Multimer: HLA-A*0201/CLGGLLTMV. 7 out of 10 participants are within "the average range" (orange columns).

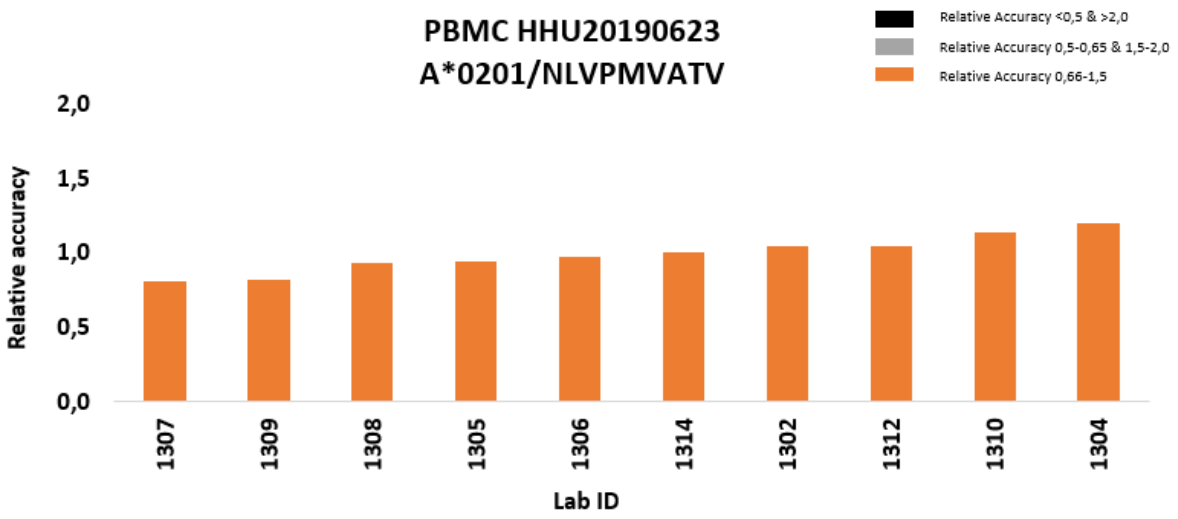

Figure 8 Relative accuracy for analysis of PBMC HHU20190623 stained with the CMV-specific Multimer: HLA-A*0201/NLVPMVATV. All participants are within "the average range" (orange columns).

PROFICIENCY PERFORMANCE

Each participant's ability to identify antigen-specific T cells were described with an overall proficiency score. For each analysis, the participants were assigned a proficiency score between 1-3 based on the relative accuracy (Table 3). The overall proficiency was then defined by the average score obtained for all four analyses. Thus, a participant with an

overall proficiency of 3 is within the average range on all four measurements and has obtained the highest possible score. Figure 9 shows the overall proficiency of all the participants.

Table 3 Proficiency score to assess the overall performance of each individual laboratory. Each laboratory got a proficiency score based on all four analyses.

Relative accuracy	***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*

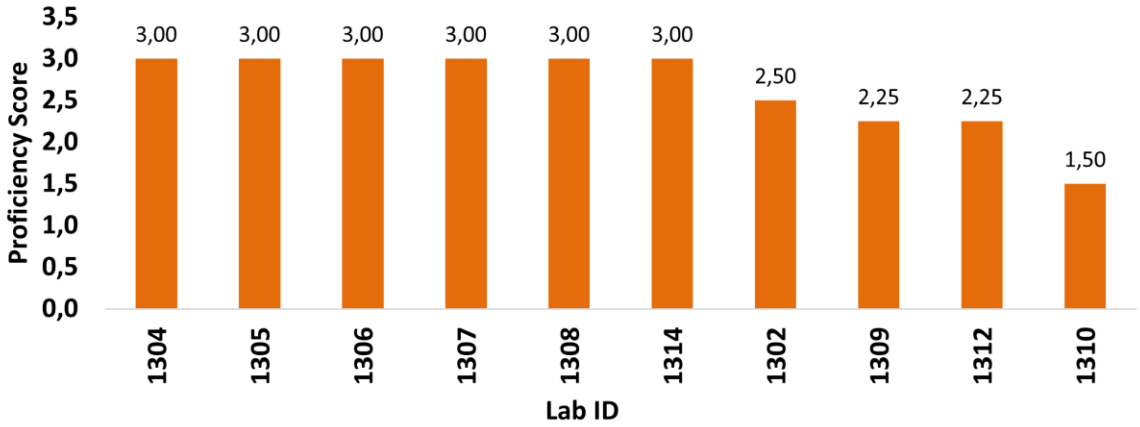

Figure 9. Overall proficiency score of the participants in the MHC Multimer Proficiency Panel 2021.

DISCUSSION

Immudex MHC Multimer Proficiency Panels provide a program for laboratories worldwide to evaluate their proficiency in monitoring antigen-specific T-cell responses using flow cytometry-based MHC Multimer assay. Evaluation of laboratory performance is essential to ensure alignment and drive research and development improvements. Harmonized laboratory performance is of high importance in multicenter trials, where clinical results from different sites are compared to evaluate treatment response in immunotherapeutic research and development.

In the MHC Multimer Proficiency Panel 2021, participants used their own laboratory-specific assay protocol to enumerate antigen-specific CD8+ T cells using MHC Dextramer® reagents in a flow cytometry-based assay. This report provides each participant with information on how aligned their results are with the rest of the participants. This critical knowledge allows each laboratory with the opportunity to evaluate their MHC Multimer assay protocol:

- to ensure and sustain their ability to identify antigen-specific T-cell responses accurately and reproducibly
- to align their assay with other researchers across sites
- to identify necessary protocol optimizations.

The results from the MHC Multimer Proficiency Panel 2021 show that almost all laboratories have equivalent duplicates with very little variation for both the positive and negative MHC multimer specificities.

To assess the performance of the participants, the relative accuracy was compared and 80% of all reported measurements were found to be in the average range (defined as a relative accuracy of 0,66 – 1,5) or in the near average range (defined as a relative accuracy of 0,50 – 0,65 and 1,6 – 2,0).

In the overall proficiency score, 9 out of the 10 participants (90%) got a proficiency score of ≥ 2.0 corresponding to being in the average or near average range. This result is very similar to what was observed in the last three MHC Multimer Proficiency Panels from 2018, 2019 and 2020, where 89%, 89% and 80% of the participants, respectively, obtained a proficiency score of ≥ 2,0. The participants results were equally harmonized in the analyses of PBMCs with low, medium, and high number of antigen-specific CD8+ T-cell responses (Mean of low (0,08%), medium (0,13% and 0,64%), and High (1,13%), respectively.

Conclusively, this Multimer Proficiency Panel shows that MHC Multimer assays are:

- well harmonized across different laboratories
- equally harmonized when looking at high-frequent T-cell responses and low-frequent T-cell responses
- 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.

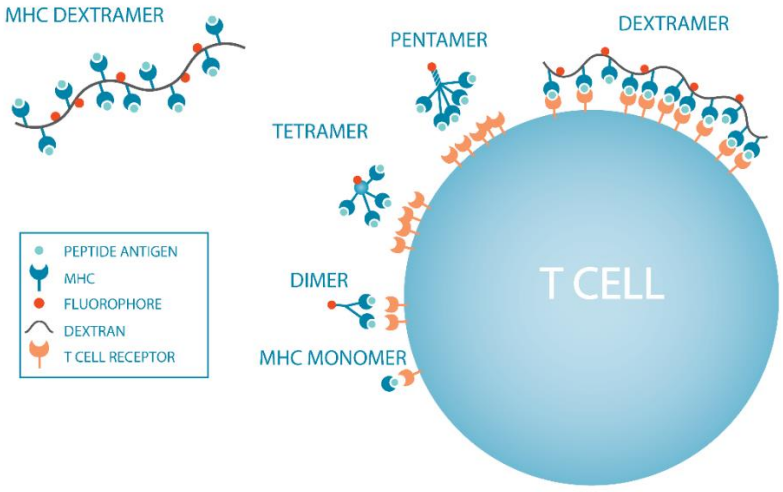

Figure 10 Schematic drawing of MHC Dextramer® and conventional MHC multimer reagents binding to T-cell receptors (TCRs) on the surface of a T cell. MHC Dextramer® reagents are fluorescent labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a strong and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

PLEASE READ INSTRUCTIONS CAREFULLY BEFORE THAWING AND STAINING THE CELLS

Introduction

Making accurate, reproducible, and state-of-the-art T-cell immune monitoring analysis is becoming increasingly important in immunotherapeutic research and development. By participating in this Proficiency Panel, you get the chance to assess how well you detect antigen-specific CD8+ T cells using predefined MHC Multimer reagents.

Please analyze the two PBMC samples according to these instructions and report your results back to Immudex. Results and performance from all participants are presented in a final report, where participant's names and affiliation are kept anonymous. In the report. you will be able to see how accurately you enumerated the antigen-specific CD8+ T cells compared to the other participating laboratories. This will provide you with the opportunity to check your MHC Multimer assay protocol – to ensure and sustain its ability to accurately identify antigen-specific T-cell responses, or to possible identity necessary protocol optimization.

We recommend you have a look at the Multimer Harmonization Guidelines (Appendix 2).

Deadlines and Immudex contact

Data submission:	December 20, 2021
Report from Immudex:	May 2022

If you have any questions, please contact the organizer by email:
proficiencypanel@immudex.com

PBMC samples

You will receive two vials of pretested PBMC samples:
PBMC HHU20181002 and PBMC HHU20190623, (each vial contains ≥ 10 million cells in 1,5ml).
Please store PBMCs at ≤ -150°C.

MHC Multimer reagents

The following MHC Multimer reagents are needed for analysis:

- HLA-A*0101/VTEHDTLLY MHC Multimer – **CMV-specific**
- HLA-A*0201/NLVPMVATV MHC Multimer – **CMV-specific**
- HLA-A*0201/CLGGLLTMV MHC Multimer – **EBV-specific**
- Negative Control MHC Multimer

If you have requested MHC Dextramer® reagents, you will receive these five PE-labeled reagents:

• WA2131-PE	HLA-A*0101/VTEHDTLLY MHC Dextramer®	10 tests
• WB2132-PE	HLA-A*0201/NLVPMVATV MHC Dextramer®	10 tests
• WB2144-PE	HLA-A*0201/CLGGLLTMV 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

We recommend you use your own choice of materials (multimer, antibodies, viability marker etc.), and protocol (staining tubes/plates, washing buffer etc.) for these MHC Multimer analyses to make it reflect routine sample analysis being conducted in your laboratory. However, it is necessary to include anti-CD8 antibody in your staining.

Experimental setup

Analyze the two PBMC samples as listed below in duplicates (summarized in Table 1). Please note, that the indicated staining ID's must be used when naming the FCS files.

Stain PBMC HHU20181002 with:

- Negative Control MHC Multimer
- HLA-A*0101/VTEHDTLLY MHC Multimer
- HLA-A*0201/NLVPMVATV MHC Multimer

Stain PBMC HHU20190623 with:

- Negative Control MHC Multimer
- HLA-A*0201/CLGGLLTMV MHC Multimer
- HLA-A*0201/NLVPMVATV MHC Multimer

Table 4 Required analysis for the MHC Multimer Proficiency Panel 2021

Staining ID	*PBMC Donor*	*MHC Multimer specificity*
R1	*PBMC HHU20181002*	*Negative control*
R2	*PBMC HHU20181002*	*Negative control*
R3	*PBMC HHU20181002*	*A*0101/VTEHDTLLY*
R4	*PBMC HHU20181002*	*A*0101/VTEHDTLLY*
R5	*PBMC HHU20181002*	*A*0201/NLVPMVATV*
R6	*PBMC HHU20181002*	*A*0201/NLVPMVATV*
R7	*PBMC HHU20190623*	*Negative control*
R8	*PBMC HHU20190623*	*Negative control*
R9	*PBMC HHU20190623*	*A*0201/CLGGLLTMV*
R10	*PBMC HHU20190623*	*A*0201/CLGGLLTMV*
R11	*PBMC HHU20190623*	*A*0201/NLVPMVATV*
R12	*PBMC HHU20190623*	*A*0201/NLVPMVATV*

Instructions for sample analysis

Cell preparation

Thaw PBMCs and count the cells. For each of the two PBMCs please record:

- The total cell numbers.
- The viability of the cells

Staining and gating

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

Preferable, acquire a minimum of 100 000 CD8+ T cells for each of the 12 required analysis. 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 reagents.

Data recording

For each of the 12 analyses, you will need to:

- Record the number of CD8+ T cells (e.g., number of events in gate R6 in Figure 1).
- Record the number of MHC multimer+ CD8+ T cells (e.g., number of events in gate R5 in Figure 1).
- Calculate the percentage of MHC multimer+ CD8+ T cells out of total CD8+ T cells (R5/R6x100 % in Figure 1). Please record all results with two decimals.

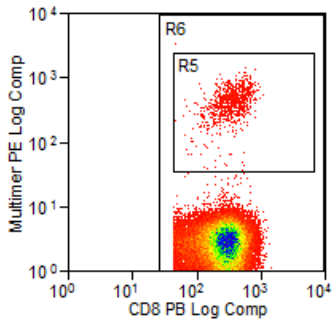

Figure 9 Example of CD8+ MHC Multimer+ cells.
Please see "PowerPoint Dot plot" (provided by email) for gating example.

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.

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. Upload data zip file here: https://immudex.sharefile.com/i/i24a3625aace41a3b
    b. Report data and results obtained from sample analysis in this survey: https://immudex.wufoo.com/forms/qkf5x7e0qtu4rc/

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 shows the doublet values reported by the participants for analysis of PBMC donor lot: HHU20181002 stained with the three different MHC multimer reagents. R1, R2: PBMC stained with Negative control multimer (NC), R3, R4: PBMC stained with CMV epitope-specific MHC multimer. R5, R6: PBMC stained with the other CMV epitope-specific MHC multimer:

Lab ID	Cell viability PBMC HHU20181002	R1 % CD8+ T cells (NC)	R2 % CD8+ T cells (NC)	R3 % CD8+ CMV-specific T cells	R4 % CD8+ CMV-specific T cells	Relative accuracy CMV-specific T cells	R5 % CD8+ CMV-specific T cells	R6 % CD8+ CMV-specific T cells	Relative accuracy CMV-specific T cells
1302	86	0,04	0,05	0,64	0,62	1,17	0,20	0,21	1,46
1304	76	0,00	0,01	0,52	0,57	1,01	0,16	0,15	1,11
1305	NA	0,00	0,00	0,56	0,51	0,99	0,13	0,12	0,90
1306	99	0,00	0,00	0,63	0,58	1,12	0,16	0,17	1,18
1307	94	0,00	0,00	0,53	0,50	0,95	0,12	0,12	0,86
1308	96	0,01	0,01	0,50	0,54	0,96	0,15	0,12	0,96
1309	63	0,00	0,00	0,23	0,27	0,46	0,16	0,13	1,04
1310	95	0,10	0,09	1,72	1,65	3,12	0,27	0,43	2,50
1312	98	0,00	0,03	99,60	99,60	184,44	0,14	0,01	0,55
1314	100	0,00	0,00	0,50	0,48	0,91	0,13	0,12	0,89

NB: Lab 1305 did not report results appropriately upon submission, so some values are missing and marked with "NA"

Table 6 shows the doublet values reported by the participants for analysis of PBMC donor lot: HHU20190623 stained with the three different MHC multimer reagents. R7, R8: PBMC stained with Negative control multimer (NC), R9, R10: PBMC stained with EBV epitope-specific MHC multimer. R11, R12: PBMC stained with CMV epitope-specific MHC multimer:

Lab ID	Cell viability PBMC HHU20190623	R7 % CD8+ T cells (NC)	R8 % CD8+ T cells (NC)	R9 % CD8+ EBV-specific T cells	R10 % CD8+ EBV-specific T cells	Relative accuracy EBV-specific T cells	R11 % CD8+ CMV-specific T cells	R12 % CD8+ CMV-specific T cells	Relative accuracy CMV-specific T cells
1302	70	0,02	0,02	0,09	0,33	5,26	1,12	1,27	1,05
1304	83	0,01	0,00	0,03	0,04	0,88	1,33	1,40	1,20
1305	NA	0,00	0,00	0,02	0,03	0,68	1,07	1,07	0,94
1306	98	0,00	0,00	0,03	0,03	0,75	1,12	1,12	0,98
1307	96	0,01	0,00	0,04	0,04	1,00	0,93	0,91	0,81
1308	98	0,00	0,01	0,04	0,04	1,00	1,05	1,07	0,93
1309	85	0,00	0,00	0,02	0,02	0,50	0,95	0,92	0,82
1310	98	0,22	0,18	0,26	0,28	6,75	1,27	1,34	1,14
1312	99	0,01	0,00	0,04	0,03	0,98	1,16	1,24	1,05
1314	100	0,01	0,00	0,04	0,04	1,00	1,16	1,14	1,01

NB: Lab 1305 did not report results appropriately upon submission, so some values are missing and marked with "NA"

APPENDIX 4: CALCULATION OF THE RELATIVE ACCURACY

Table 7 Example of relative accuracy calculation on PBMC donor HHU20181002 stained with the HLA-B*0801/FLRGRAYGL.

Lab ID	*EBV - specific multimer*	*EBV - specific multimer*	*Mean value of Lab ID 1105, EBV-specific multimer*	*Median for all participants, EBV-specific multimer*	*Relative Accuracy (mean/median) for Lab ID 1105 on the EBV-specific Multimer*
1105	*0,24*	*0,26*	*0,25*	*0,24*	$\frac{0,25}{0,24} = 1,04$

Instructions for MHC Multimer Proficiency Panel 2021

PLEASE READ THE INSTRUCTIONS CAREFULLY BEFORE THAWING AND STAINING THE CELLS

Introduction

Making accurate, reproducible, and state-of-the-art T-cell immune monitoring analysis is becoming increasingly crucial in immunotherapeutic research and development. Supported by the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC of CRI) and the Association for Cancer Immunotherapy (CIMT), Immudex conducts Proficiency Panels annually, allowing laboratories to assess their performance in monitoring antigen-specific T-cell responses. By participating in this Proficiency Panel, you get the chance to evaluate how accurately you detect antigen-specific CD8+ T cells using predefined MHC Multimer reagents.

Each participant is asked to analyze the two PBMC samples according to these instructions but following their own protocol. We encourage participants to analyze samples with their own protocol to reflect routine sample analysis. We also recommend that participants 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. All participants' results and performance are presented in a final report, where participants' names and affiliations are kept anonymous. In the report, you will see how accurately you enumerated the antigen-specific CD8+ T cells compared to the other participating laboratories. This will provide you with the opportunity to check your MHC Multimer assay protocol – to ensure and sustain its ability to accurately identify antigen-specific T-cell responses, or to possibly identify necessary protocol optimizations.

Deadlines and Immudex Contact

Data submission: December 20, 2021

Report from Immudex: June, 2022

If you have any questions, please get in touch with the Proficiency Panel Coordinator at:

proficiencypanel@immudex.com

PBMC Samples

You will receive two vials of pretested PBMC samples:

PBMC HHU20181002 and PBMC HHU20190623 (each vial contains ≥ 10 million cells in 1.5 ml).

Please store PBMC samples at ≤ -150°C.

Multimer reagents

The following MHC Multimer reagents are needed for analysis:

- HLA-A*0101/VTEHDTLLY MHC Multimer – CMV-specific
- HLA-A*0201/NLVPMVATV MHC Multimer – CMV-specific
- HLA-A*0201/CLGGLLTMV MHC Multimer – EBV-specific
- Negative Control MHC Multimer

If you have requested MHC Dextramer® reagents, you will receive these four PE-labeled reagents:

▪ WA02131 PE 10	HLA-A*0101/VTEHDTLLY MHC Dextramer®	10 tests
▪ WB02132 PE 15	HLA-A*0201/NLVPMVATV MHC Dextramer®	15 tests
▪ WB02144-PE 10	HLA-A*0201/CLGGLLTMV MHC Dextramer®	10 tests
▪ WB02666-PE 15	Neg. Control MHC Dextramer®	15 tests

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

Additional reagents needed for analysis

We recommend you use your own choice of materials (multimers, antibodies, viability marker, etc.), and protocol (staining tubes/plates, washing buffer, etc.) for these MHC Multimer analyses to make it reflect routine sample analysis conducted in your laboratory. However, it is necessary to include anti-CD8 antibodies in your staining.

Experimental setup

Analyze the two PBMC samples as listed below in duplicates (summarized in Table 1). Please note that the Lab ID (provided by email) and the indicated staining ID's must be used when naming the FCS files (e.g "Lab ID_1010_R1").

Stain PBMC HHU20181002 with:

- Negative Control MHC Multimer
- HLA-A*0101/VTEHDTLLY MHC Multimer
- HLA-A*0201/NLVPMVATV MHC Multimer

Stain PBMC HHU20190623 with:

- Negative Control MHC Multimer
- HLA-A*0201/CLGGLLTMV MHC Multimer
- HLA-A*0201/NLVPMVATV MHC Multimer

Table 8 Required analyses for the MHC Multimer Proficiency Panel 2021

Staining ID	PBMC Donor	MHC Multimer specificity
R1	PBMC HHU20181002	Negative control
R2	PBMC HHU20181002	Negative control
R3	PBMC HHU20181002	A*0101/VTEHDTLLY
R4	PBMC HHU20181002	A*0101/VTEHDTLLY
R5	PBMC HHU20181002	A*0201/NLVPMVATV
R6	PBMC HHU20181002	A*0201/NLVPMVATV
R7	PBMC HHU20190623	Negative control
R8	PBMC HHU20190623	Negative control
R9	PBMC HHU20190623	A*0201/CLGGLLTMV
R10	PBMC HHU20190623	A*0201/CLGGLLTMV
R11	PBMC HHU20190623	A*0201/NLVPMVATV
R12	PBMC HHU20190623	A*0201/NLVPMVATV

Instructions for sample analysis

Cell Preparation

Thaw PBMC samples and count the cells. For each of the two PBMC samples, please record:

- The total cell numbers
- The viability of the cells

Staining and gating

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

Preferably, acquire a minimum of 100,000 CD8+ T cells for each of the 12 required analyses. 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 reagents.

Data recording

For each of the 12 analyses, you will need to:

- Record the number of CD8+ T cells (e.g. number of events in gate R6 in Figure 1).
- Record the number of MHC multimer+ CD8+ T cells (e.g. number of events in gate R5 in Figure 1).
- Calculate the percentage of MHC multimer+ CD8+ T cells out of total CD8+ T cells (R5/R6 x 100% in Figure 1). Please record all results with two decimals.

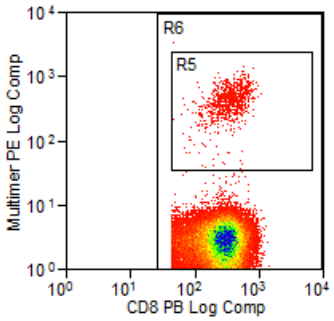

Figure 10 Example of CD8+ MHC Multimer+ cells.
Please see "PowerPoint Dot plot" (provided by email) for gating example.

Data reporting

4. 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.

5. Prepare and share the following files with us by using the link provided by email:
    a. The filled-in "PowerPoint Dot plot".
    b. The 12 FCS files, with Lab ID and staining ID included in the file name as described in Table 1, e.g. "Lab_1010_R1".
    c. If acquired, include your single-color compensation files.

6. Data reporting
    a. Upload data files using the link provided by email.
    b. Report data and results obtained from sample analysis in this survey: https://immudex.wufoo.com/forms/r1fmm8j804trpoi/

Appendix I

Assay harmonization guidelines

These guidelines are provided by the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC of CRI) and the Association for Cancer Immunotherapy (CIMT) for optimizing 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 final 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 Let only experienced personnel (per lab SOP) conduct assay.