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MHC MULTIMER PROFICIENCY PANEL 2017

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FOR MORE INFORMATION
www.proficiencypanel.com

MHC MULTIMER PROFICIENCY PANEL 2017

This report summarizes the results of the MHC Multimer Proficiency Panel 2017. The report provides individual test results for each participating laboratory that participated in the MHC Multimer proficiency panel 2017, as well as an anonymized overview of the other participants' test results.

18 laboratories from 10 countries participated in the MHC Multimer Proficiency Panel.

The proficiency panel services offered by Immudex are open to any laboratory, independent on geographic location or field of interest, with a need to perform accurate and reproducible quantification of antigen-specific T cells.

The report is provided using European numeration.

Immudex has taken over the MHC Multimer and Elispot proficiency panels from the CIC (Cancer Immunotherapy Consortium of the Cancer Research Institute, USA) and the CIMT (Association for Cancer Immunotherapy, Europe).

The proficiency panels conducted by Immudex are non-profit services offered with the intent of testing and ensuring a high level of proficiency and reliability among the researchers and clinicians that perform the immune monitoring assays.

The current MHC Multimer Proficiency Panel is therefore not a harmonization panel, but rather a proficiency testing service. Consequently, harmonization and standardization is not addressed in this report.

The next MHC Multimer Proficiency Panel will be held in 2018.

Contents

INFORMATION ON PARTICIPANTS, PROTOCOLS, REAGENTS, CELL SAMPLES

- 18 laboratories participated in the proficiency panel.
- Each participating laboratory was assigned a confidential participant Identification Number (Lab Id), only known by the laboratory itself and Immudex.
- Each participant received two vials of PBMCs (human peripheral blood mononuclear cells), named PBMC 2010113381 and PBMC 2010113382, respectively.
- The PBMCs were shipped in liquid nitrogen. A temperature logger was included in the shipment, allowing observation of vial temperature from packaging to delivery.
- MCH Dextramers were provided upon request by Immudex. 17 laboratories used MHC Dextramers.
- The three MHC multimer specificities tested were CMV HLA-A*0201/NLVPMVATV, FLU HLA-A*0201/GILGFVFTL and EBV HLA-A*0201/CLGGLLTMV.
- A negative control MHC multimer carrying an irrelevant peptide was also included.
- Each laboratory performed the Multimer assay according to their own preferred operating procedure.
- Instructions (see Appendix 1) including Harmonization Guidelines, were provided to all participants.

Prior to the shipping of the PBMCs to the participants, the PBMCs were pretested by two labs at separate locations, in order to verify the uniformity of the cell samples. Thus, the Multimer assay was performed on a total of 6 vials for each PBMC, using the three MHC multimer specificities provided. No variability was observed in cell samples vials from same batch with respect to cell viability and frequencies of antigen-specific T cells.

The PBMCs tested included a range of frequencies of antigen-specific T cells, from about 0,08 % MHC multimer$^+$ CD8$^+$ cells of CD8$^+$ cells ("Low responder") to about 0,76 % MHC multimer$^+$ CD8$^+$ cells of CD8$^+$ cells ("High responder").

ANALYSES PERFORMED BY THE PARTICIPANTS

Each participant received detailed instructions for carrying out the proficiency test; see Instructions (Appendix 1).

The participants were asked to report back the following experimental data for PBMC 2010113381 and PBMC 2010113382 from the final flow cytometry plots. See instructions for details (Appendix 1):

- Number of $CD8^+$ cells counted
- Number of CMV HLA-A*0201/NLVPMVATV-specific $CD8^+$ cells counted (PBMC 2010113381 and PBMC 2010113382)
- Number of FLU HLA-A*0201/GILGFVFTL-specific $CD8^+$ cells counted (PBMC 2010113381)
- Number of EBV HLA-A*0201/CLGGLLTMV $CD8^+$ cells counted (PBMC 2010113382)
- Number of negative control multimer$^+$ $CD8^+$ cells counted
- From the reported number of $CD8^+$ cells and multimer$^+$ $CD8^+$ cells for each measurement, the percentage of multimer$^+$ $CD8^+$ cells of the total number of $CD8^+$ cells were calculated and reported as the result

All measurements should be done in duplicate.

PRESENTATION OF DATA

The results obtained are shown in Figures 1-4 and Appendices 2-3.

The median of the results for each PBMC/multimer combination represents the "average value" for all the participants for that particular PBMC/multimer combination. The median of all the participants' results for a particular PBMC/multimer combination was used to calculate the Relative Accuracy of a given participant's result.

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

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

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

Any result below or above 2,0 times the median, corresponding to a relative accuracy of below 0,5 and above 2,0 is considered "far from avarage"

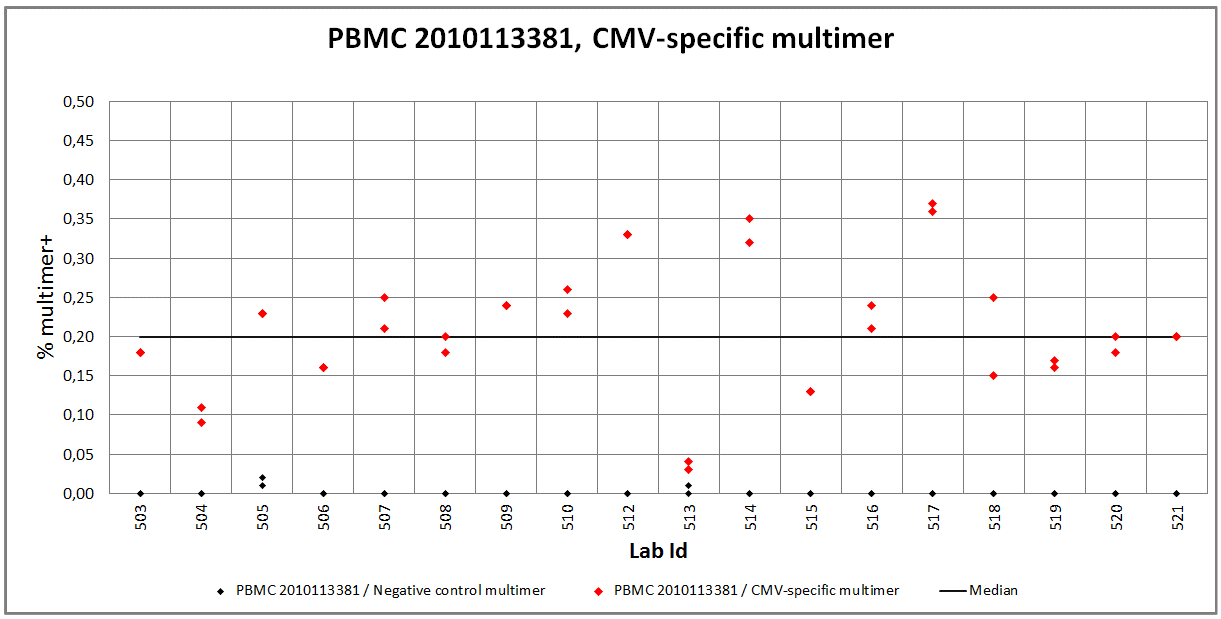
PROFICIENCY TESTING RESULTS

The results obtained by the 18 participants of the MHC Multimer Proficiency Panel 2017 are shown in Figures 1-4 and Appendices 2-3.

Each of Figures 1-4 has an upper and a lower panel.

The upper panel shows for each lab the percentage of antigen-specific cells (red diamonds), and the percentage of negative control multimer$^+$ cells, i.e. background staining found (black diamonds). The data are presented in order of increasing Lab Id from left to right.

The lower panel shows the relative accuracy, defined as percentage of antigen-specific cells determined by a given participant, divided by the median percentage of antigen-specific cells determined by all the participants. Relative accuracies of 0,66 – 1,5 are considered "in the average range" and are represented by filled black columns; relative accuracies of 0,50 – 0,65 or 1,6 – 2,0 are considered "near average" and are represented by hatched columns; relative accuracies below 0,50 or above 2,0 are considered "far from average" and are represented by open columns. The data are presented in order of increasing relative accuracy from left to right. A relative accuracy of 1,0 indicates full agreement with the "average" result.



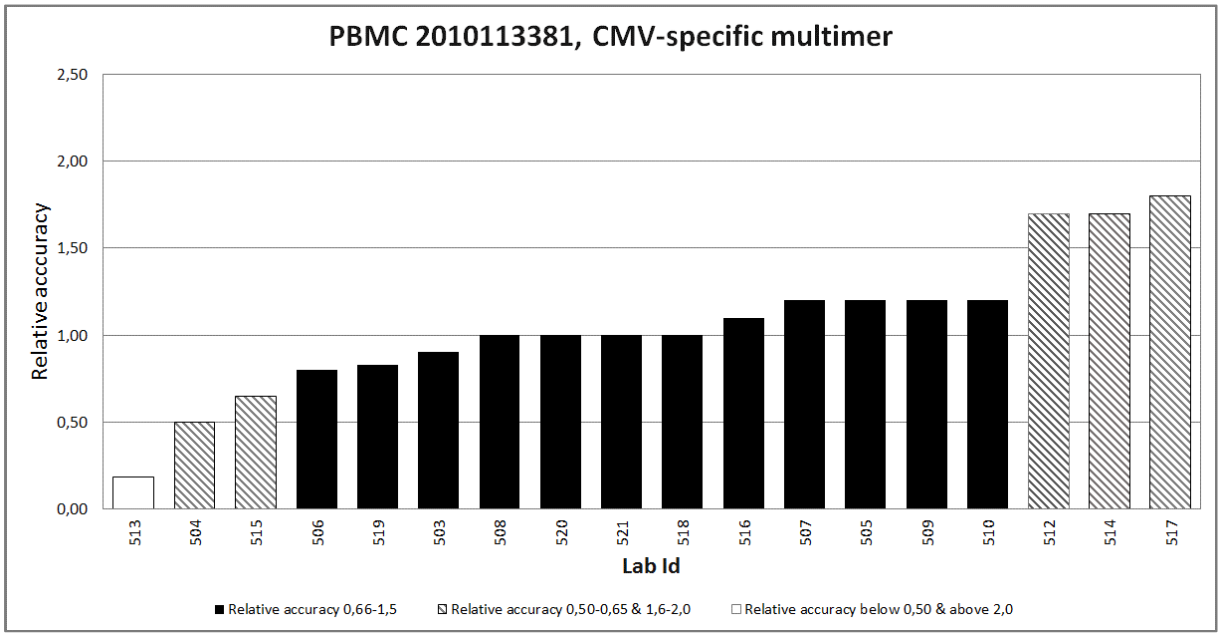


Figure 1. CMV-specific cells of PBMC 2010113381. *Percentage of CMV-specific CD8⁺ cells of total CD8⁺ cells, determined by the 18 participants.*

<u>Upper panel:</u> Duplicate measurements of the percentage of CMV-specific CD8⁺ cells (red diamonds) and the percentage of negative control multimer⁺ CD8⁺ cells (black diamonds).The median value (0,20 %) for CMV-specific CD8⁺ cells is indicated with a black horizontal line.
<u>Lower panel:</u> Relative accuracy for the measurement of CMV-specific CD8⁺ cells.

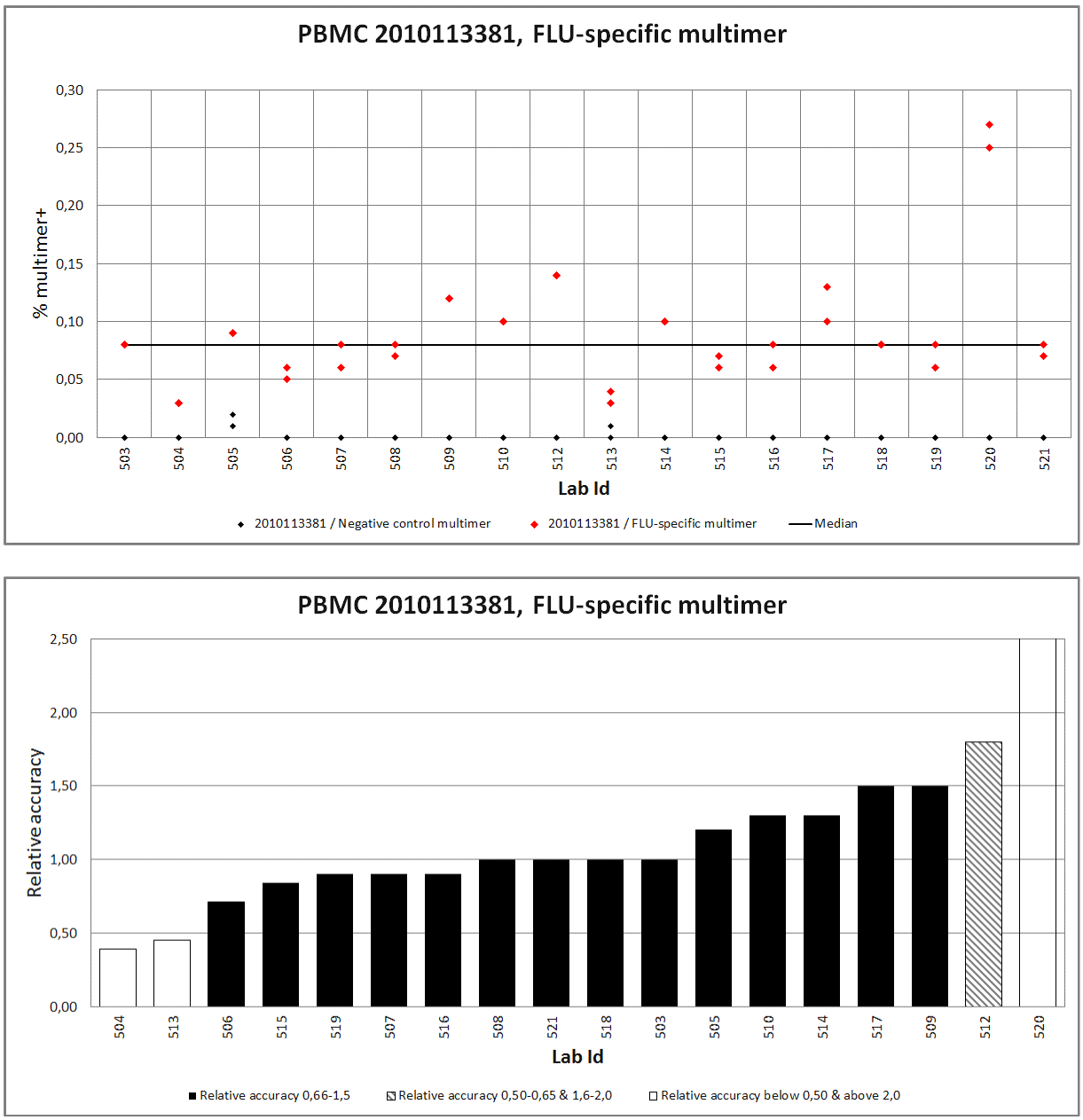


Figure 2. FLU-specific cells of PBMC 2010113381. *Percentage of FLU-specific CD8⁺ cells of total CD8⁺ cells, determined by the 18 participants.*

<u>Upper panel:</u> Duplicate measurements of the percentage of FLU-specific CD8⁺ cells (red diamonds) and the percentage of negative control multimer⁺ CD8⁺ cells (black diamonds). The median value (0,08 %) for FLU-specific CD8⁺ cells is indicated with a black horizontal line.
<u>Lower panel:</u> Relative accuracy for the measurement of FLU-specific CD8⁺ cells. The relative accuracy for Lab Id 520 is 3,4.

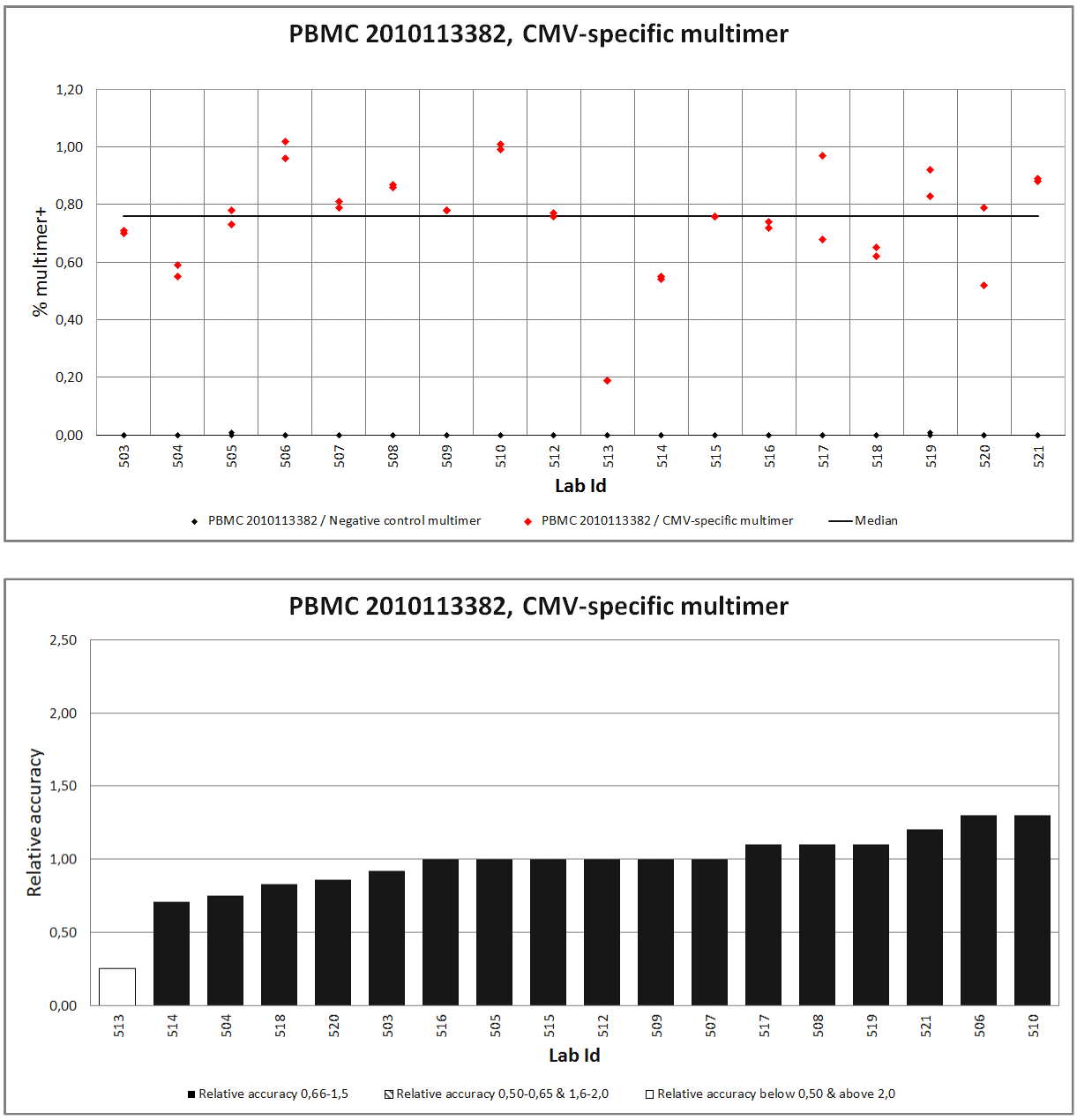


Figure 3. CMV-specific cells of PBMC 2010113382. *Percentage of CMV-specific CD8⁺ cells of total CD8⁺ cells, determined by the 18 participants.*

<u>Upper panel:</u> Duplicate measurements of the percentage of CMV-specific CD8⁺ cells (red diamonds) and the percentage of negative control multimer⁺ CD8⁺ cells (black diamonds). The median value (0,76 %) for CMV-specific CD8⁺ cells is indicated with a black horizontal line.
<u>Lower panel:</u> Relative accuracy for the measurement of CMV-specific CD8⁺ cells.

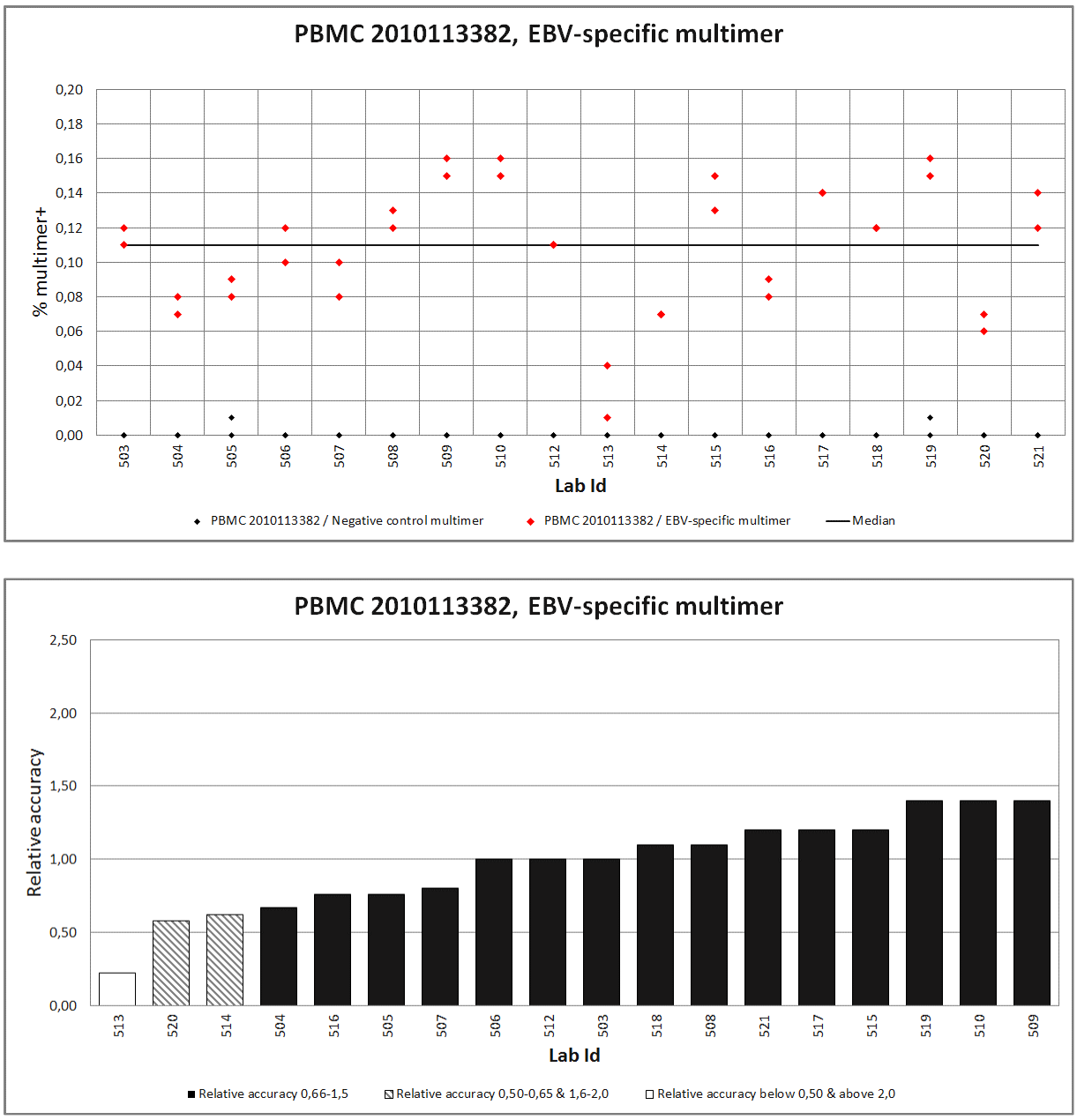


Figure 4. EBV-specific cells of PBMC 2010113382. *Percentage of EBV-specific CD8⁺ cells of total CD8⁺ cells, determined by the 18 participants.*

<u>Upper panel:</u> Duplicate measurements of the percentage of CMV-specific CD8⁺ cells (red diamonds) and the percentage of negative control multimer⁺ CD8⁺ cells (black diamonds). The median value (0,11 %) for EBV-specific CD8⁺ cells is indicated with a black horizontal line.
<u>Lower panel:</u> Relative accuracy for the measurement of EBV-specific CD8⁺ cells.

OVERALL PROFICIENCY

In order to describe the Overall Proficiency of each participating laboratory in enumerating the MHC multimer$^+$ CD8$^+$ cells, a score was assigned to each laboratory for each of the 4 measurements performed. The score "3" was assigned to results in the average range (i.e. Relative Accuracy between 0,66 and 1,5), the score "2" was assigned to results near average (i.e. Relative Accuracy 0,50-0,65 or 1,6-2,0), and finally, the score "1" was assigned to results far from average (i.e. Relative Accuracy below 0,50 or above 2,0).

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

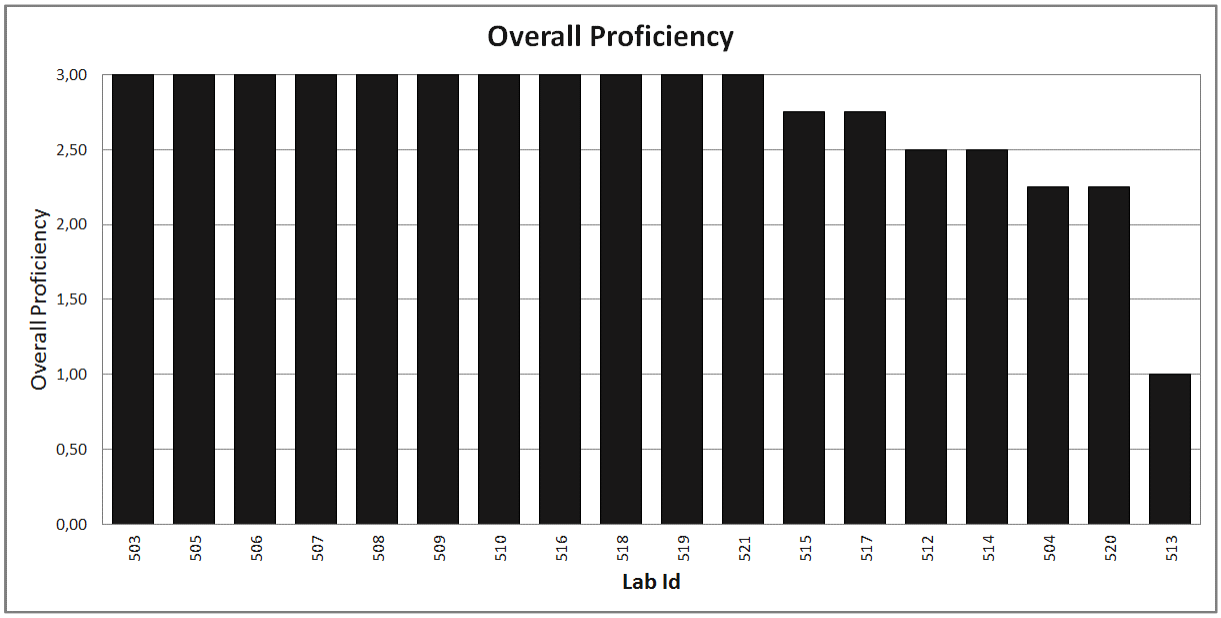


Figure 5. Overall Proficiency. *The laboratories' proficiency in performing the Multimer measurements is shown. An Overall Proficiency of "3" represents the highest possible proficiency score; an Overall Proficiency of "1" represents the lowest possible Overall proficiency score. A score of 3" indicates that this laboratory was "in average" on all four measurements. A score of "1" indicates that this laboratory was "far from average" on all four measurements.*

ACKNOWLEDGEMENTS

We thank Cryoport for sponsoring shipping and temperature logger expenses at a reduced price.

ABOUT IMMUDEX

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex provides MHC Dextramers® for the monitoring of antigen-specific T cells. Under an agreement with the US Cancer Immunotherapy Consortium (CIC) and the European Cancer Immunotherapy Consortium (CIMT), Immudex also provides MHC Multimer and Elispot proficiency panel services worldwide.

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

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

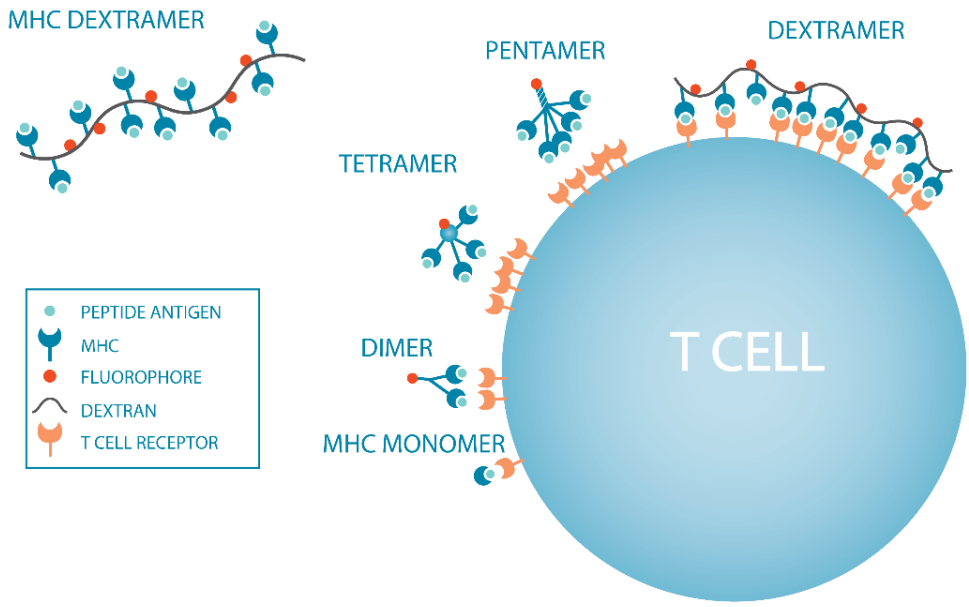


Figure 1 Schematic drawing of the MHC Dextramer® and the conventional MHC multimers binding to T-cell receptors (TCRs) on the surface of a T cell. MHC Dextramers® are fluorescent labeled MHC multimers that can bind simultaneously to multiple TCRs on a single T cell. This provides a strong and stable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

APPENDIX 1: INSTRUCTIONS

FOR THE MHC MULTIMER PROFICIENCY PANEL 2017

General Introduction to the MHC Multimer panel

All participants will receive two pre-tested PBMC donor samples. All participants must determine the percentage of CMV-, FLU- and EBV-specific T cells for both donor samples using predefined MHC Multimer reagents. Analyses are done by flow cytometry.

PLEASE READ ALL THE BELOW INSTRUCTIONS CAREFULLY BEFORE THAWING AND STAINING THE CELLS.

If you have any questions, please contact the organizer:

Charlotte Halgreen
Coordinator of Proficiency Panels
email: ProficiencyPanel@immudex.com
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Materials and Reagents:

Each participant receives 2 vials each comprising a donor sample, and named PBMC 2010113381 and PBMC 2010113382, respectively. Each vial contains 1,5ml, 10 x 10^6 PBMCs Please store samples in liquid nitrogen upon arrival.

MHC Multimer reagents needed for analysis:

- CMV HLA-A*0201/NLVPMVATV MHC Multimer
- EBV HLA-A*0201/CLGGLLTMV MHC Multimer
- FLU HLA-A*0201/GILGFVFTL MHC Multimer
- Negative Control MHC Multimer

Participants who requested MHC Dextramers will receive the following 4 PE-labeled Dextramers:

- WB2132-PE HLA-A*0201/NLVPMVATV MHC Dextramer 15 tests
- WB2144-PE HLA-A*0201/CLGGLLTMV MHC Dextramer 10 tests
- WB2161-PE HLA-A*0201/GILGFVFTL MHC Dextramer 10 tests
- WB2666-PE Neg. Control MHC Dextramer 15 tests

Dextramers should be stored in the dark at 2-8°C until use.

Overview of Required Staining reactions:

Each participant must perform a total of 12 analysis, corresponding to 6 analysis on each of the 2 supplied donor samples (PBMC 2010113381 and PBMC 2010113382).

Analyze the 2 supplied donor samples as follows:

PBMC-2010113381

- Negative control; staining with Negative control MHC Multimer.
- Measurement of CMV-specific CD8+ T cells using CMV (HLA-A*0201/NLVPMVATV) MHC Multimer
- Measurement of FLU-specific CD8+ T cells using FLU (HLA-A*0201/GILGFVFTL) MHC Multimer.

PBMC-2010113382

- Negative control; staining with Negative control MHC Multimer.
- Measurement of CMV-specific CD8+ T cells using CMV (HLA-A*0201/NLVPMVATV) MHC Multimer.
- Measurement of EBV-specific CD8+ T cells using EBV (HLA-A*0201/CLGGLLTMV) MHC Multimer.

All analysis are made in duplicates and should in addition to MHC Multimers include anti-CD8 antibody and relevant antibody marker(s) useful for exclusion or inclusion of specific cell population (e.g. anti-CD4 antibody, anti-CD3 antibody, or DEAD cell dyes) during data analysis.

Below is a table with an overview of the required analysis. Indicated staining ID's must be used for naming of fcs files and for reporting results of the proficiency panel.

Staining ID	Donor sample	MHC Multimer
R1-113381-Neg	PBMC-2010113381	Negative control
R2-113381-Neg	PBMC-2010113381	Negative control
R3-113381-CMV	PBMC-2010113381	CMV (A0201/NLVPMVATV)
R4-113381-CMV	PBMC-2010113381	CMV (A0201/NLVPMVATV)
R5-113381-FLU	PBMC-2010113381	FLU (A0201/GILGFVFTL)
R6-113381-FLU	PBMC-2010113381	FLU (A0201/GILGFVFTL)
R7-113382-Neg	PBMC-2010113382	Negative control
R8-113382-Neg	PBMC-2010113382	Negative control
R9-113382-CMV	PBMC-2010113382	CMV (A0201/NLVPMVATV)
R10-113382-CMV	PBMC-2010113382	CMV (A0201/NLVPMVATV)
R11-113382-EBV	PBMC-2010113382	EBV (A0201/CLGGLLTMV)
R12-113382-EBV	PBMC-2010113382	EBV (A0201/CLGGLLTMV)

Instructions for cell preparation and staining:

1) Thawing and Counting

Thaw both vials of donor sample. Count and record total cell number after thawing and the number of viable cells for each vial.

2) Staining

Please see Appendix A: Multimer Harmonization Guidelines to Optimize Assay Performance

Use your own Standard Operating Procedure (SOP) for staining and gating of MHC multimer-specific CD8+Tcells.
In addition to the three virus-specific MHC, and the negative control Multimer reagents (indicated in the table overview page 2), the SOP must also include:

- anti-CD8 antibody staining
- Optionally, additional marker(s) for exclusion or inclusion of specific cell population(s) (e.g. anti-CD4 antibody, anti-CD3 antibody, or dead cell dyes) can be included.

You are free to choose buffers, tubes, staining volume, incubation time, and use of dead cell markers in the assay. Record staining and washing conditions. You will have to perform the six staining reactions per donor outlined above, preferably collecting a minimum of 100.000 CD8+ T cells. In order to achieve this minimum, it is recommended that you stain at least 1,5 x 10^6 viable cells per staining.

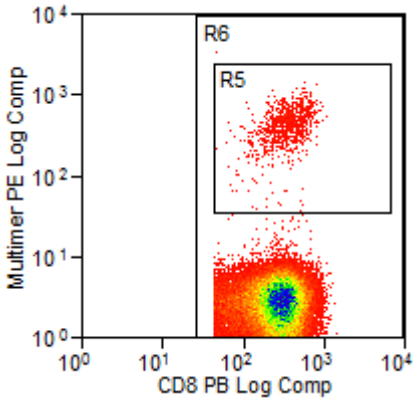
Note: If using MHC-Dextramer reagents, please read the Staining Protocol that comes with the Dextramers.
In particular, staining and incubation with Dextramers prior to addition of antibodies (anti-CD8, etc.) is essential for optimal staining of the antigen-specific T cells.

3) Data acquisition and analysis

All fcs files (flow data files) must be named exactly as described in the table on page 2.

Your analysis must end with a dot plot showing the CD8-staining on the x-axis and the MHC Multimer staining on the y-axis, as exemplified below.

Example of dot plot showing CD8-staining and MHC Multimer staining:



Record results as follows:

- Number of CD8-positive T cells (number of events in gate R6, in the above example).
- Number of MHC multimer-positive T cells (number of events in gate R5 in the above example).
- Calculate the percentage of MHC multimer-positive T cells of total CD8-positive T cells (R5/R6x100 % in above example). Record result with two decimals.

Reporting of data

1. Fill-in the “PowerPoint Dot plot” slide (sent by email to all participants) with your own dot plot and gating strategy.

2. Create a Zip file, name it with your Lab ID, and include the following files:
    a. The filled-in “PowerPoint Dot plot”.
    b. The 12 fcs files, labeled as described in the table page 2
    c. If acquired, include you single color compensation.

3. Proficiency data reporting
    a. Go to » Proficiency panel data registration
        i. If prompt to, select your region
    b. Upload you data Zip file, as described
    c. Report Proficiency Panel Data (use link to » Data report form)
        i. Fill-in the survey as described.

Links and documents relating to Proficiency Panels can be found on www.proficiencypanel.org

Appendix A

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-positive cells (e.g. irrelevant Multimer or autofluorescence).

A3. Establish adequate measures to reduce the amount of non-specific binding of Multimer in the CD8-positive 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 Only let experienced personnel (per lab SOP) conduct assay.

APPENDIX 2: PBMC 2010113381 REPORTED NUMBERS

PBMC 2010113381, Negative control multimer (Neg)

PBMC 2010113381, CMV-specific multimer

PBMC 2010113381, FLU-specific multimer

% multimer$^+$ CD8$^+$ of CD8$^+$ cells and relative accuracy from data reported

Lab Id	R1-2010 113381-Neg %	R2-2010 113381-Neg %	R3-2010 113381-CMV %	R4-2010 113381-CMV %	2010 113381-CMV Relative accuracy	R5-2010 113381-FLU %	R6-2010 113381-FLU %	2010 113381-FLU Relative accuracy
503	0,00	0,00	0,18	0,18	0,90	0,08	0,08	1,0
504	0,00	0,00	0,11	0,09	0,50	0,03	0,03	0,39
505	0,02	0,01	0,23	0,23	1,2	0,09	0,09	1,2
506	0,00	0,00	0,16	0,16	0,80	0,05	0,06	0,71
507	0,00	0,00	0,25	0,21	1,2	0,06	0,08	0,90
508	0,00	0,00	0,18	0,20	1,0	0,08	0,07	1,0
509	0,00	0,00	0,24	0,24	1,2	0,12	0,12	1,5
510	0,00	0,00	0,26	0,23	1,2	0,10	0,10	1,3
512	0,00	0,00	0,33	0,33	1,7	0,14	0,14	1,8
513	0,01	0,00	0,04	0,03	0,18	0,03	0,04	0,45
514	0,00	0,00	0,35	0,32	1,7	0,10	0,10	1,3
515	0,00	0,00	0,13	0,13	0,65	0,06	0,07	0,84
516	0,00	0,00	0,24	0,21	1,1	0,08	0,06	0,90
517	0,00	0,00	0,37	0,36	1,8	0,10	0,13	1,5
518	0,00	0,00	0,15	0,25	1,0	0,08	0,08	1,0
519	0,00	0,00	0,17	0,16	0,83	0,06	0,08	0,90
520	0,00	0,00	0,18	0,20	1,0	0,25	0,27	3,4
521	0,00	0,00	0,20	0,20	1,0	0,07	0,08	1,0

APPENDIX 3: PBMC 2010113382 REPORTED NUMBERS

PBMC 2010113382, Negative control multimer (Neg)

PBMC 2010113382, CMV-specific multimer

PBMC 2010113382, EBV-specific multimer

% multimer$^+$ CD8$^+$ of CD8$^+$ cells and relative accuracy from data reported

Lab Id	R7-2010 113382-Neg %	R8-2010 113382-Neg %	R9-2010 113382-CMV %	R10-2010 113382-CMV %	2010 113382-CMV Relative accuracy	R11-2010 113382-EBV %	R12-2010 113382-EBV %	2010 113382-EBV Relative accuracy
503	0,00	0,00	0,71	0,70	0,92	0,12	0,11	1,0
504	0,00	0,00	0,59	0,55	0,75	0,07	0,08	0,67
505	0,00	0,01	0,78	0,73	1,0	0,08	0,09	0,76
506	0,00	0,00	1,02	0,96	1,3	0,10	0,12	1,0
507	0,00	0,00	0,79	0,81	1,0	0,08	0,10	0,80
508	0,00	0,00	0,86	0,87	1,1	0,12	0,13	1,1
509	0,00	0,00	0,78	0,78	1,0	0,15	0,16	1,4
510	0,00	0,00	1,01	0,99	1,3	0,16	0,15	1,4
512	0,00	0,00	0,77	0,76	1,0	0,11	0,11	1,0
513	0,00	0,00	0,19	0,19	0,25	0,04	0,01	0,22
514	0,00	0,00	0,54	0,55	0,71	0,07	0,07	0,62
515	0,00	0,00	0,76	0,76	1,0	0,15	0,13	1,2
516	0,00	0,00	0,74	0,72	1,0	0,08	0,09	0,76
517	0,00	0,00	0,97	0,68	1,1	0,14	0,14	1,2
518	0,00	0,00	0,62	0,65	0,83	0,12	0,12	1,1
519	0,00	0,01	0,92	0,83	1,1	0,16	0,15	1,4
520	0,00	0,00	0,79	0,52	0,86	0,06	0,07	0,58
521	0,00	0,00	0,88	0,89	1,2	0,14	0,12	1,2