

# MHC MULTIMER PROFICIENCY PANEL 2015

July 2015

# MHC MULTIMER PROFICIENCY PANEL

## 2015

This report summarizes the results of the MHC Multimer Proficiency Panel 2015. The report provides individual test results for each participating laboratory that participated in the MHC Multimer proficiency panel 2015, as well as an anonymized overview of the other participants' test results.

30 laboratories from 13 countries participated in the MHC Multimer Proficiency Panel. Participants included researchers and clinicians active in such diverse fields as Cancer, HIV, CMV, and Diabetes.

Immudex has taken over the MHC Multimer and Elispot proficiency panels from the CIC (Cancer Immunotherapy Consortium of the Cancer Research Institute, USA) and the CIMT (Association for Cancer Immunotherapy, Europe). The proficiency panel services offered by Immudex are open to any laboratory, 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.

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. It is the hope and expectation that better immune monitoring assays will lead to better and more efficient immunotherapies.

The next MHC Multimer proficiency panel will be held in 2016.

[30 laboratories from 13 countries participated in the MHC Multimer Proficiency Panel](#)

[Participants are active in such diverse fields as Cancer, HIV, CMV, and Diabetes](#)

[Next MHC Multimer proficiency panel to be held in 2016](#)

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## **INFORMATION ON PARTICIPANTS, PROTOCOLS, REAGENTS, CELL SAMPLES**

- 30 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), termed PBMC 13075 and HBC 525, 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. All laboratories used MHC Dextramers.
- The four multimer specificities tested were EBV HLA-A\*0201/GLCTLVAML, EBV HLA-A\*0201/CLGGLLTMV, EBV HLA-B\*0702/RPPIFIRRL and CMV HLA-B\*0702/TPRVTGGGAM.
- A negative control 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 PBMC vials. Thus, the Multimer assay was performed on a total of 6 vials for each PBMC, using the four multimer specificities provided. The observed variability between different PBMC vials of the same cell sample, as regards cell viability and number of antigen-specific T cells, was insignificant.

PBMC samples were thoroughly pretested prior to shipping and sample temperature was monitored from packaging to delivery

The PBMCs tested included a range of frequencies of antigen-specific T cells, from about 0,06 % multimer<sup>+</sup> CD8<sup>+</sup> cells ("Low responder") to about 0,80 % multimer<sup>+</sup> CD8<sup>+</sup> 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 13075 and HBC 525:

- Number of CD8<sup>+</sup> cells counted

- Number of EBV HLA-A\*0201/GLCTLVAML-specific CD8<sup>+</sup> cells counted (PBMC 13075)
- Number of EBV HLA-A\*0201/CLGGLLTMV-specific CD8<sup>+</sup> cells counted (PBMC 13075)
- Number of EBV HLA-B\*0702/RPPIFIRRL-specific CD8<sup>+</sup> cells counted (HBC 525)
- Number of CMV HLA-B\*0702/TPRVTGGAM-specific CD8<sup>+</sup> cells counted (HBC 525)
- Number of negative control multimer<sup>+</sup> CD8<sup>+</sup> cells counted
- From the reported number of CD8<sup>+</sup> cells and multimer<sup>+</sup> CD8<sup>+</sup> cells for each measurement, the percentage of multimer<sup>+</sup> CD8<sup>+</sup> cells of the total number of CD8<sup>+</sup> 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 30 participants' data comprised significant outliers for each of the four PBMC/multimer combinations tested. It was therefore decided to use the median, rather than the average, of the reported results (including all outliers) to represent the "average" result.

Thus, in the following the median of the results for each PBMC/multimer combination represents the "average value" for all the participants for that particular PBMC/multimer combination. It should be emphasized that the "average value" (the median) is not necessarily the "true" value, as no golden standard exists for the MHC Multimer assay. Nevertheless, 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 (see below).

The median, rather than the average, of the reported results represents the "average" result

## **PROFICIENCY TESTING RESULTS**

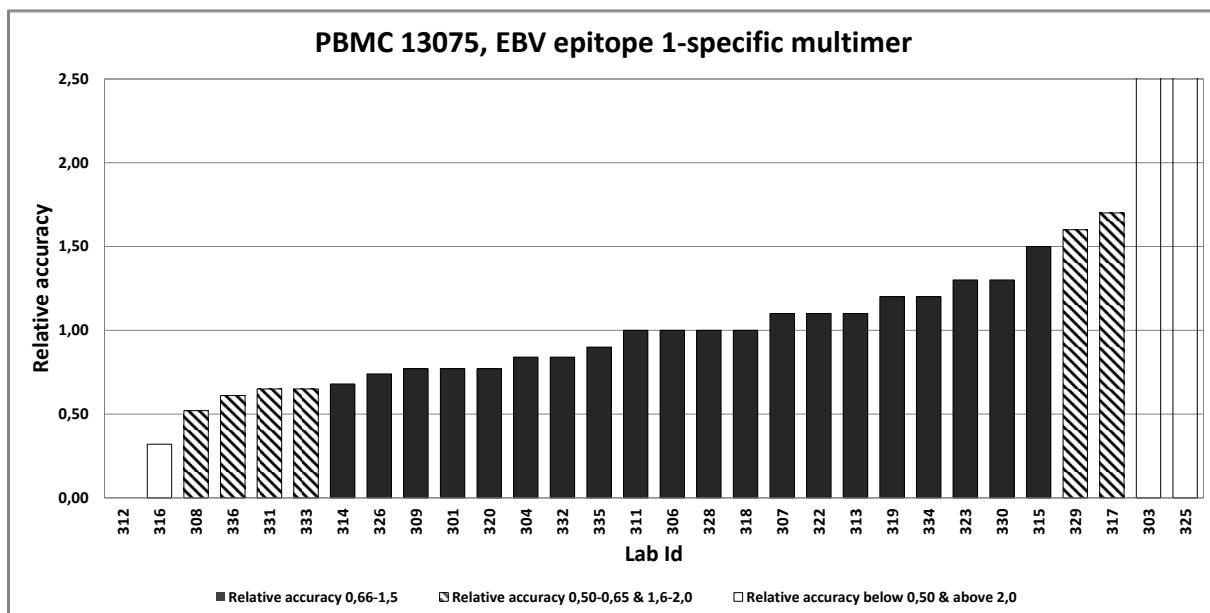
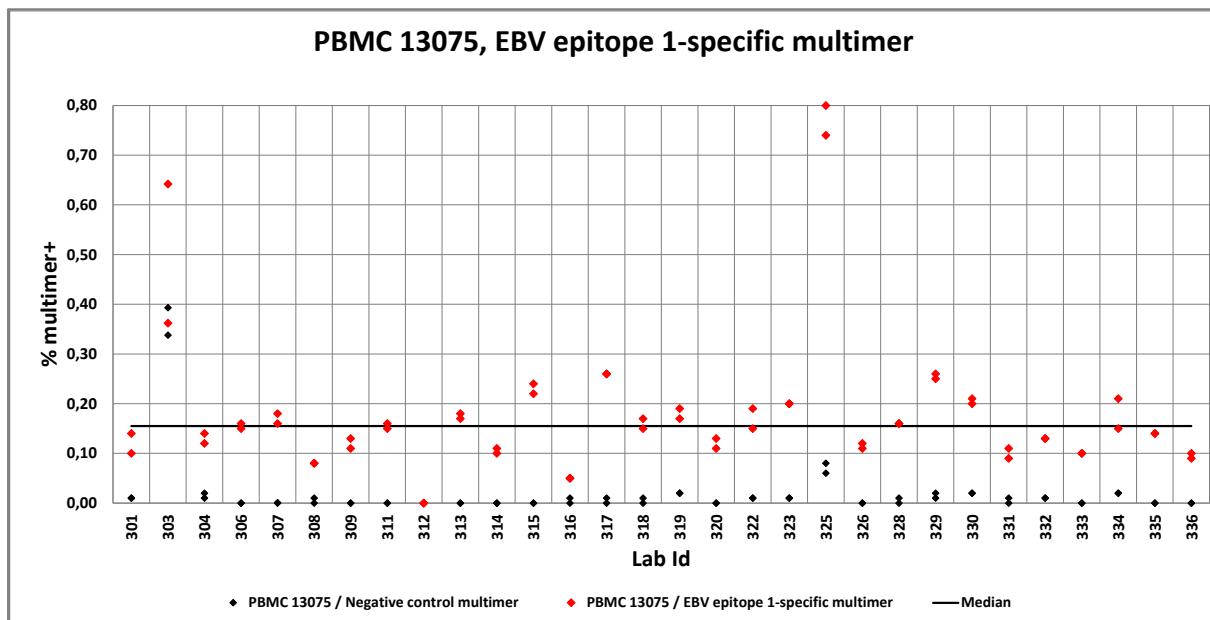
The results obtained by the 30 participants of the MHC Multimer Proficiency Panel 2015 are shown in Figures 1-4 and Appendices 2-3.

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

The upper panel shows the percentage of antigen-specific cells (duplicate analysis indicated with two red diamonds), and the percentage of negative control multimer<sup>+</sup> cells, i.e. background

staining (duplicate analysis indicated with two black diamonds). The data are presented in order of increasing Lab Id from left to right.

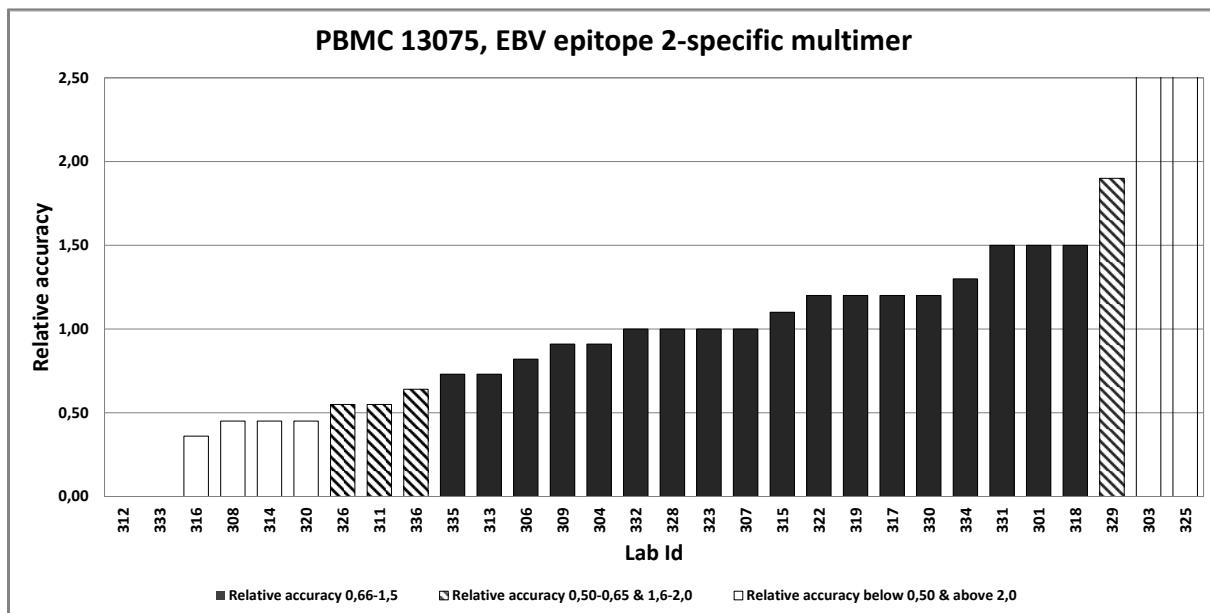
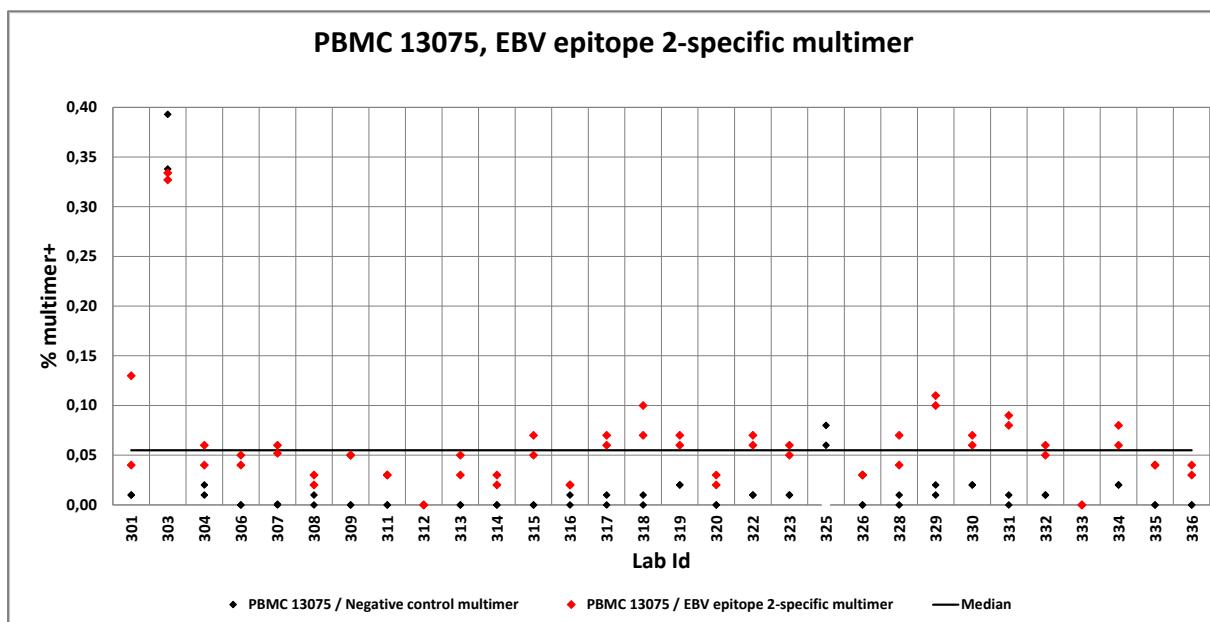
The lower panel shows the relative accuracy, defined as percentage of antigen-specific cells determined by a given participant, divided by the median percentage of antigen-specific cells determined by all the participants. Relative accuracies of 0.66 – 1.5 are considered “in the average range” and are 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. EBV epitope 1-specific cells of PBMC 13075.** Percentage of EBV epitope 1-specific CD8<sup>+</sup> cells of total CD8<sup>+</sup> cells, determined by the 30 participants.

Upper panel: Duplicate measurements of the percentage of EBV epitope 1-specific CD8<sup>+</sup> cells (red diamonds) and the percentage of negative control multimer<sup>+</sup> CD8<sup>+</sup> cells (black diamonds). The median value (0,16 %) for EBV epitope 1-specific CD8<sup>+</sup> cells is indicated with a black horizontal line.

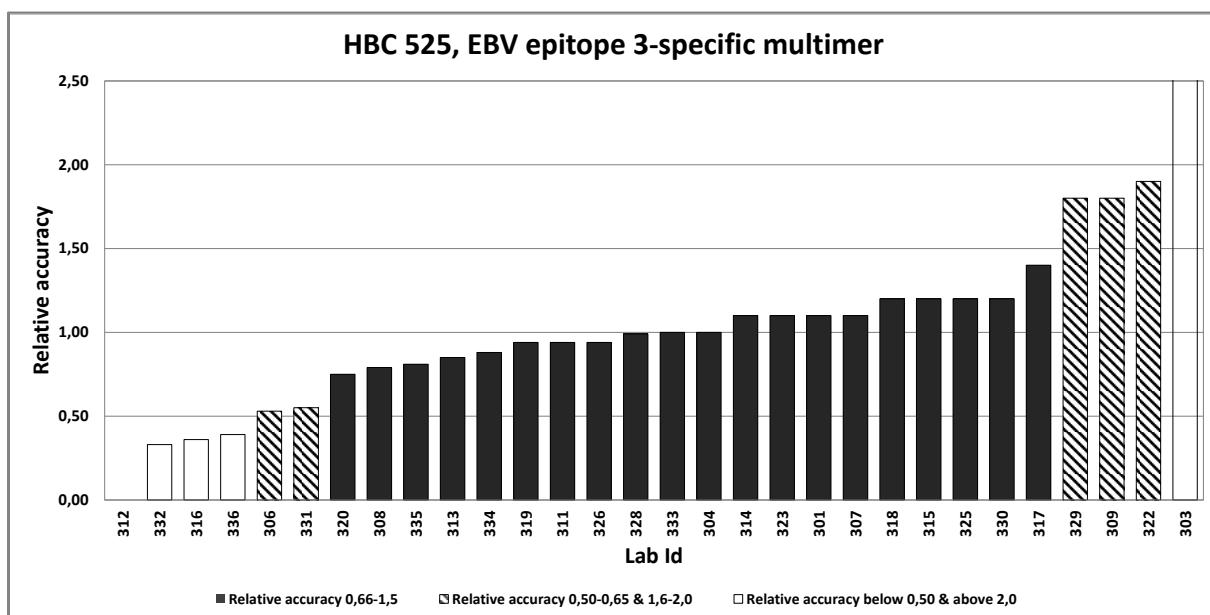
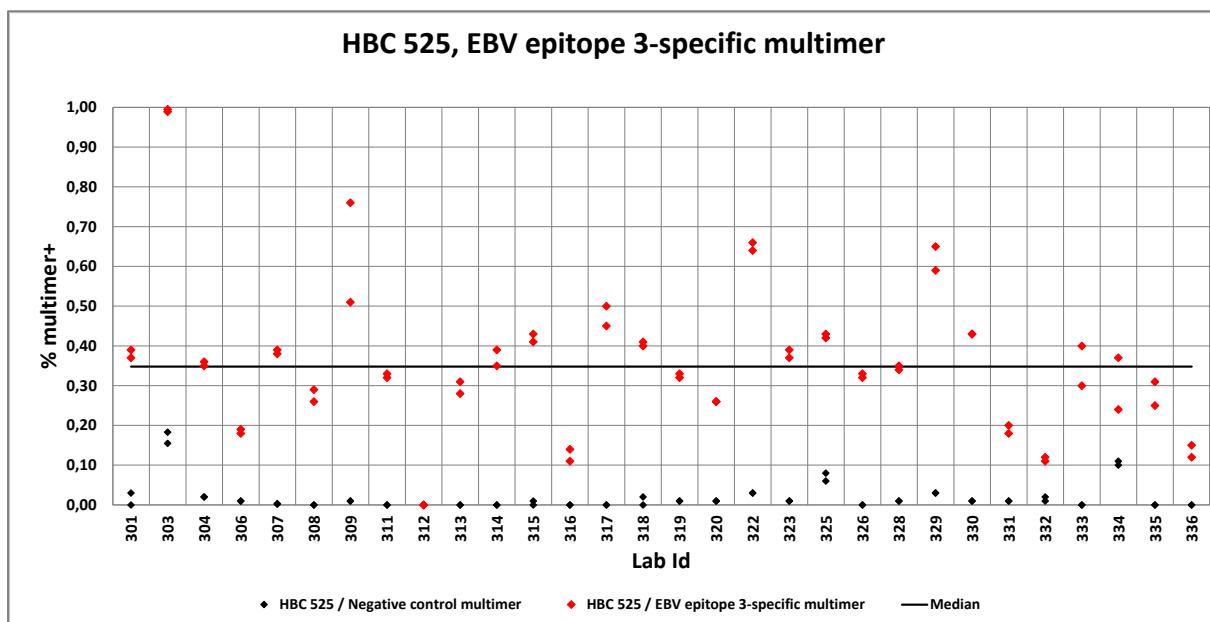
Lower panel: Relative accuracy for the measurement of EBV epitope 1-specific CD8<sup>+</sup> cells. The relative accuracy for Lab Id 312, 303 and 325 are 0, 3,2 and 5,0, respectively.



**Figure 2. EBV epitope 2-specific cells of PBMC 13075.** Percentage of EBV epitope 2-specific CD8<sup>+</sup> cells of total CD8<sup>+</sup> cells, determined by the 30 participants.

Upper panel: Duplicate measurements of the percentage of EBV epitope 2-specific CD8<sup>+</sup> cells (red diamonds) and the percentage of negative control multimer<sup>+</sup> CD8<sup>+</sup> cells (black diamonds). Lab Id 325 only reported one measurement with the result 1,92 % for the EBV epitope 2-specific CD8<sup>+</sup> cells. The median value (0,06 %) for EBV epitope 2-specific CD8<sup>+</sup> cells is indicated with a black horizontal line.

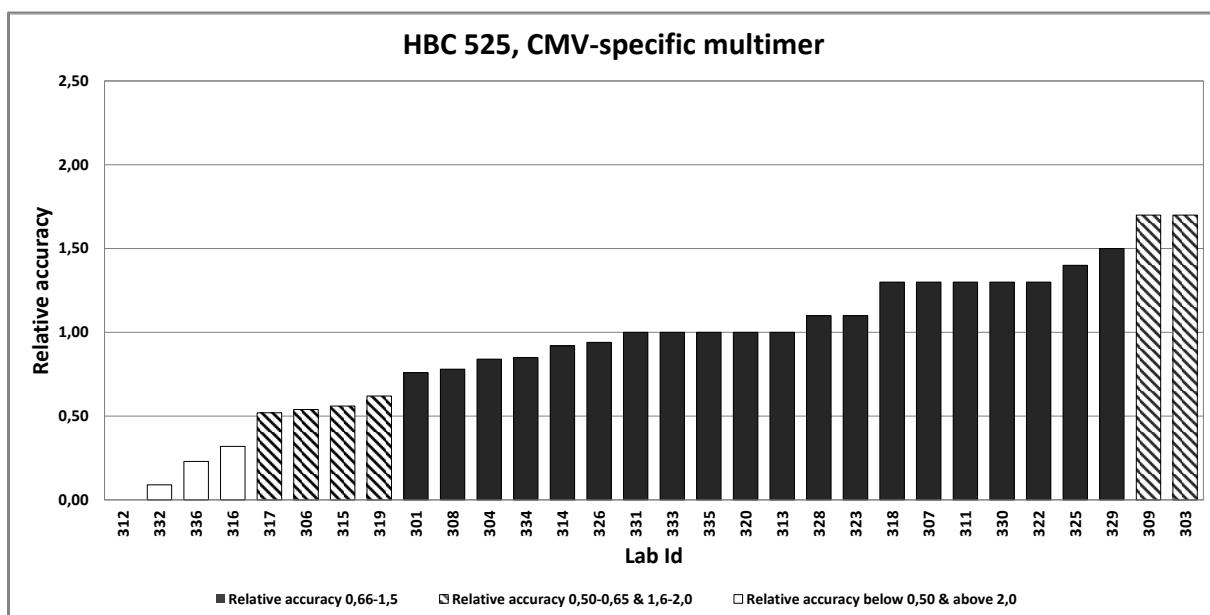
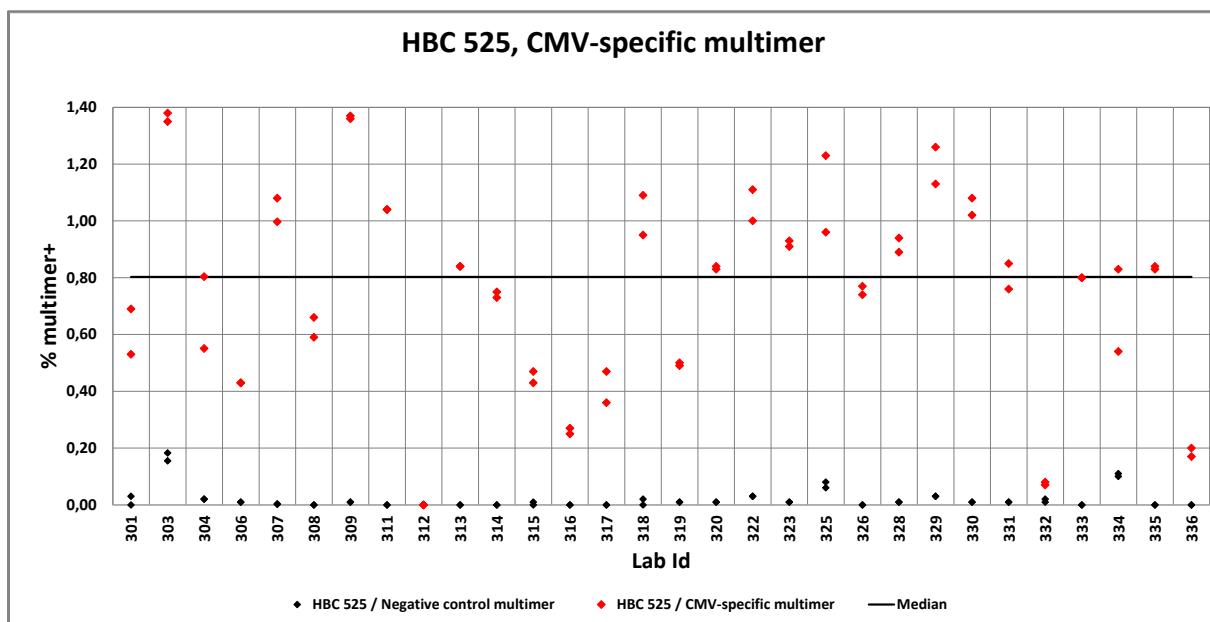
Lower panel: Relative accuracy for the measurement of EBV epitope 2-specific CD8<sup>+</sup> cells. The relative accuracy for Lab Id 312, 333, 303 and 325 are 0, 0, 6,0 and 35, respectively.



**Figure 3. EBV epitope 3-specific cells of HBC 525.** Percentage of EBV epitope 3-specific CD8<sup>+</sup> cells of total CD8<sup>+</sup> cells, determined by the 30 participants.

Upper panel: Duplicate measurements of the percentage of EBV epitope 3-specific CD8<sup>+</sup> cells (red diamonds) and the percentage of negative control multimer<sup>+</sup> CD8<sup>+</sup> cells (black diamonds). The median value (0,35 %) for EBV epitope 3-specific CD8<sup>+</sup> cells is indicated with a black horizontal line.

Lower panel: Relative accuracy for the measurement of EBV epitope 3-specific CD8<sup>+</sup> cells. The relative accuracy for Lab Id 312 and 303 are 0 and 2,9, respectively.



**Figure 1. CMV-specific cells of HBC 525.** Percentage of CMV-specific CD8<sup>+</sup> cells of total CD8<sup>+</sup> cells, determined by the 30 participants.

Upper panel: Duplicate measurements of the percentage of CMV-specific CD8<sup>+</sup> cells (red diamonds) and the percentage of negative control multimer<sup>+</sup> CD8<sup>+</sup> cells (black diamonds). The median value (0,80 %) for CMV-specific CD8<sup>+</sup> cells is indicated with a black horizontal line.

Lower panel: Relative accuracy for the measurement of CMV-specific CD8<sup>+</sup> cells. The relative accuracy for Lab Id 312 is 0.

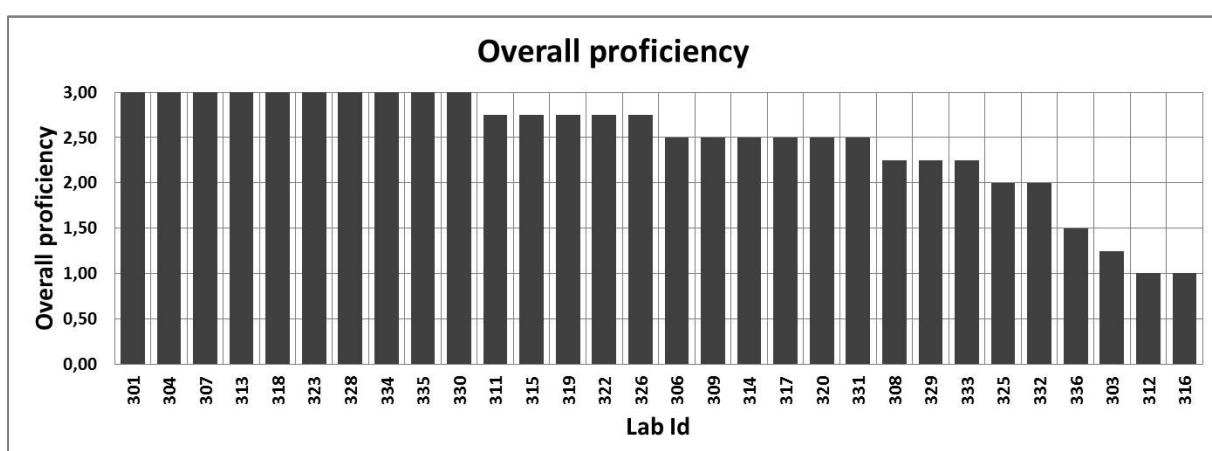
## OVERALL PROFICIENCY

In order to describe the Overall Proficiency of each participating laboratory in enumerating the multimer<sup>+</sup> CD8<sup>+</sup> 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.

Overall Proficiency is shown in Figure 5. As can be seen, 10 out of 30 laboratories (33 %) are in the average range on all four measurements, and thus have the highest possible Overall Proficiency score of "3".

33 % of participants had results in "the average range" for 4 out of 4 measurements.



**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.

## **GENERAL COMMENTS**

The proficiency panel series was initiated by the CIC and CIMT in 2006. One of the goals of past proficiency panels was to harmonize procedures across laboratories. Harmonization is now considered finalized, and the responsibility of conducting the proficiency panel was handed over to Immudex in 2013.

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.

A survey was carried out in connection with the proficiency panel execution. The full set of reported data and information will be published separately.

### **General observations and conclusions.**

The MHC Multimer assays performed in this proficiency panel included a range of frequencies of antigen-specific T cells from about 0,06 % multimer<sup>+</sup> CD8<sup>+</sup> cells ("Low responder") to about 0,80 % multimer<sup>+</sup> CD8<sup>+</sup> cells ("High responder").

- For each of the four measurements, about 60 % of the participants had a Relative Accuracy of between 0,66 and 1,5. In other words, for a given measurement, about 60 % of the participants had results "in the average range".
- 33 % of the participants were "in the average range" for four out of four measurements.
- For a given measurement, the 75 % of the participants that were closest to the "average" (median value) had results differing from 2 to 3 fold. For example, for the PBMC 13075 / EBV epitope 1 multimer combination, the 75 % of participants (i.e. 23 out of 30 participants) that were closest to the median value, detected from 0,10 % to 0,23 % multimer<sup>+</sup> CD8<sup>+</sup> cells, and thus differed 2,3 fold.

For each measurement performed, ~60 % of the participants obtained a value close to average

33 % of the participants were close to average for 4 out of 4 measurements

Among the 75 % of participants closest to the "average", the lowest and highest percentage of multimer<sup>+</sup> CD8<sup>+</sup> cells detected differed 2-3 fold

## **ACKNOWLEDGEMENTS**

We thank Tina Jakobsen/Dako for performing the pretesting of cell samples, Steffen Walter for providing PBMCs and Sine Reker and Marij Schoenmaekers-Welters for providing helpful advice.

## **ABOUT IMMUDEX**

Based in Copenhagen, Denmark, with North American operations based in Fairfax, Virginia, Immudex provides MHC Dextramer products for the monitoring of antigen-specific T cells, as well as provides MHC multimer and Elispot immune monitoring proficiency panel services. Immudex recognizes the need for accuracy and reproducibility in scientific and clinical research, and patient care, and has a number of research- and clinical products on the market. The goal is to enable simple, accurate and reliable monitoring of antigen-specific cellular immunity, and to promote the routine use of these technologies in diagnostics and research, and in all steps of immunotherapeutics development.

[www.immudex.com](http://www.immudex.com)

**APPENDIX 1: INSTRUCTIONS**  
**FOR THE MHC MULTIMER PROFICIENCY PANEL 2015**

**General Introduction to the MHC Multimer panel**

All participants will receive two pre-tested donor samples. All participants must determine the percentage of CMV- and EBV-specific T cells for both donor samples using predefined MHC Multimer specificities. 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](mailto:ProficiencyPanel@immudex.com)

P: +45 3917 9772

**Materials and Reagents:**

Each participant receives 2 vials each comprising a donor sample, and named HBC-525 and PBMC 13075, respectively. HBC-525 contains 1ml,  $20 \times 10^6$  PBMCs; PBMC 13075 contains 1ml,  $10 \times 10^6$  PBMCs.

Please store samples in liquid nitrogen upon arrival.

MHC Multimer specificities needed for analysis:

- EBV HLA-A\*0201/GLCTLVAML MHC Multimer
- EBV HLA-A\*0201/CLGGLLTMV MHC Multimer
- EBV HLA-B\*0702/RPPIFIRRL Multimer
- CMV HLA-B\*0702/TPRVTGGGAM Multimer
- Negative Control MHC Multimer

Participants who requested MHC Dextramers will receive the following 5 PE-labeled Dextramers:

- |             |                                      |          |
|-------------|--------------------------------------|----------|
| • WB2130-PE | HLA-A*0201/GLCTLVAML MHC Dextramer   | 6 tests  |
| • WB2144-PE | HLA-A*0201/CLGGLLTMV MHC Dextramer   | 6 tests  |
| • WH2166-PE | HLA-B*0702/RPPIFIRRL MHC Dextramer   | 6 tests  |
| • WH2136-PE | HLA-B*0702/TPRVTGGGAM MHC Dextramer  | 6 tests  |
| • NI3233-PE | General / Neg. Control MHC Dextramer | 10 tests |

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

## **Overview of Required Staining reactions:**

Each participant has to perform a total of 12 staining reactions, corresponding to 6 staining reactions on each of the 2 supplied donor samples (HBC-525 and PBMC-13075).

The 2 supplied donor samples should be analyzed as follows:

### **PBMC-13075**

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

### **HBC-525**

- Negative control; staining with Negative control MHC Multimer.
- Measurement of EBV-specific CD8+ T cells using EBV (HLA-B\*0702/RPPIFIRRL) MHC Multimer.
- Measurement of CMV-specific CD8+ T cells using CMV (HLA-B\*0702/TPRVTGGGAM) MHC Multimer.

All analyses 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 overview of the required staining reactions. Indicated staining ID's must be used for naming of fcs files and for reporting results of the proficiency panel.

<b>Staining ID</b>	<b>Donor sample</b>	<b>MHC Multimer</b>
R1-13075-Neg	PBMC-13075	Negative control
R2-13075-Neg	PBMC-13075	Negative control
R3-13075-EBV1	PBMC-13075	EBV (A0201/GLCTLVAML)
R4-13075-EBV1	PBMC-13075	EBV (A0201/GLCTLVAML)
R5-13075-EBV2	PBMC-13075	EBV (A0201/CLGGLLTMV)
R6-13075-EBV2	PBMC-13075	EBV (A0201/CLGGLLTMV)
R7-525-Neg	HBC-525	Negative control
R8-525-Neg	HBC-525	Negative control
R9-525-EBV3	HBC-525	EBV (B0702/RPPIFIRRL)
R10-525-EBV3	HBC-525	EBV (B0702/RPPIFIRRL)
R11-525-CMV	HBC-525	CMV (B0702/TPRVTGGGAM)
R12-525-CMV	HBC-525	CMV (B0702/TPRVTGGGAM)

## **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 SOP (protocol) for staining and gating of MHC multimer-specific CD8+ T cells. In addition to the four virus-specific MHC, and the negative control Multimer reagents (indicated in the table overview above), 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 with a minimum of 100.000 CD8+ T cells. In order to achieve this minimum, it is recommended that you stain at least  $1,5 \times 10^6$  viable cells per staining.

Note: If using MHC-Dextramer reagents, please read the Staining Protocol that came with the Dextramers.

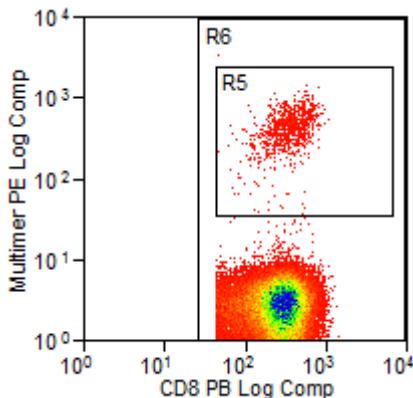
In particular, staining and incubation with Dextramers prior to addition of antibodies (anti-CD8, etc.) is essential for optimal staining of the antigen-specific T cells.

### **3) Data acquisition and analysis**

All fcs files (flow 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.

Dot plot showing CD8-staining and MHC Multimer staining example:



Results are recorded as:

- 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 ( $R5/R6 \times 100\%$  in above example). Result must be recorded 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, too.
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](http://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 13075 REPORTED NUMBERS

### **PBMC 13075, Negative control multimer (Neg)**

### **PBMC 13075, EBV epitope 1-specific multimer (EBV1)**

### **PBMC 13075, EBV epitope 2-specific multimer (EBV2)**

**% multimer<sup>+</sup> CD8<sup>+</sup> of CD8<sup>+</sup> cells and relative accuracy from data reported**

<b>Lab Id</b>	<b>R1- 13075- Neg %</b>	<b>R2- 13075- Neg %</b>	<b>R3- 13075- EBV1 %</b>	<b>R4- 13075- EBV1 %</b>	<b>13075- EBV1 Relative accuracy</b>	<b>R5- 13075- EBV2 %</b>	<b>R6- 13075- EBV2 %</b>	<b>13075- EBV2 Relative accuracy</b>
<b>301</b>	0,01	0,01	0,14	0,10	0,77	0,13	0,04	1,5
<b>303</b>	0,34	0,39	0,64	0,36	3,2	0,33	0,33	6,0
<b>304</b>	0,01	0,02	0,12	0,14	0,84	0,06	0,04	0,91
<b>306</b>	0,00	0,00	0,15	0,16	1,0	0,04	0,05	0,82
<b>307</b>	0,00	0,00	0,16	0,18	1,1	0,06	0,05	1,0
<b>308</b>	0,00	0,01	0,08	0,08	0,52	0,02	0,03	0,45
<b>309</b>	0,00	0,00	0,13	0,11	0,77	0,05	0,05	0,91
<b>311</b>	0,00	0,00	0,15	0,16	1,0	0,03	0,03	0,55
<b>312</b>	0,00	0,00	0,00	0,00	0	0,00	0,00	0
<b>313</b>	0,00	0,00	0,18	0,17	1,1	0,03	0,05	0,73
<b>314</b>	0,00	0,00	0,11	0,10	0,68	0,03	0,02	0,45
<b>315</b>	0,00	0,00	0,24	0,22	1,5	0,07	0,05	1,1
<b>316</b>	0,01	0,00	0,05	0,05	0,32	0,02	0,02	0,36
<b>317</b>	0,00	0,01	0,26	0,26	1,7	0,06	0,07	1,2
<b>318</b>	0,01	0,00	0,15	0,17	1,0	0,07	0,10	1,5
<b>319</b>	0,02	0,02	0,17	0,19	1,2	0,07	0,06	1,2
<b>320</b>	0,00	0,00	0,13	0,11	0,77	0,03	0,02	0,45
<b>322</b>	0,01	0,01	0,15	0,19	1,1	0,07	0,06	1,2
<b>323</b>	0,01	0,01	0,20	0,20	1,3	0,05	0,06	1,0
<b>325</b>	0,08	0,06	0,80	0,74	5,0	ANR	1,92	35
<b>326</b>	0,00	0,00	0,12	0,11	0,74	0,03	0,03	0,55
<b>328</b>	0,00	0,01	0,16	0,16	1,0	0,04	0,07	1,0
<b>329</b>	0,02	0,01	0,26	0,25	1,6	0,10	0,11	1,9
<b>330</b>	0,02	0,02	0,21	0,20	1,3	0,07	0,06	1,2
<b>331</b>	0,00	0,01	0,11	0,09	0,65	0,08	0,09	1,5
<b>332</b>	0,01	0,01	0,13	0,13	0,84	0,05	0,06	1,0
<b>333</b>	0,00	0,00	0,10	0,10	0,65	0,00	0,00	0
<b>334</b>	0,02	0,02	0,15	0,21	1,2	0,08	0,06	1,3
<b>335</b>	0,00	0,00	0,14	0,14	0,90	0,04	0,04	0,73
<b>336</b>	0,00	0,00	0,10	0,09	0,61	0,03	0,04	0,64

### APPENDIX 3: HBC 525 REPORTED NUMBERS

#### **HBC 525, Negative control multimer (Neg)**

#### **HBC 525, EBV epitope 3-specific multimer (EBV3)**

#### **HBC 525, CMV-specific multimer (CMV)**

**% multimer<sup>+</sup> CD8<sup>+</sup> of CD8<sup>+</sup> cells and relative accuracy from data reported**

<b>Lab Id</b>	<b>R7-525-Neg %</b>	<b>R8-525-Neg %</b>	<b>R9-525-EBV3 %</b>	<b>R10-525-EBV3 %</b>	<b>525-EBV3 Relative accuracy</b>	<b>R11-525-CMV %</b>	<b>R12-525-CMV %</b>	<b>525-CMV Relative accuracy</b>
<b>301</b>	0,03	0,00	0,37	0,39	1,1	0,53	0,69	0,76
<b>303</b>	0,16	0,18	0,99	1,00	2,9	1,35	1,38	1,7
<b>304</b>	0,02	0,02	0,35	0,36	1,0	0,80	0,55	0,84
<b>306</b>	0,01	0,01	0,19	0,18	0,53	0,43	0,43	0,54
<b>307</b>	0,00	0,00	0,39	0,38	1,1	1,00	1,08	1,3
<b>308</b>	0,00	0,00	0,26	0,29	0,79	0,66	0,59	0,78
<b>309</b>	0,01	0,01	0,76	0,51	1,8	1,36	1,37	1,7
<b>311</b>	0,00	0,00	0,33	0,32	0,94	1,04	1,04	1,3
<b>312</b>	0,00	0,00	0,00	0,00	0	0,00	0,00	0
<b>313</b>	0,00	0,00	0,31	0,28	0,85	0,84	0,84	1,0
<b>314</b>	0,00	0,00	0,39	0,35	1,1	0,75	0,73	0,92
<b>315</b>	0,00	0,01	0,41	0,43	1,2	0,47	0,43	0,56
<b>316</b>	0,00	0,00	0,11	0,14	0,36	0,25	0,27	0,32
<b>317</b>	0,00	0,00	0,45	0,50	1,4	0,36	0,47	0,52
<b>318</b>	0,02	0,00	0,41	0,40	1,2	0,95	1,09	1,3
<b>319</b>	0,01	0,01	0,32	0,33	0,94	0,50	0,49	0,62
<b>320</b>	0,01	0,01	0,26	0,26	0,75	0,84	0,83	1,0
<b>322</b>	0,03	0,03	0,64	0,66	1,9	1,00	1,11	1,3
<b>323</b>	0,01	0,01	0,37	0,39	1,1	0,93	0,91	1,1
<b>325</b>	0,08	0,06	0,43	0,42	1,2	0,96	1,23	1,4
<b>326</b>	0,00	0,00	0,33	0,32	0,94	0,77	0,74	0,94
<b>328</b>	0,01	0,01	0,34	0,35	0,99	0,94	0,89	1,1
<b>329</b>	0,03	0,03	0,59	0,65	1,8	1,26	1,13	1,5
<b>330</b>	0,01	0,01	0,43	0,43	1,2	1,02	1,08	1,3
<b>331</b>	0,01	0,01	0,18	0,20	0,55	0,76	0,85	1,0
<b>332</b>	0,01	0,02	0,12	0,11	0,33	0,07	0,08	0,09
<b>333</b>	0,00	0,00	0,40	0,30	1,0	0,80	0,80	1,0
<b>334</b>	0,10	0,11	0,37	0,24	0,88	0,83	0,54	0,85
<b>335</b>	0,00	0,00	0,31	0,25	0,81	0,84	0,83	1,0
<b>336</b>	0,00	0,00	0,12	0,15	0,39	0,20	0,17	0,23