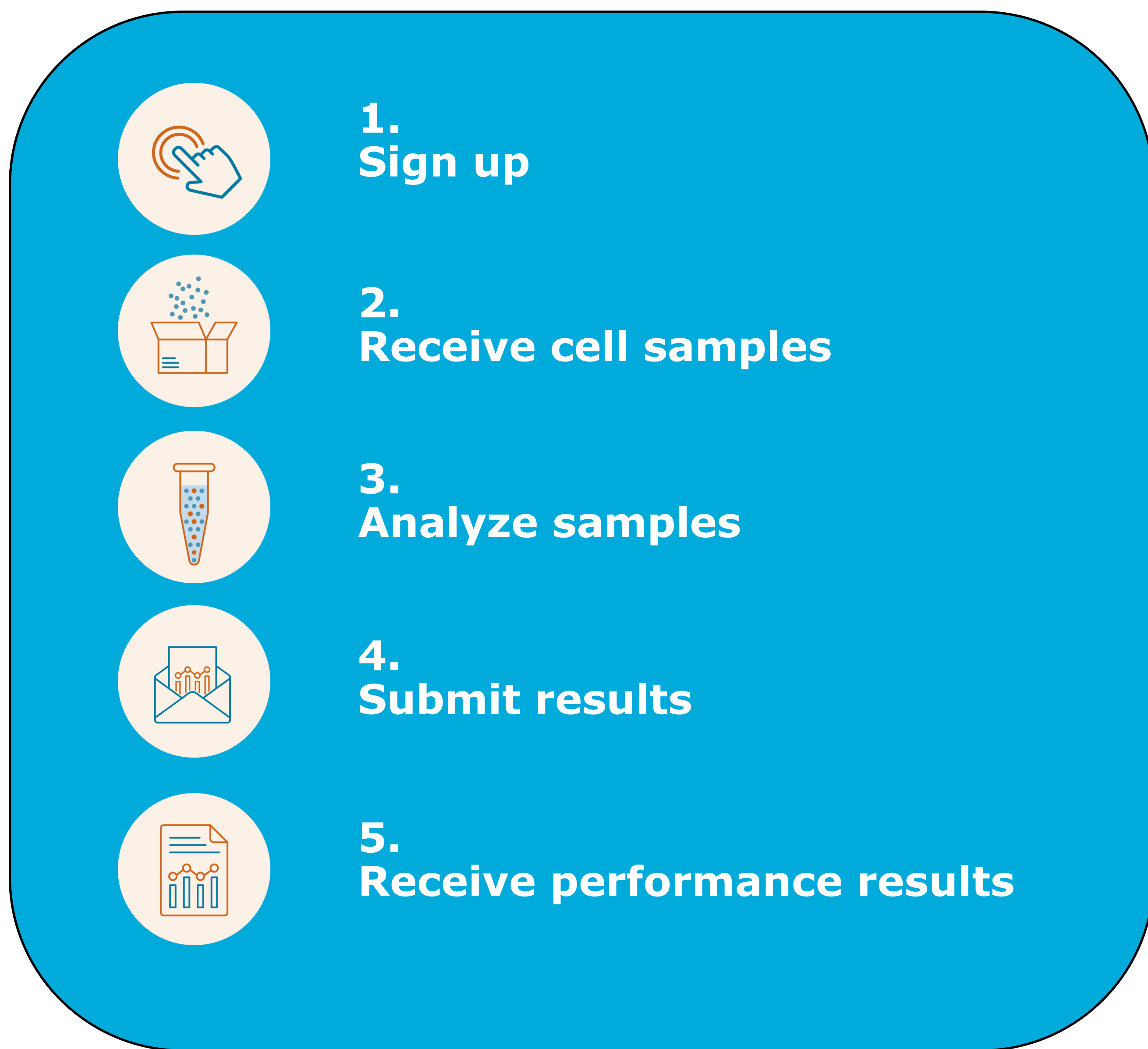


Evaluation of T-cell assay performance between laboratories worldwide

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Proficiency testing, also known as interlaboratory comparison, can provide you with a measurement of your assay performance. The annual Proficiency Panels conducted by Immudex is a non-profit service open to any laboratory, independent of location or field of interest, who wish to compare T-cell immune monitoring assay performance with peers in the field. Identical cells samples were sent to the participating laboratories. Each laboratory measured the samples according to a given set of instructions and reported their results back to Immudex. Here we present results of the two recent Proficiency Panels.

Step-by-step of a Proficiency Panel



Proficiency Panel Details

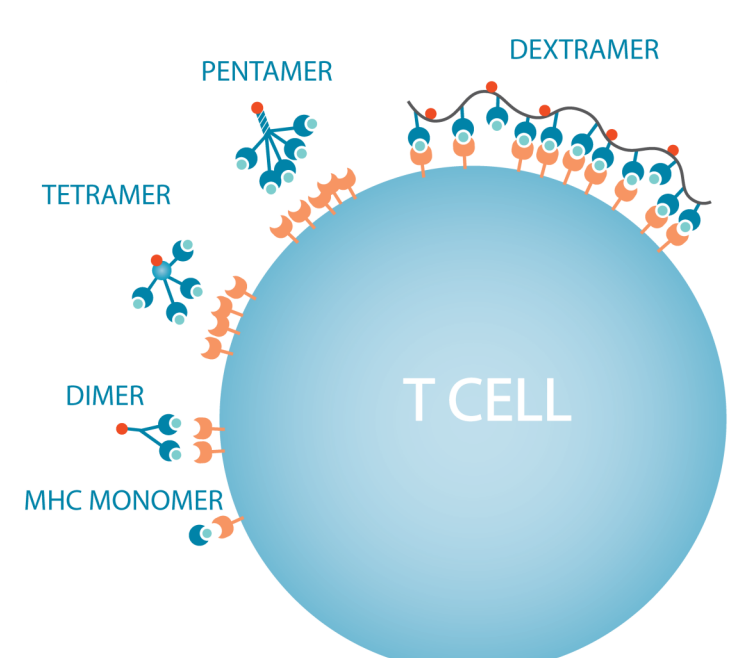
MHC Multimer Proficiency Panel

- In this panel, participants received two pretested PBMCs and were asked to quantify the number of antigen-specific T cells with their MHC Multimer type of choice.

MHC Multimer specificities used
HLA-A*0201/NLVPNVATV (CMV)
HLA-B*3501/IPSINVHHY (CMV)
HLA-A*0201/GILGFVFTL (FLU)
HLA-B*3501/HPVGEADYFEY (EBV)

- Each participant performed the Multimer assay according to a given set of instructions, but with their own choice of materials (multimer, antibodies, viability marker ect), and following their own protocol.

Participants reported back the percentage of MHC multimer+ CD8+ T cells for all MHC multimer specificities. All analyses were performed by flow cytometry.



MHC Multimer reagents will identify the antigen-specific T cells by binding to the T-cell receptors.

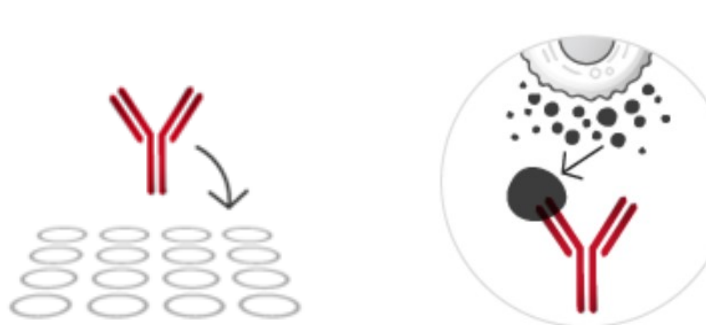
T-cell Elispot Proficiency Panel

- In this panel, participants received two pretested PBMCs and two predefined peptide pools, and were asked to stimulate and then quantify the number of IFN- γ secreting T cells.

Predefined peptide pools used
Reagent 1: CMV peptide pool (provider: JPT).
Reagent 2: CEFX peptide pool (provider: JPT).
Reagent 3: PBS/DMSO.

- Each participant performed the Elispot assay according to a given set of instructions, but with their own choice of materials and procedure (e.g. Counting method and Elispot Reader) and following their own protocol.

Participants reported back the number IFN- γ secreted T cells by reading the number of spots per well for each PBMC batch/reagent combination.



T-cell Elispot. Specific antibodies on the surface will capture cytokine IFN- γ secreted by the T-cells. Figure modified from Mabtech.

Science behind Proficiency Panel

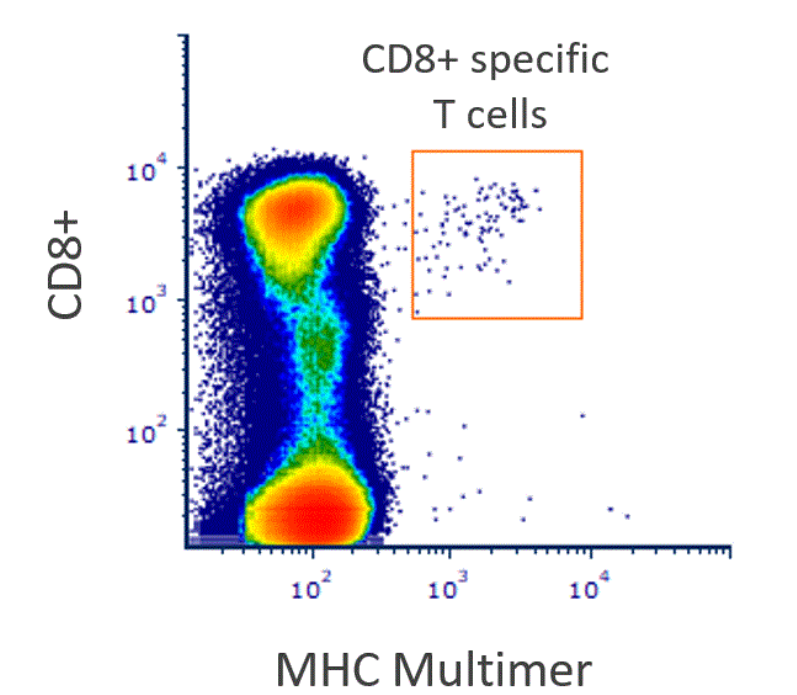
- T-cell immune monitoring Proficiency Panels are conducted in collaboration with CIC (the US Cancer Immunotherapy Consortium of the CRI) and CIMT (the European Association for Cancer Immunotherapy).

Originally, CIC/CRI and CIMT established panel programs to offer an external validation of assay performance and to enhance harmonization. Over the years more than hundred laboratories have participated, and harmonization guidelines for MHC multimer and T-cell Elispot assays have been established. Participants in Immudex's Proficiency Panel are recommended to follow these guidelines in the proficiency testing.



MHC Multimer Proficiency Panel Results (2018)

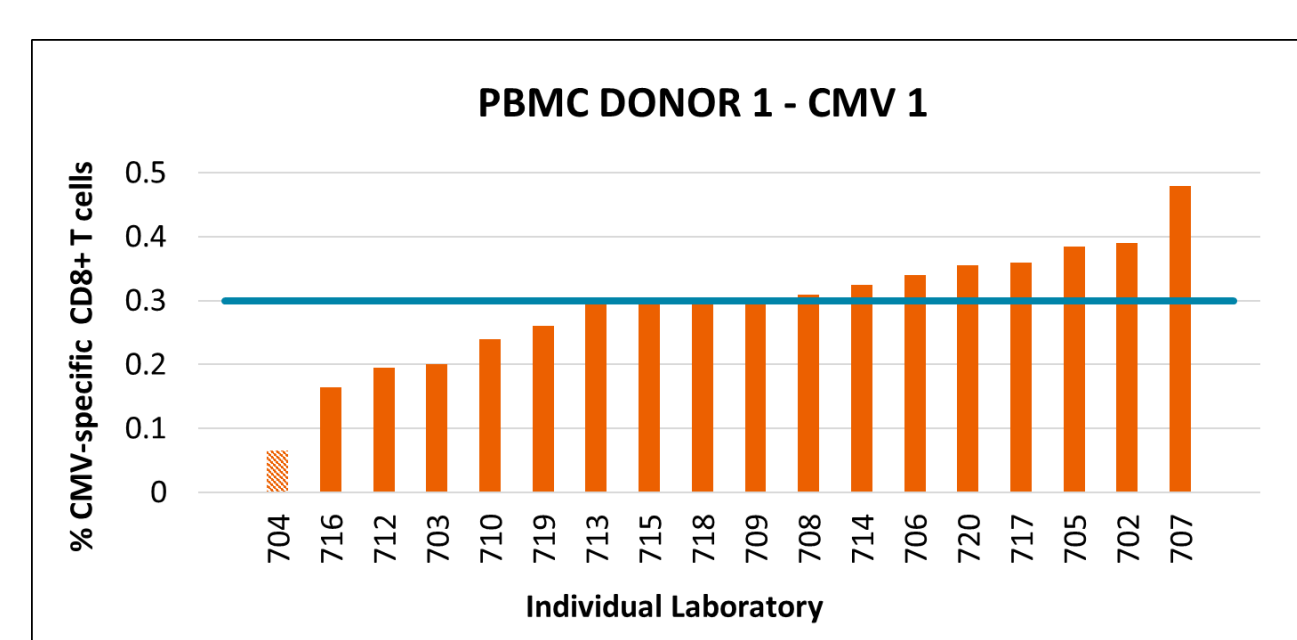
- 18 laboratories from 8 countries participated. 17 participants used MHC Dextramer reagents.
- Each participant was assigned a confidential participant identification number (702, 703, 704.....)
- The percentage of CMV-, FLU- and EBV-specific T cells for both donor samples (PBMCs) were reported.
- Approximately 90% of the participants' results were in the average or near the average range (Figure 1-4). All measurements were made in duplicates, and here presented as mean values.



MHC Dextramer identifying CD8+ specific T cells. The sample analysis was performed by flow cytometry.

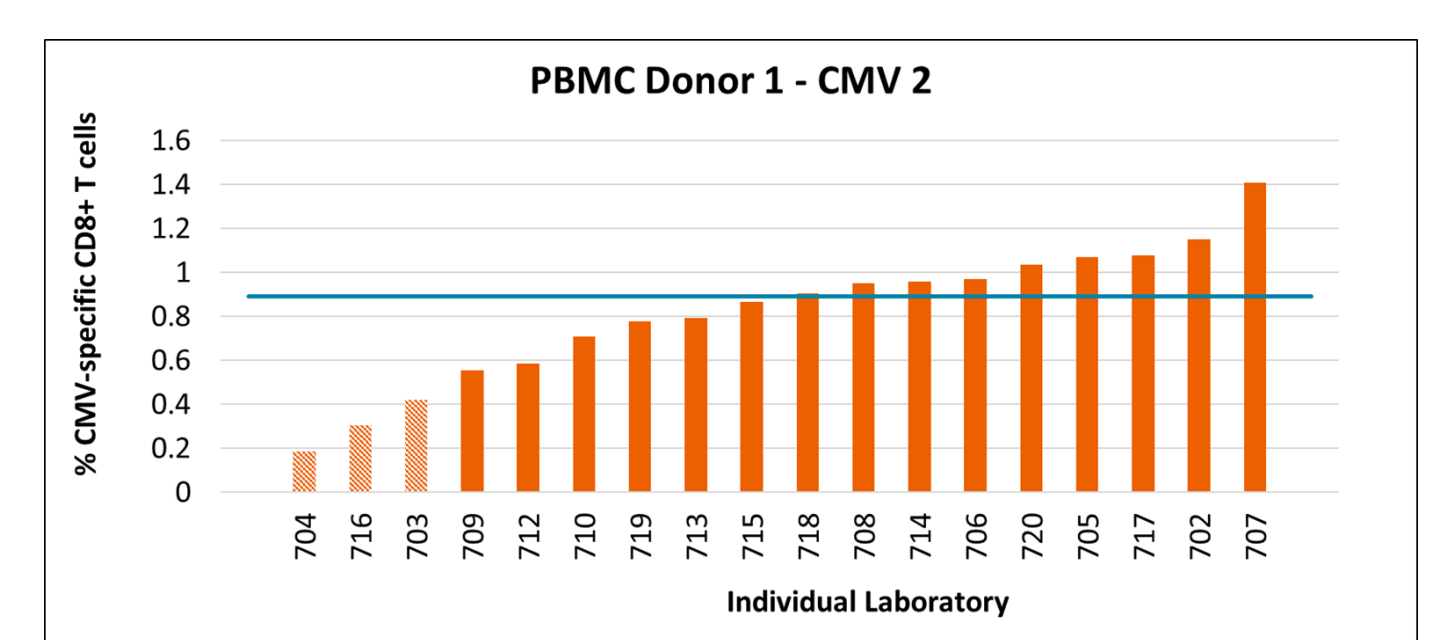
Dark orange columns illustrate participants with results "in the average range" or "near the average range", and the blue line shows the median. Average range: Any result from 1,5 times lower to 1,5 times higher than the median of all results. Near average range: Any results from 1,6 to 2,0 times higher than the median of all results, and result from 0,50 to 0,65 times lower than the median of all results.

Figure 1



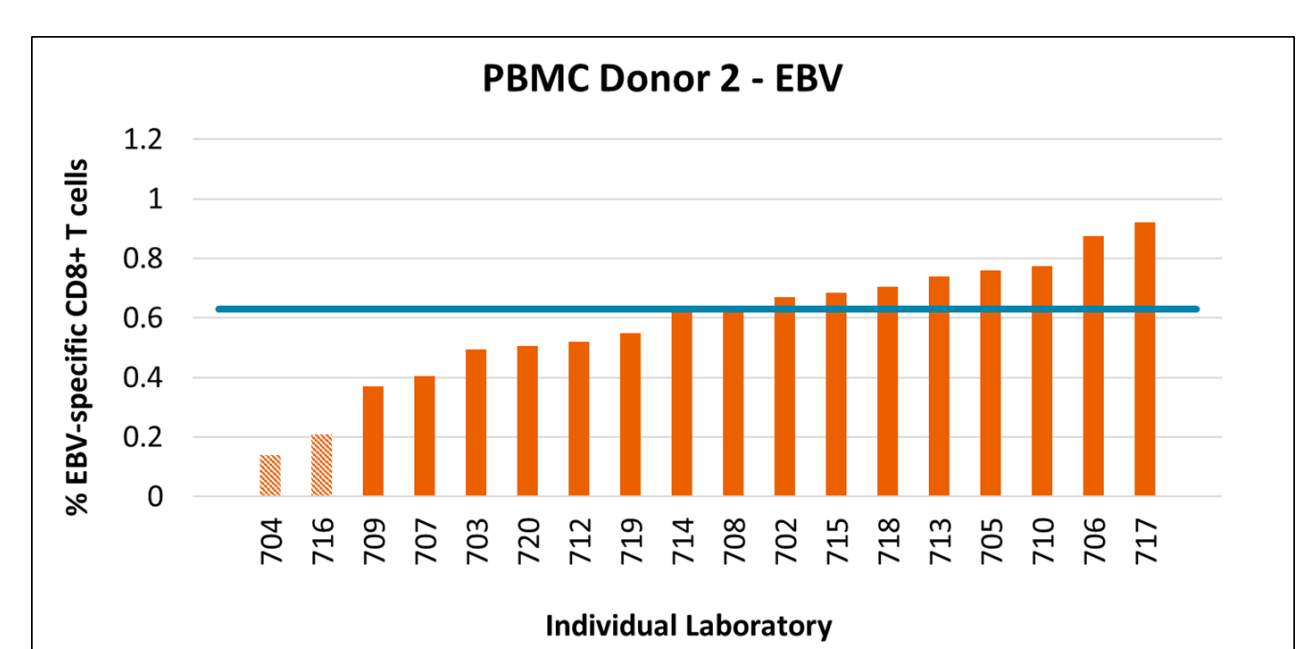
Median: 0,30 % CMV-specific CD8+ T cells.

Figure 2



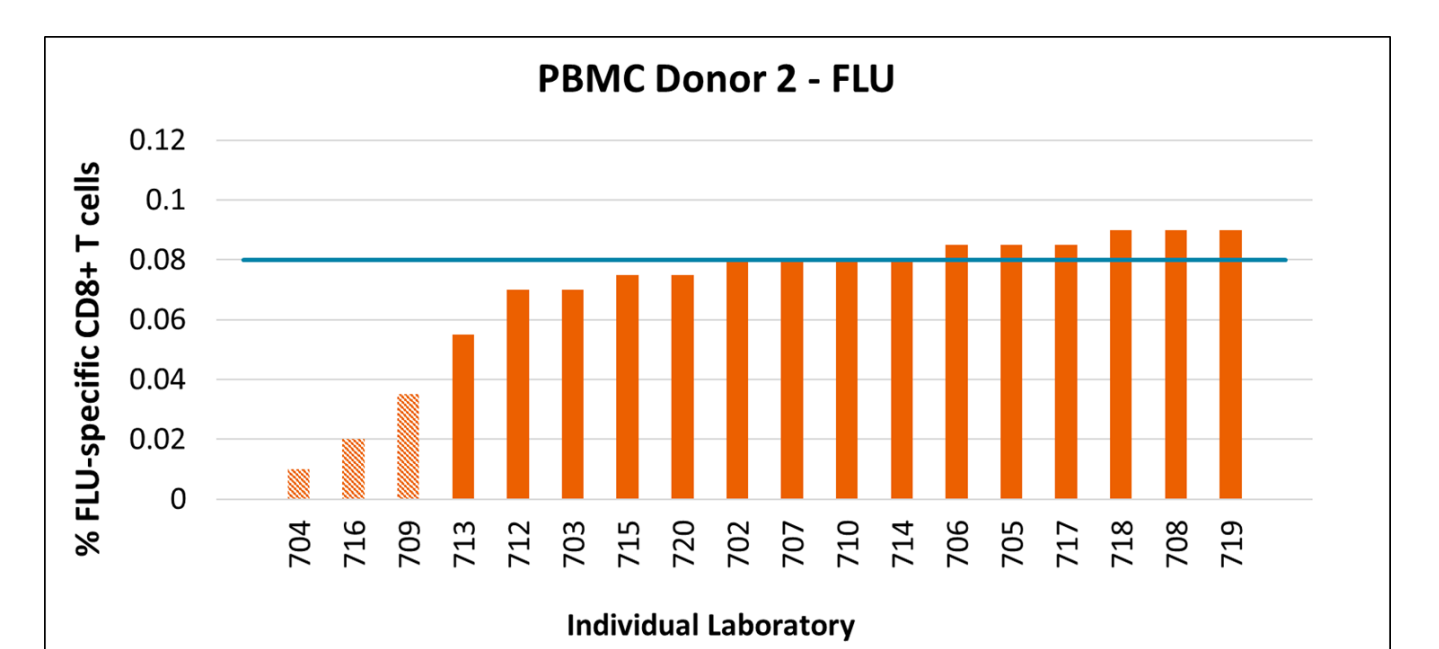
Median: 0,89 % CMV-specific CD8+ T cells.

Figure 3



Median: 0,63% EBV-specific CD8+ T cells.

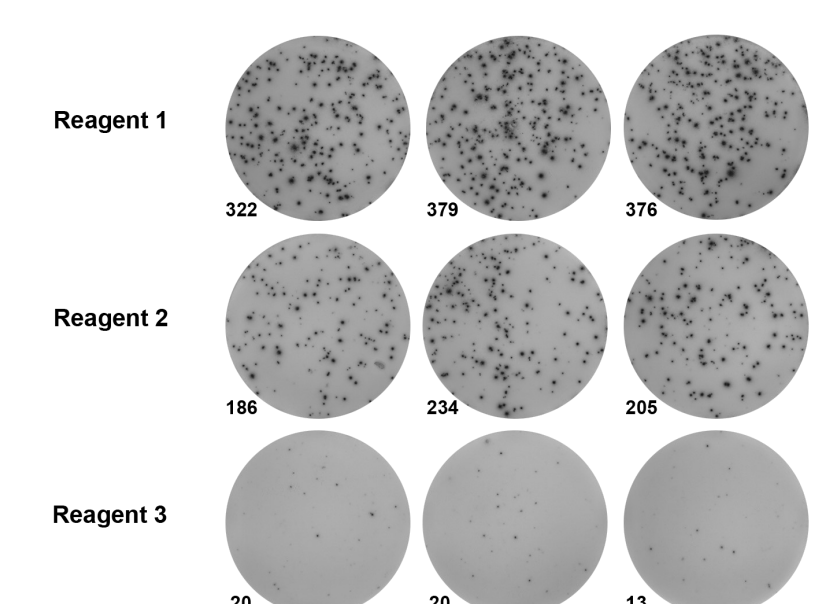
Figure 4



Median: 0,08 % FLU-specific CD8+ T cells.

T-cell Elispot Proficiency Panel Results (2019)

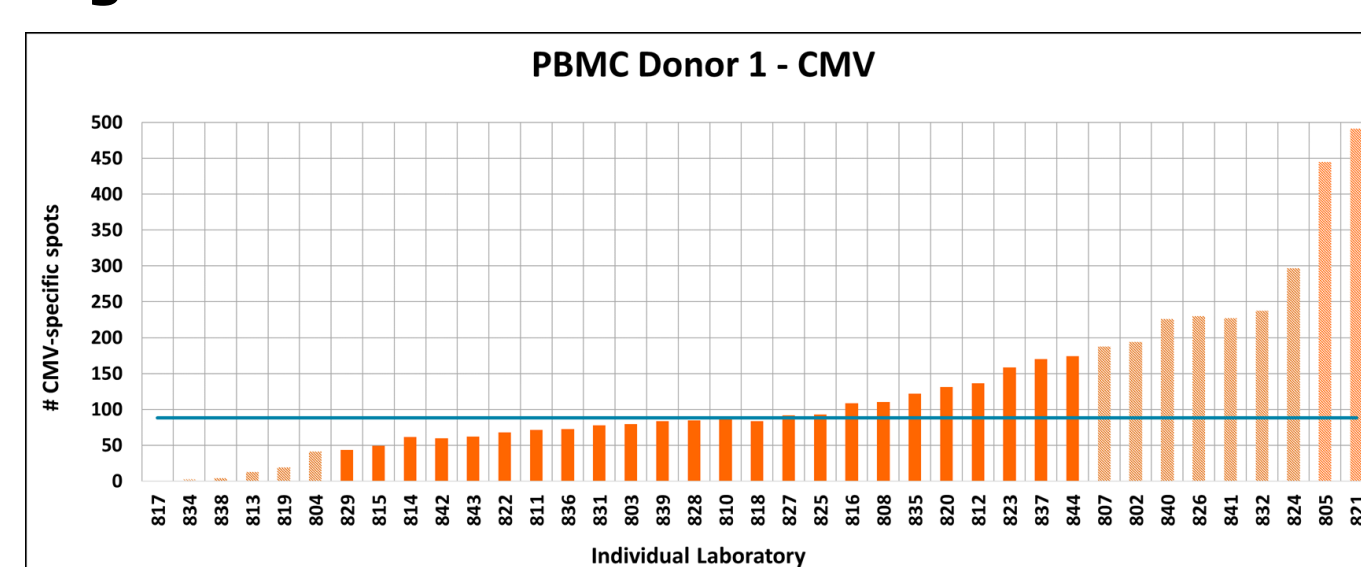
- 43 laboratories from 13 countries participated.
- Each participant was assigned a confidential participant identification number (802, 803, 804,)
- The number of spots for each peptide pool were reported for both donor samples (PBMCs).
- Approximately 65% of the participants' results were in the average or near the average range (Figure 5-8). All measurements were made in triplicates, and here presented as mean values.



Elispot plates analyzed with Reagent 1, Reagent 2 or Reagent 3. The spots were analyzed with the Mab-tech IRIS ELISpot/FluoroSpot reader.

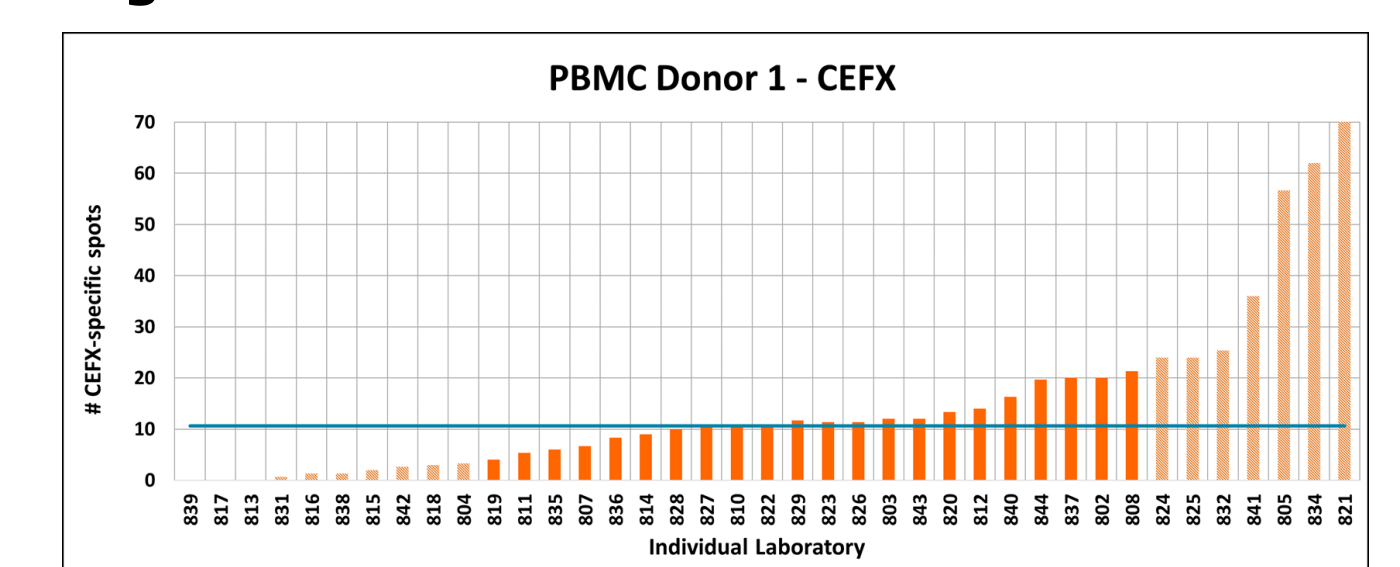
Dark orange columns illustrate participants with results "in the average range" or "near the average range", and the blue line shows the median. Average range: Any result from 1,5 times lower to 1,5 times higher than the median of all results. Near average range: Any results from 1,6 to 2,0 times higher than the median of all results, and result from 0,50 to 0,65 times lower than the median of all results.

Figure 5



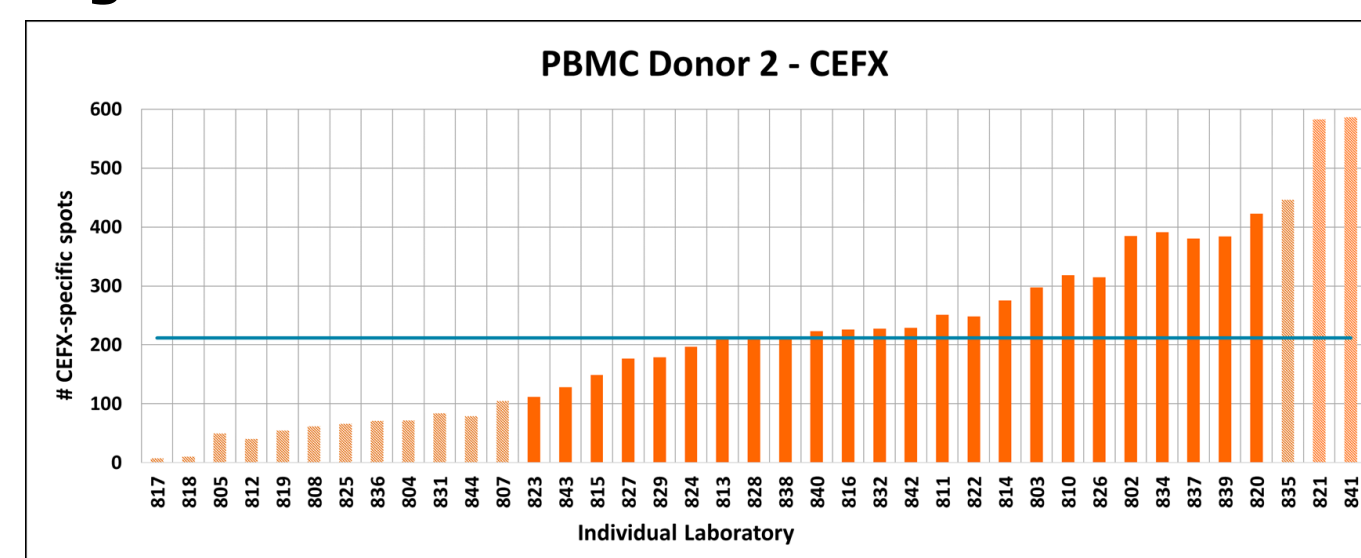
Median: 88 CMV-specific spots/200.000 PBMC's.

Figure 6



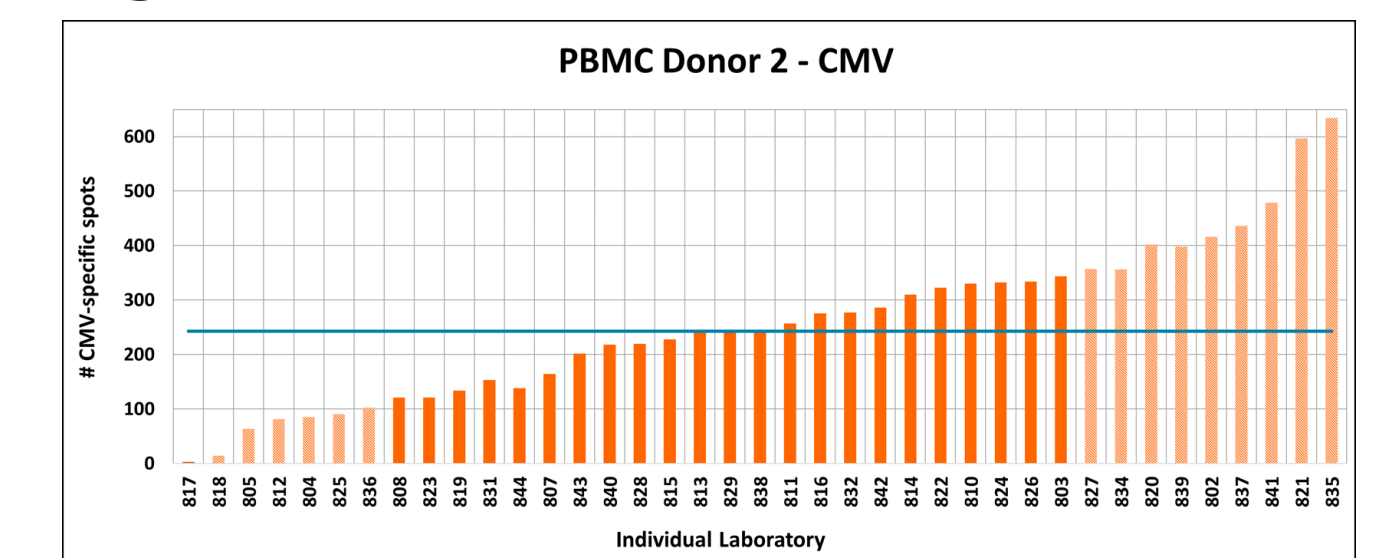
Median: 11 CEFX-specific spots/200.000 PBMC's.

Figure 7



Median: 211 CEFX-specific spots/200.000 PBMC's.

Figure 8



Median: 243 CMV-specific spots/200.000 PBMC's.

Conclusion Participants in both panels showed good reproducibility and provided equivalent replicates for their measurements. For the MHC multimer analysis 90% of the participants' results were in the average or near average range, and for the T-cell Elispot it was 65% of the participants. Thus MHC Multimer analysis is more standardized across laboratories compared to the T-cell Elispot analysis. If multicenter research trials are conducted, proficiency testing among collaborating parties could be a valuable tool to ensure high consistency in data generation between laboratories.