T-cell Elispot Proficiency Panel 2019

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Background

The T-cell Elispot Proficiency Panel 2019 conducted by Immudex is a non-profit service offered with the intent of testing and ensuring a high level of proficiency and reliability among the researchers and clinical laoboratories that perform this type of immune monitoring assay.
The proficiency panel services offered by Immudex are open to any laboratory, independent of geographic location or field of interest, with a need to evaluate if they perform accurate and reproducible quantification of antigen-specific T cells using T-cell Elispot. Here we present preliminary results.

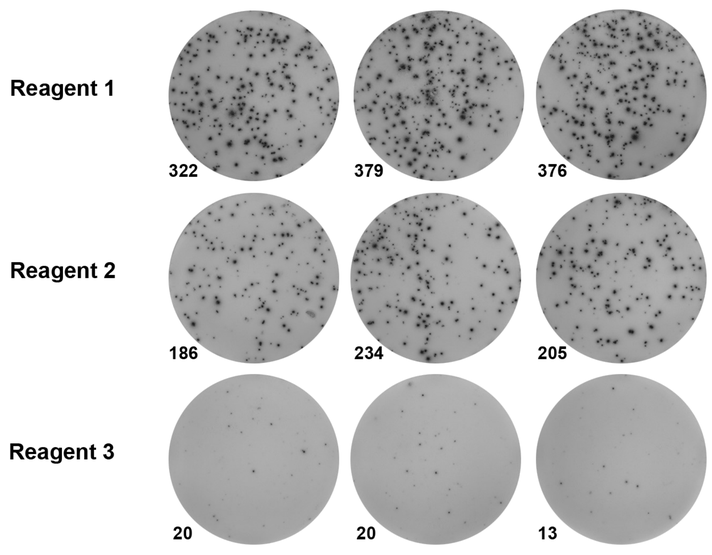
Elispot Proficiency Panel Design

- 43 laboratories from 13 countries participated in the Elispot proficiency panel. Here we present data from 37 laboratories.
- Each participant was assigned a confidential participant Identification Number (Id).
- Each participant received two cell samples comprising PBMC's from two different donors and three vials of reagents: Reagent 1 (CMV peptide pool), Reagent 2 (CEFX peptide pool) and Reagent 3 (PBS/DMSO).
- All vials were shipped in liquid nitrogen. A temperature logger was included in the shipment to observe the temperature from packaging to delivery.
- Each laboratory performed the T-cell Elispot assay and reported the results according to "Instruction for the Elispot Proficiency Panel 2019".

Elispot proficiency panel assay instructions

- Participants were recommended to take into consideration previously established Elispot harmonization guidelines, based on the CIC/CRI and CIMT Elispot panel programs. These guidelines was provided to all participants.
- Participants were requested to use their own protocol for Direct Human IFNγ Elispot Assays, including antibodies, plates, enzymes, equipment, medium and other chemicals and tools necessary to perform the assay.
- Participants were instructed to thaw both vials of PBMC and count and record the number and percentage of viable cells.
- 200.000 viable cells/well in 50µL medium were plated in a 96 well plate together with diluted Reagent 1, 2 and 3 in 50µL to a total volume of 100µL in each well. All measurements were requested in triplicates.

Figure 1



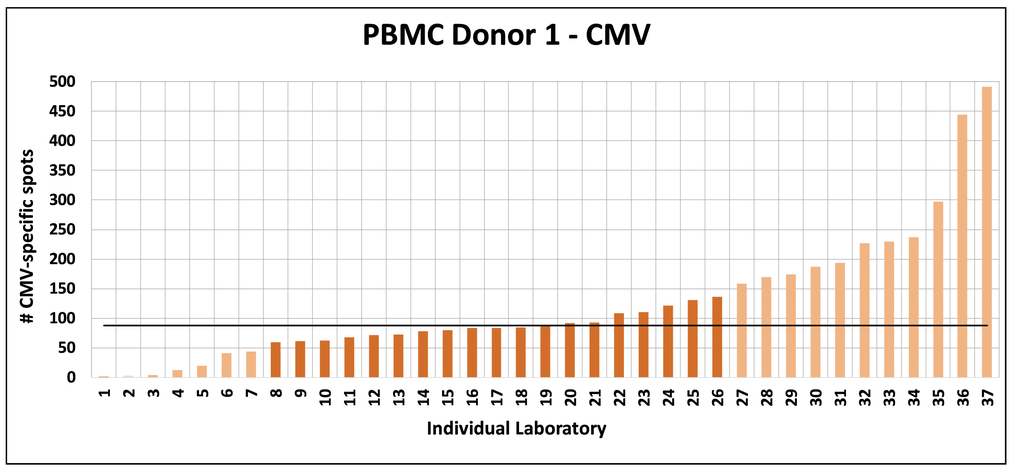
Elispot plates treated with Reagent 1, Reagent 2 or Reagent 3.
The spots were analysed with the Mabtech IRIS ELISpot/FluoroSpot reader.

Results

Figures 2-5 illustrate results obtained by participating laboratories. All measurements were made in triplicates, and here presented as mean values. Number of spots per 200.000 PBMC's are shown.

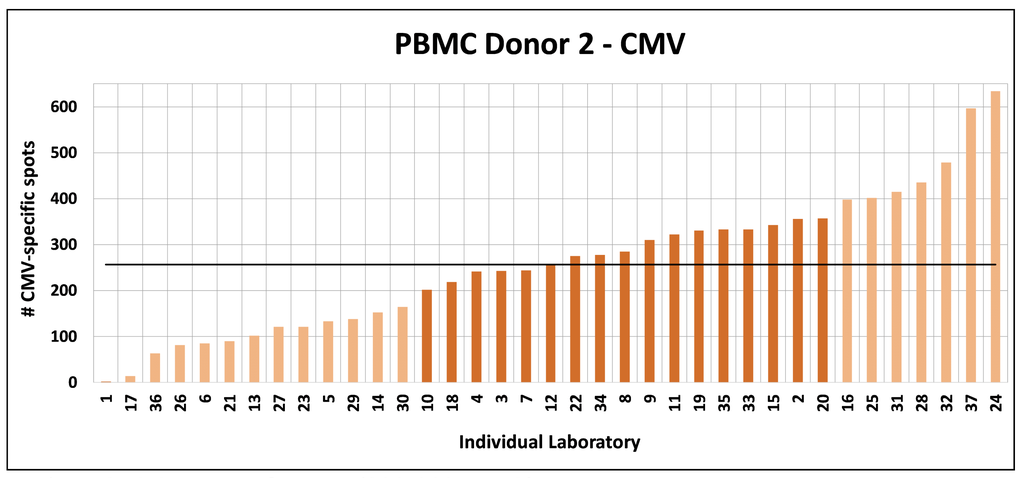
The median result from all laboratories are shown as a black line. Dark orange columns indicate participants with results in the average range. "Average range" is defined as 1,5 times lower or higher than the median.

Figure 2



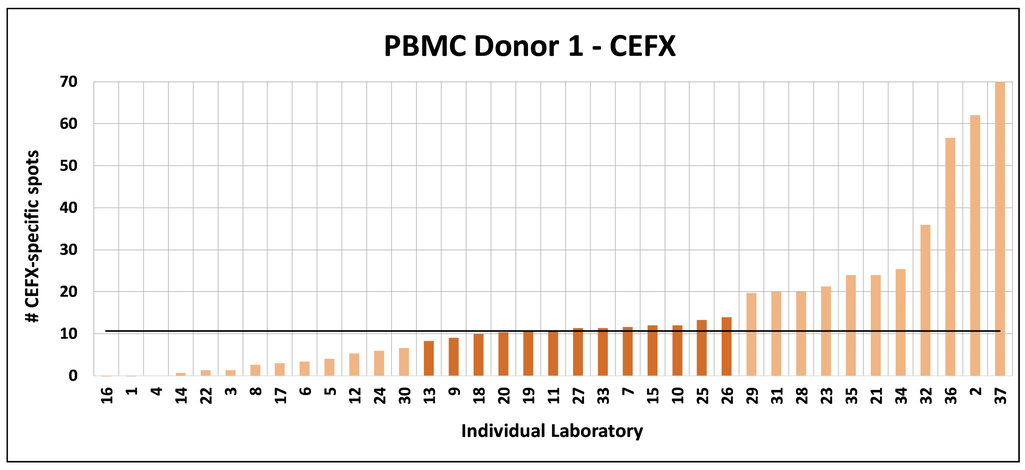
Median: 88 CMV-specific spots/200.000 PBMC's.

Figure 3



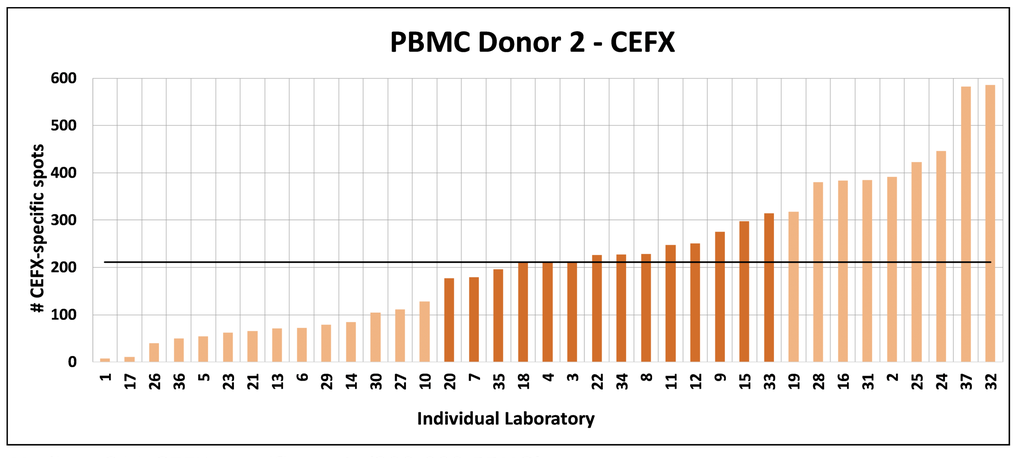
Median: 256 CMV-specific spots/200.000 PBMC's.

Figure 4



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

Figure 5



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

Conclusion

Approximately, 42% of the participants' results for all measurements were in the average range. All participating laboratories showed good reproducibility and provided equivalent values of their triplicates for Reagent 1, Reagent 2 and Reagent 3. Overall, the Elispot Proficiency Panel is a valuable tool to evaluate performance between laboratories worldwide.

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