ImmuMap
We Map Cellular Immunity

Human IFNγ ELISpot assay

Assay robustness

Aim
Establish reliability of assay

Experimental approach
Ex vivo IFNγ ELISpot of human peripheral blood mononuclear cells (hPBMCs). Experimental setup and SOP has been established in concordance with harmonization guidelines (See ref 1). Initial experiment (day 1) was performed on 5 donors against 2-5 cytomegalovirus (CMV), Epstein Barr virus (EBV) or influenza derived peptide epitopes each and tested in triplicates (data shown for 2 selected donors). Experiments on day 2 and 3 were performed on two selected donors against one HLA-matched CMV-derived peptide each and tested in 6 replicates.

Assay Conditions
- Two donors
- Three to six replicates per donor
- Independent performance on three separate days

Limit of detection (spot count)

2 x median background

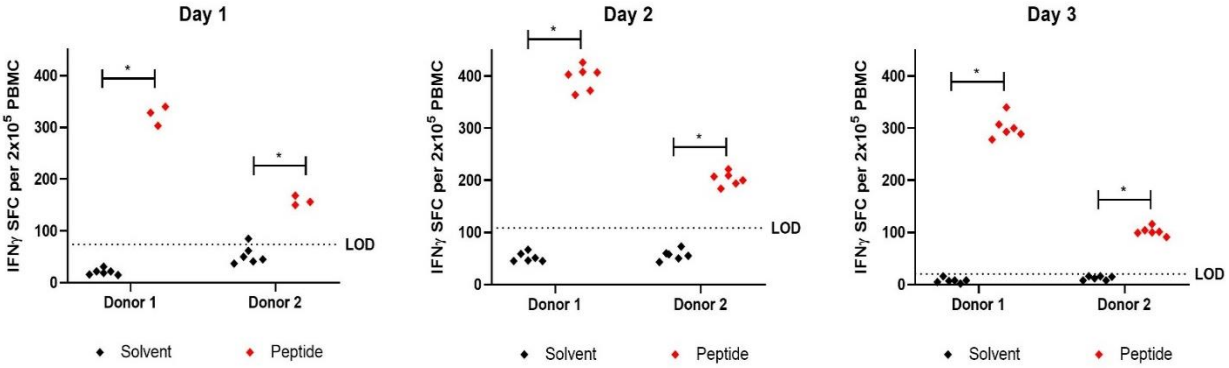

Figure 1. Establishing the limit of detection (LOD) from two donors across three independent days. Scatter plot of IFNγ spot forming cells (SFC) per $2x10^5$ PBMC on day 1 (left), day 2 (middle) and day 3(right). Solvent spots (background) (black) in PBMC stimulated with medium inclusive peptide solvent (DMSO), peptide spots (red) in PBMC stimulated with a HLA-A*02:01-restricted CMV peptide (donor 1) or a HLA-A*01:01-restricted CMV peptide (donor 2). * $p<0.05$

The limit of detection (LOD) (dotted line) has been established for each separate day as follows: LOD = 2 x the median background reactivity. LOD(day 1) = 74.2 , LOD(day 2) = 108.7 , LOD(day 3) = 20.2

The statistical significance of the responses was calculated using the distribution free resampling method (DFR(eq)) (See ref. 2).

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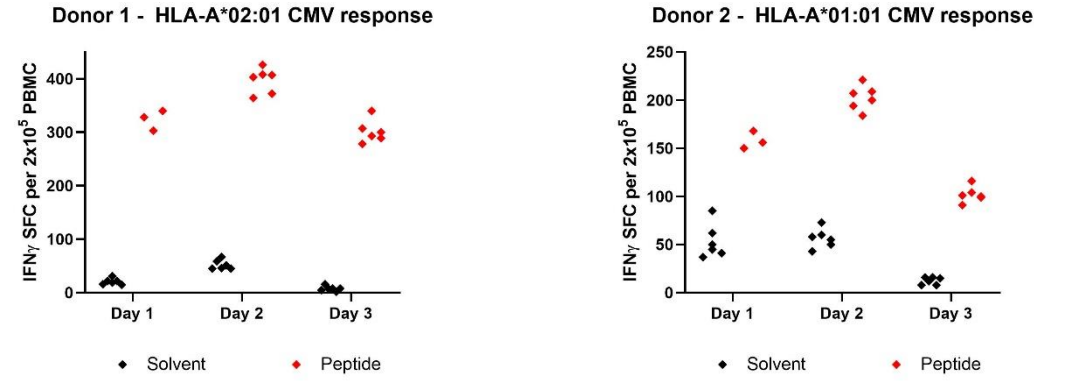
Intra-day variation



Donor 1	Day 1	Day 2	Day 3
Peptide	323.7±18.9 (5.8%)	396.7 ±23.7 (6.0%)	301.2±21.4 (7.1%)
Solvent	20.8±5.8 (27.7%)	52.2±9.0 (17.3%)	7.7±4.7 (61.0%)

Intra-day mean, standard deviation and CV% of 3 to 6 replicates for donor 1 on days 1, 2 and 3. Number in parentheses is the CV% calculated as: CV = (std dev/mean) x 100

Donor 2	Day 1	Day 2	Day 3
Peptide	158.0±9.2 (5.8%)	202.5±12.8 (6.3%)	101.8±8.2 (8.0%)
Solvent	53.3±17.8 (33.3%)	56.5±10.1 (17.9%)	12.5±3.8 (30.3%)

Intra-day mean, standard deviation and CV% of 3 to 6 replicates for donor 2 on days 1, 2 and 3. Number in parentheses is the CV% calculated as: CV = (std dev/mean) x 100

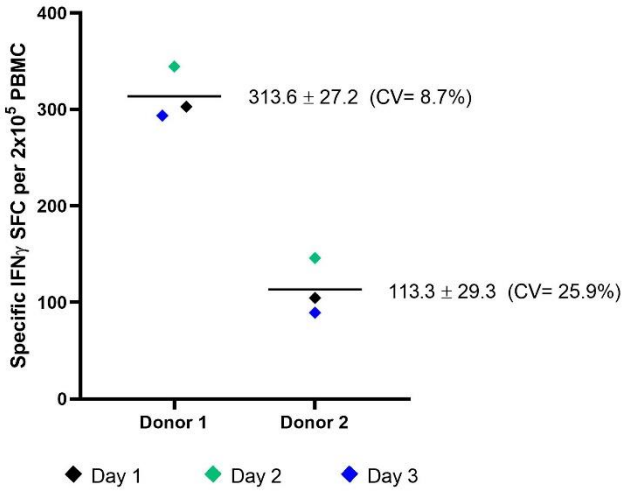
Figure 2. Results from analysis of two donors across three independent days. Scatter plot of IFNγ spot forming cells (SFC) per 2x10^5 PBMC from Donor 1 (left) and Donor 2 (right). Solvent spots (background) (black) in PBMC stimulated with medium inclusive peptide solvent (DMSO), peptide spots (red) in PBMC stimulated with an HLA-A*02:01-restricted CMV peptide (donor 1) or an HLA-A*01:01-restricted CMV peptide (donor 2).

The intra-day mean, standard deviations and CV for donor replicates are shown in the tables.

Inter-day variation

Figure 3. Mean of peptide-specific spots from two donors across three independent days. Scatter plot of the average number of IFNγ spot forming cells (SFC) per 2x10^5 PBMC from Donor 1 (left) and Donor 2 (right). Background spots are subtracted from peptide-stimulated spots to enumerate the number of peptide-specific spots. The average number of peptide specific spots for day 1 (black), day 2 (green) and day 3 (blue) as well as the mean value across all three days (black line) are depicted.

Values for the inter-day mean, standard deviation and CV% for donor replicates are listed.



References:1) S. Janetzki et al., Nature Protocols (2015) 10(7): 1098-1115 2) Z. Moodie et al., Cancer Immunol Immunother (2010) 59:1489-1501