

COVID-19, FFX T cell testing

First T-cell tests on PBMCs of (potential) COVID-19 infected donors.

ELISPOT

PBMC vs CD56 depleted PBMC

Regular stimulation vs costimulation (CD28)

Stimulation:

- DMSO (negative) control
- Spike PEPMIX (Cov-2)
- Spike protein (CoV-2)
- SARS-CoV-2 (inactive)
- PHA (positive) control

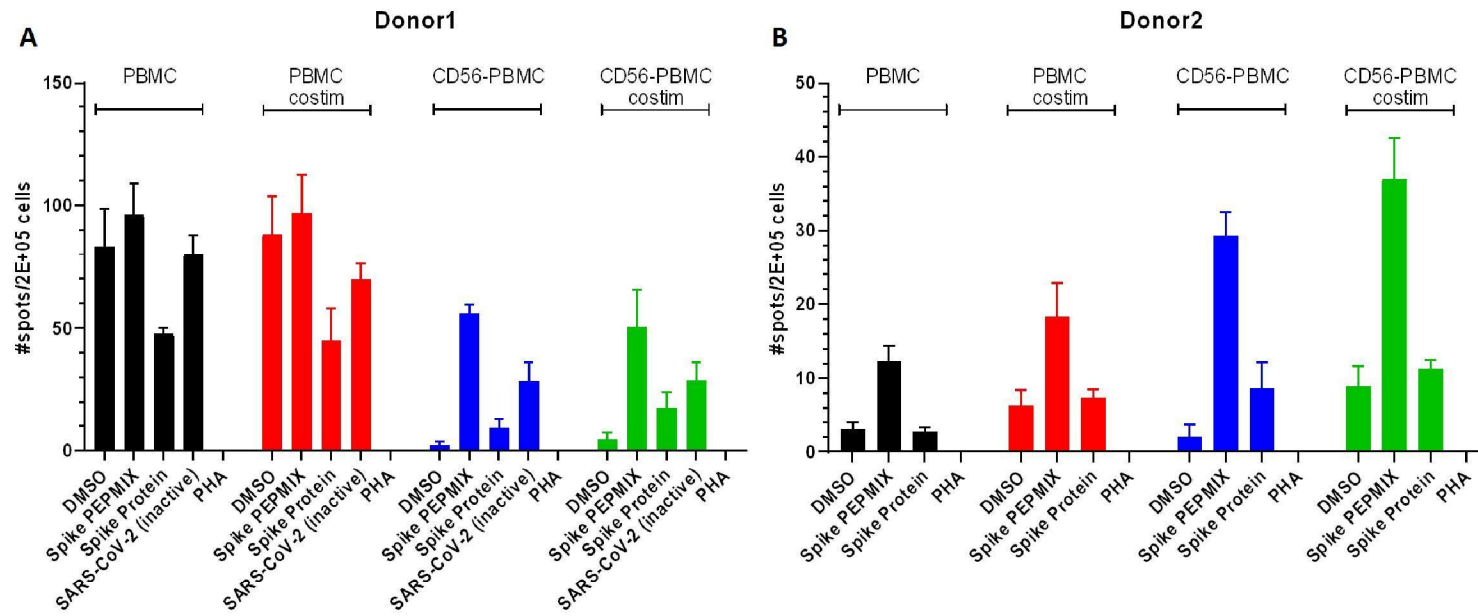
FACS

Use leftovers for stimulation (6 and 24 hours)

ICS / activationmarkers

COVID19 T-exp1ELISPOT IFN γ

+/-20 hours



PBMCs or CD56 depleted PBMC (CD56-PBMCs) of (A) donor1 and (B) donor 2 are stimulated with Spike1 PEPMIX (0,1 μ M/peptide) or DMSO (equal amount as pepmix) as negative control, Spike protein (1 μ g/ml, UU), SARS-CoV-2 MOI-0,1 (inactive, IDS) and PHA as positive control for 20 hours. 200.000 cells/well are plated. CD28 (0,1 μ g/ml) is used for costimulation

A high background when using PBMCs (very small spots), not when CD56 is depleted.

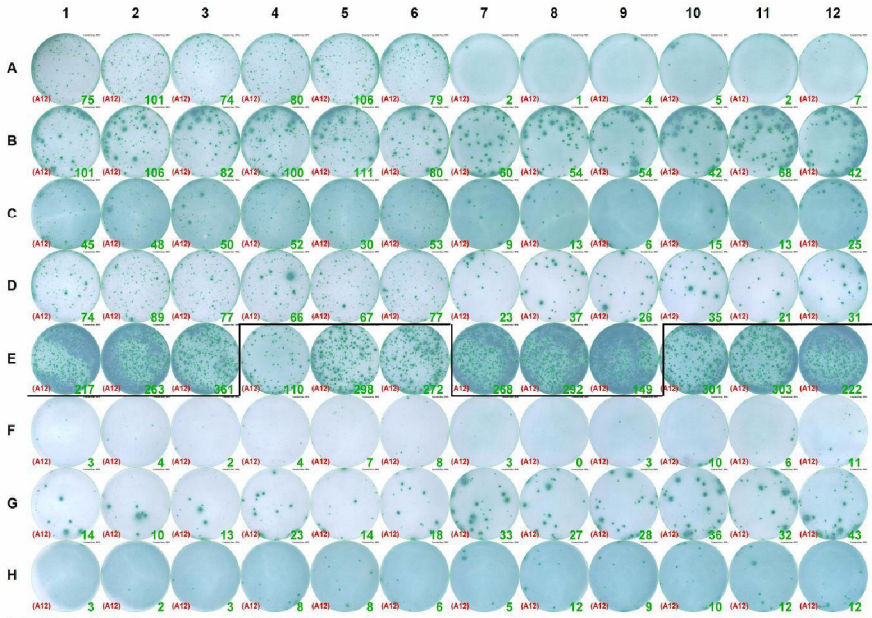
B No (high) background when using PBMCs

COVID19 T-exp1

ELISPOT IFN γ

+/-20 hours

plate1

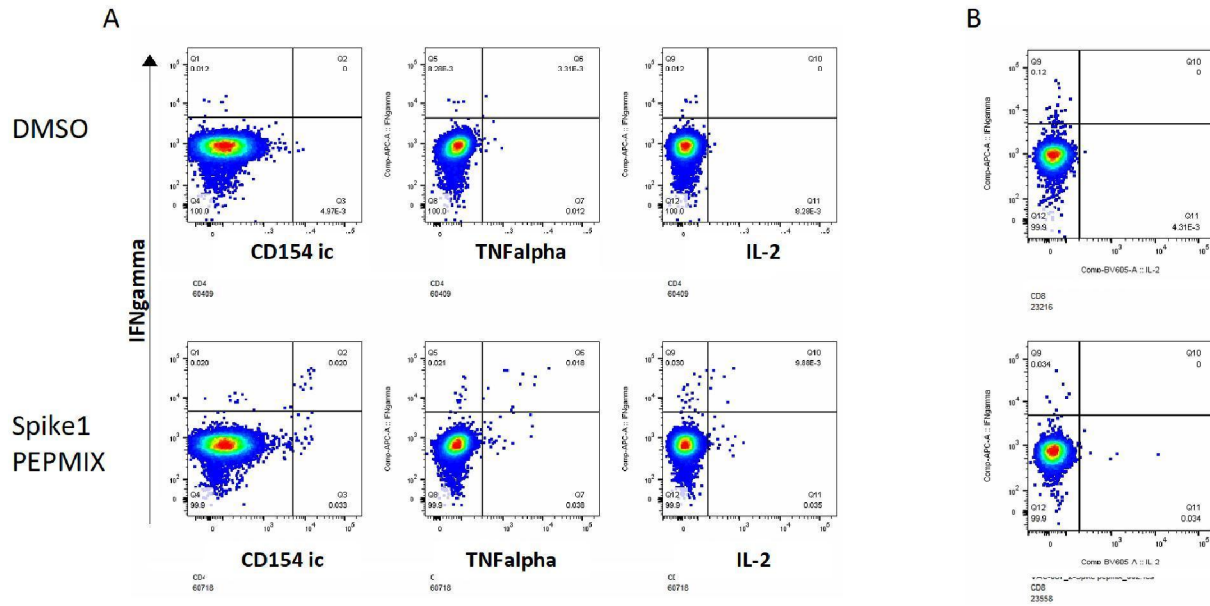


Quality Controlled using ImmunoSpot 7.0.17.0

CTL

ELISPOT		1-3	4-6	7-9	10-12
Donor1	A	DMSO control	DMSO control	DMSO control	DMSO control
	B	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)
	C	CoV-2-(S protein)	CoV-2-(S protein)	CoV-2-(S protein)	CoV-2-(S protein)
	D	CoV (inactive)	CoV (inactive)	CoV (inactive)	CoV (inactive)
Donor2	E	pos control	pos control	pos control	pos control
	F	DMSO control	DMSO control	DMSO control	DMSO control
	G	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)	CoV-2-(Spike1 pept)
	H	CoV-2-(S protein)	CoV-2-(S protein)	CoV-2-(S protein)	CoV-2-(S protein)
		CD28 costim		CD28 costim	
		0,2E+06 PBMC/well		0,2E+06 CD56- PBMC/well	

COVID19 T-exp2
 6 hours stimulation
 Donor 1



PBMCs (1E+06) of donor1 are stimulated with Spike1 PEPMIX (0,1 μM/peptide) or DMSO (equal amount as pepmix) as control for 6 hours. During the last 4 hours, a mixture of BrefeldinA and Monensin was co-cultured. Cells were stained for surface and intracellular markers.

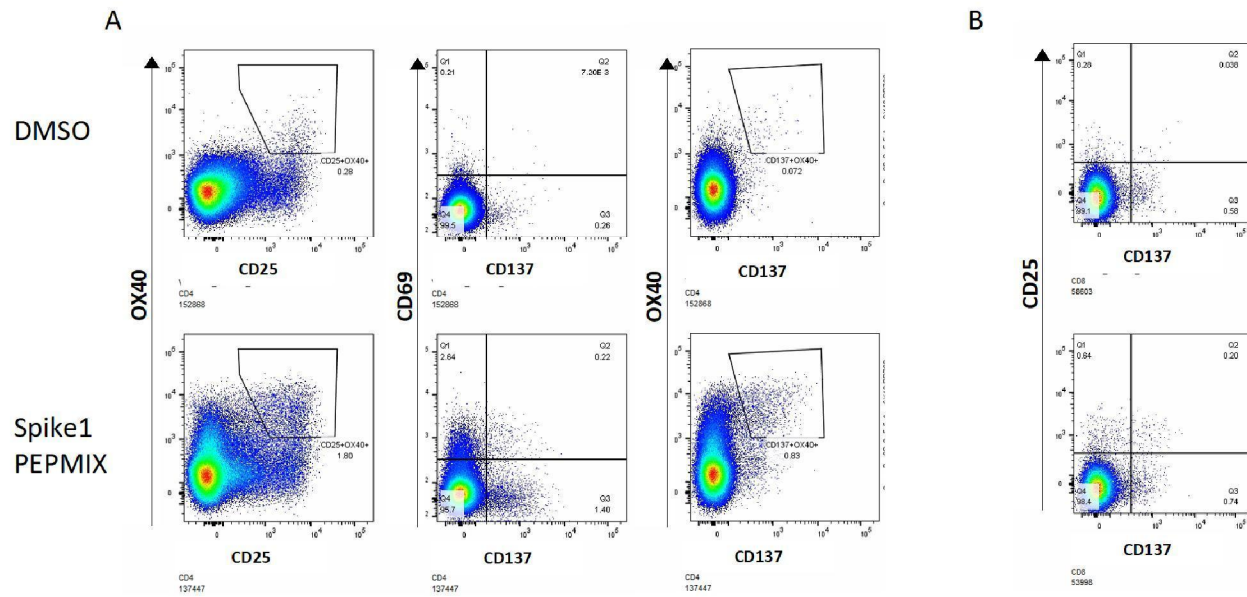
A In the CD4 cells CD154, IFN γ , TNF α and IL-2 are upregulated/produced. Looking in more depth, mainly the Tcm cells are activated.

B IN the CD8 cells, only a minimal response of IL-2. This could be the Temra cells.

COVID19 T-exp2

24 hours stimulation

Donor 1



PBMCs ($1E+06$) of donor1 are stimulated with Spike1 PEPmix ($0,1 \mu\text{M}/\text{peptide}$) or DMSO (equal amount as pepmix) as control for 24 hours. Cells were stained for surface markers.

A In the CD4 cells OX40, CD25, CD137 and CD69 are upregulated. CD25+OX40+ and CD137+OX40+ double positive cells show high responses. Looking in more depth, mainly the Tcm and Tem cells are activated.

B In the CD8 cells, CD25 and CD137 (CD137+CD25+/CD137-CD25+) are upregulated.

summary

ELISPOT

- First clear reponse on Spike PEPMIX en SARS-CoV-2 (inactive)
- High background in 1 donor when using PBMC
 - CD56 depleting prevents high background
- Use ELISPOT software to eliminate the small spots

FACS

- Specific T cells can be detected by FACS after 6 and 24 hours
 - Only Spike PEPMIX stimulation
- Mainly CD4 response (Tcm - Tem)
 - 6 hours: CD154, IFNg, TNFa and IL-2
 - 24 hours: CD137, OX40, CD25 and (CD69)
- Very minor CD8 response (Temra?)
 - 6 hours: IL-2
 - 24 hours: CD137 and CD25