Proof of principle for the use of the T cell ELISpot in clinical trial settings

Optimization strategies and considerations

Julian J. Freen-van Heeren, PhD.

Scientist Immunomonitoring Services

j.freenvanheeren@sanquin.nl

immunomonitoring@sanquin.nl

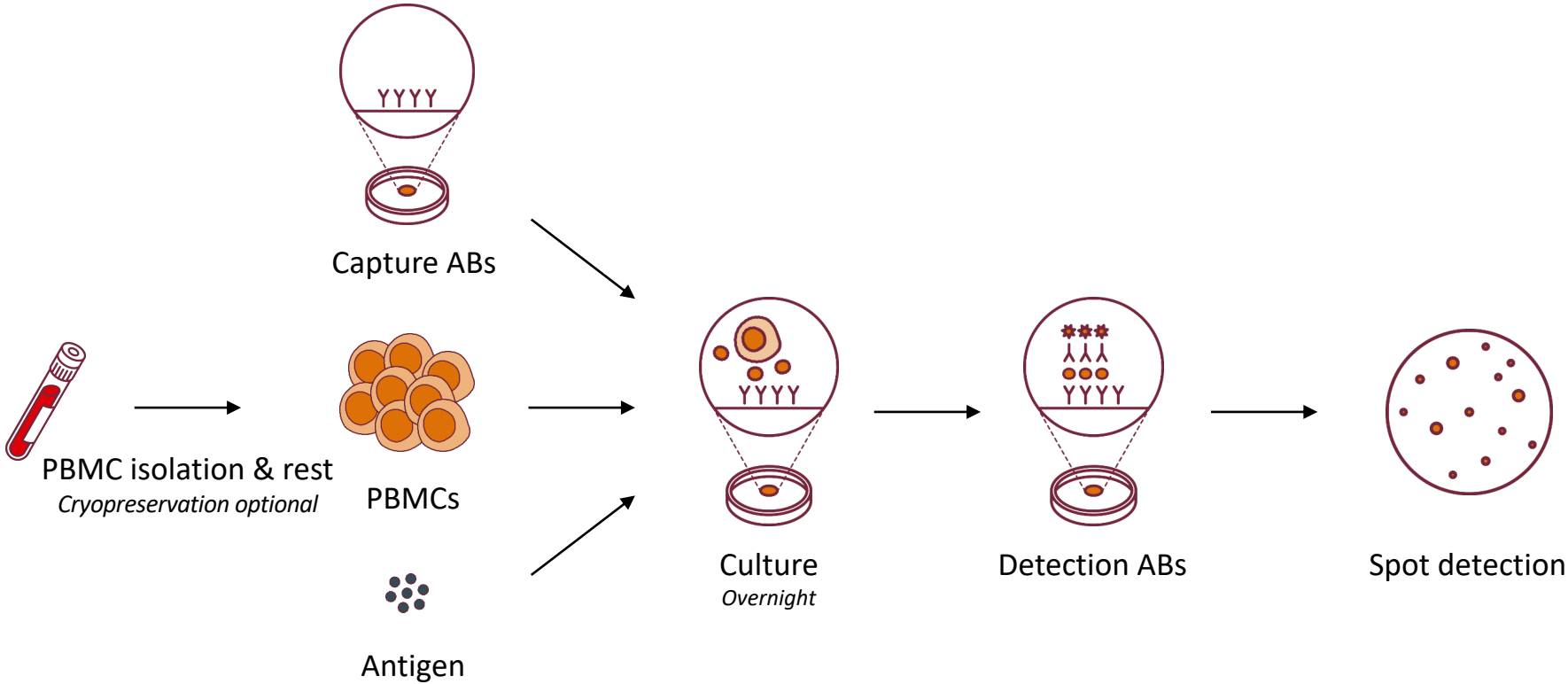
Sanquin

a not for profit organization

ELISpot assays

- The enzyme-linked immunospot (ELISpot) assay is a highly sensitive test to determine the frequency of antigen-specific cells by determining effector molecule secretion at a single cell level
- Can be used to detect singular production of a single effector molecule of interest (ELISpot) or a combination of multiple effector molecules (FluoroSpot)
  - IFN-γ, granzyme B, perforin, TNF-α – cytotoxic $CD8^+$ T cells
  - IL-2, IL-10, TNF-α – helper $CD4^+$ T cells
  - Antibody (and isotypes) – B cells
- Highly sensitive and high throughput
- Requires fewer cells than typical approaches (i.e. ELISA or flow cytometric readout of intracellular cytokine production)

How do ELISpots work?



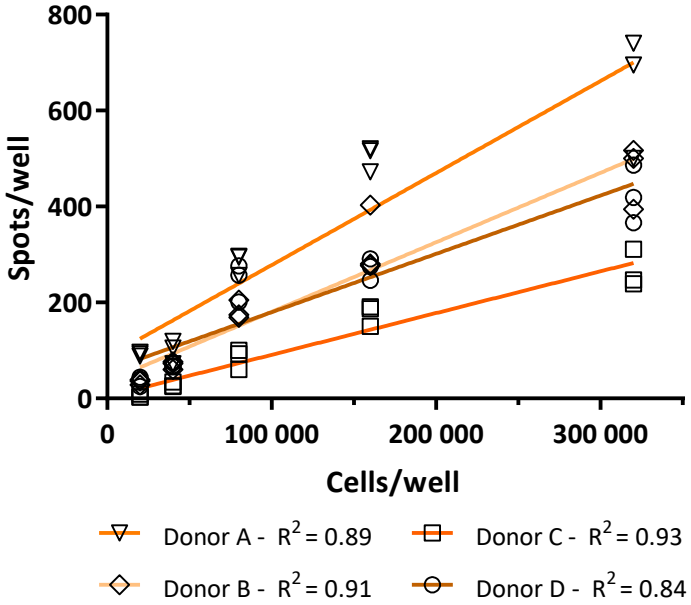
Variables that can influence ELISpot results

- Before assay
  - Donor variation
  - Quality of cells and cell source
    - *Thawing and optional rest steps*
- Stimulation-related
  - Background
  - Cell numbers
  - Medium used
    - *Use of serum*
  - Number of replicates
  - Type of stimulation and positive control(s)
- Measurement-related
  - Spot contrast/intensity, size and shape
  - For FluoroSpot: laser voltage and maintenance (when applicable)

Variables that can influence ELISpot results

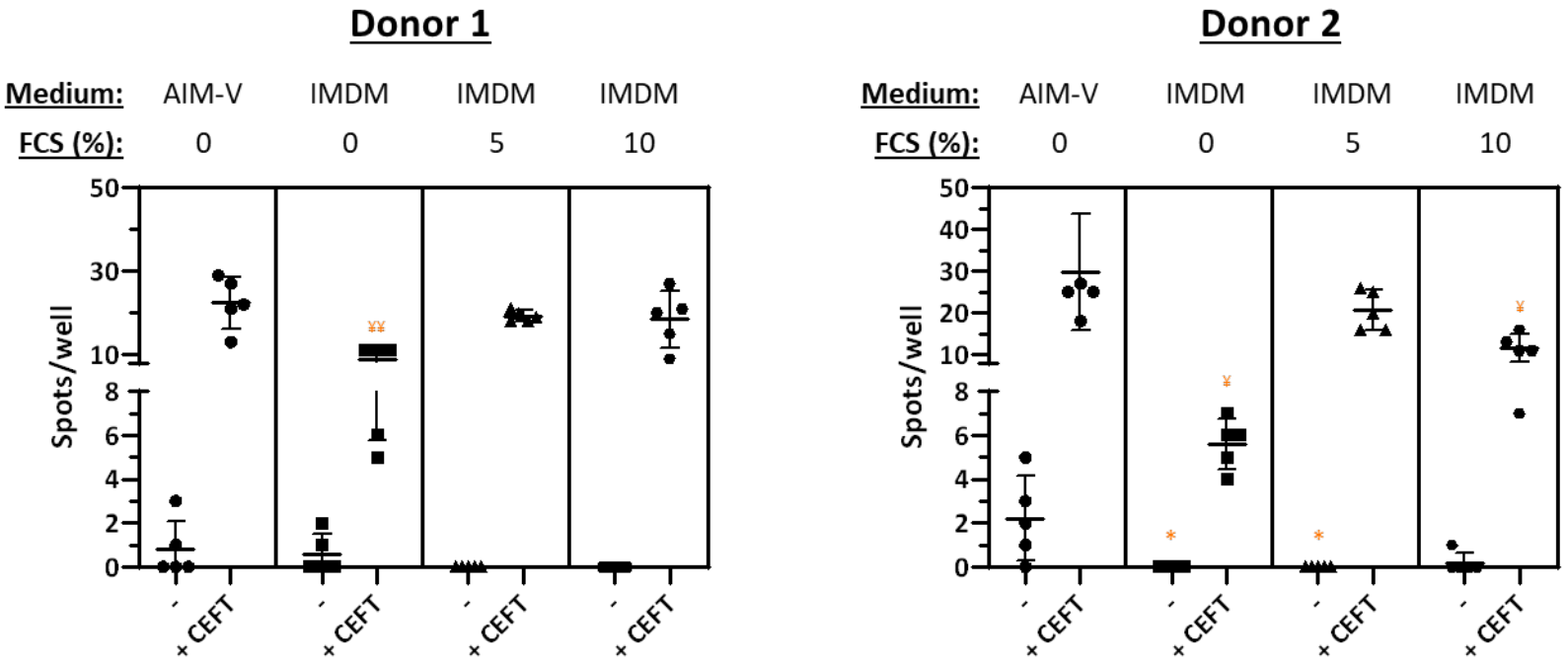
- Before assay
  - Donor variation
  - Quality of cells and cell source
    - *Thawing and optional rest steps*
- Stimulation-related
  - Background
  - Cell numbers
  - Medium used
    - *Use of serum*
  - Number of replicates
  - Type of stimulation and positive control(s)
- Measurement-related
  - Spot contrast/intensity, size and shape
  - For FluoroSpot: laser voltage and maintenance (when applicable)

Influence of cell numbers, background and donor variation



Cells were stimulated in triplicate with 1 ug SEB/mL overnight, and IFN-γ producing cells were assessed by ELISpot.

Influence of medium choice and serum addition

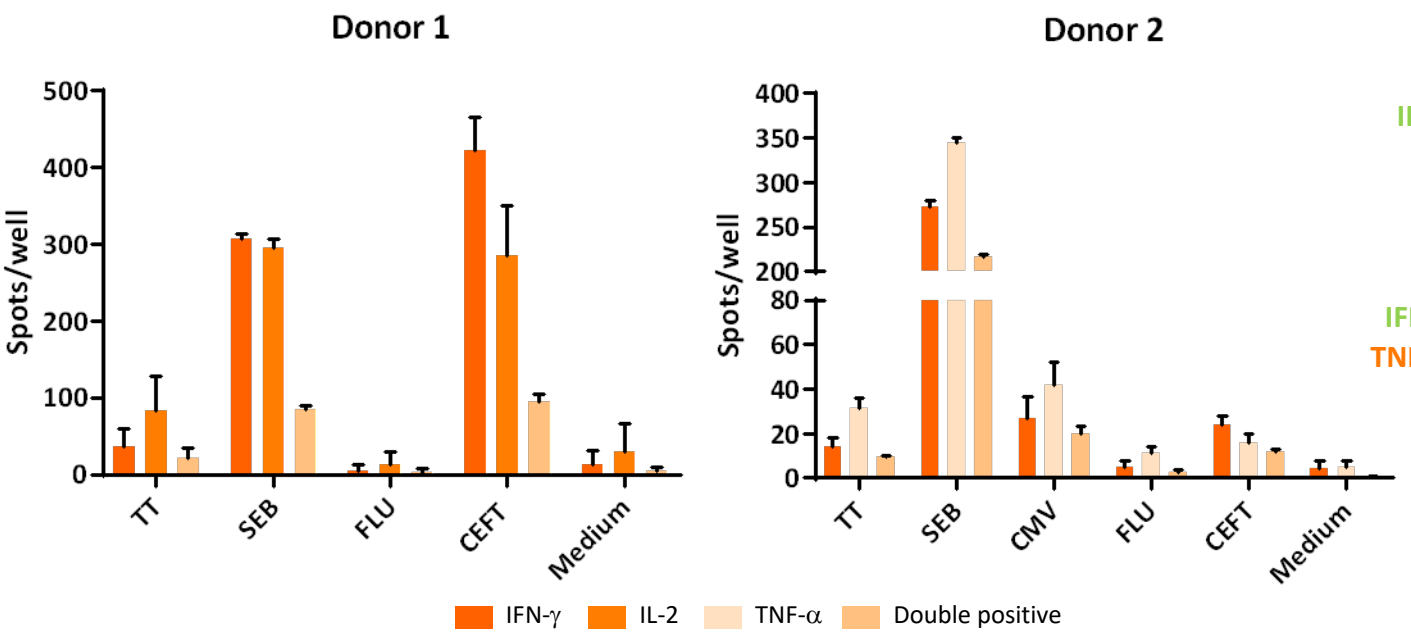


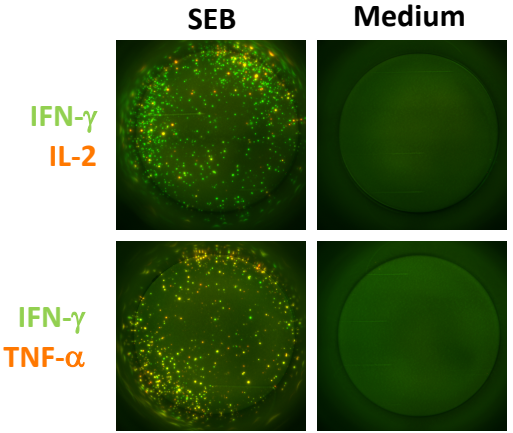
Cells were stimulated in triplicate with 1 μM CEFT overnight, and IFN-γ producing cells were assessed by ELISpot.

* indicates significantly different compared to non-stimualted AIM-V (P<0.05)

¥/¥¥ indicates significantly different compared to CEFT-stimulated AIM-V (P<0.05/<0.01)

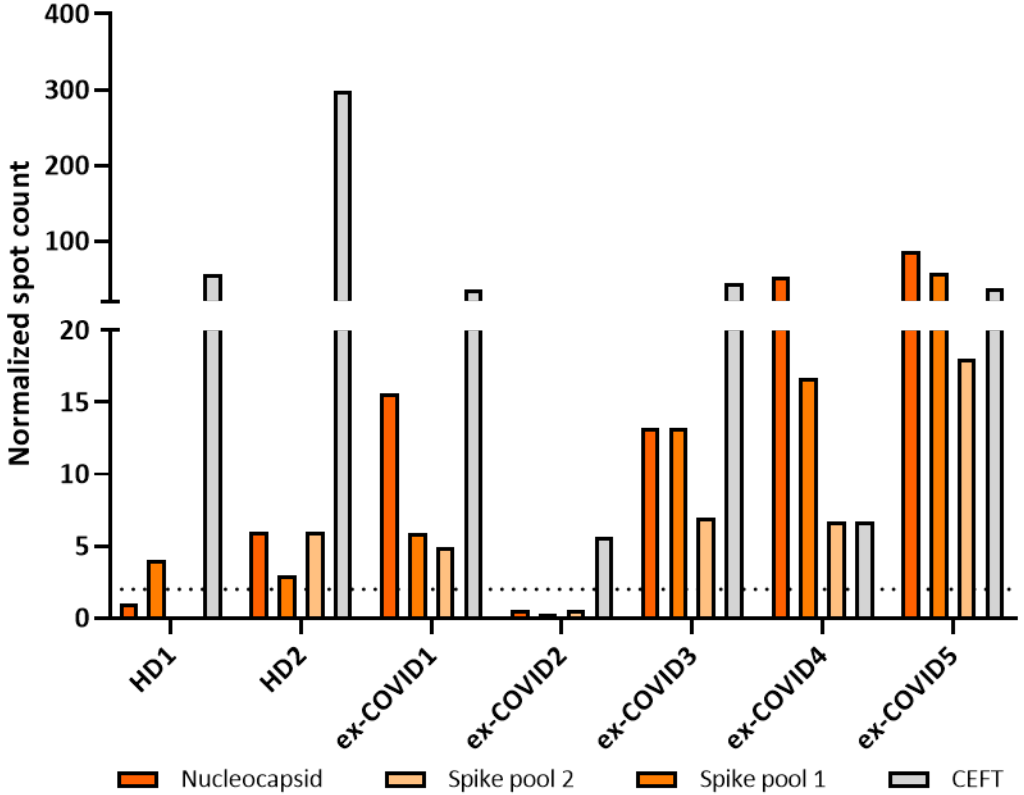
FluoroSpot to determine multiple parameters

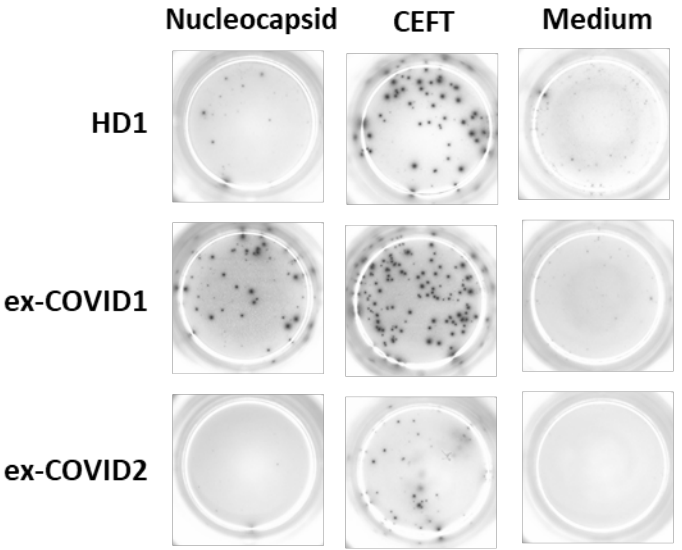




Cells were stimulated in triplicate with 1 μM peptide overnight, and IFN-γ producing cells were assessed by ELISpot.

Using ELISpot to determine SARS-CoV-2 cellular immunity





Cells from healthy donors (n=2) or ex-COVID patients (n=5) (both self reported) were stimulated in triplicate with 1 ug/mL SARS-CoV-2 nucleocapsid and spike peptide pools, after which IFN-γ producing cells were assessed via ELISpot. CEFT was used as a positive control.

Conclusion

- Many variables can be of influence on ELISpot results, therefore qualified personnel and standardization are of importance.

- ELISpot assays are useful tools for high-throughput screening of patient/donor samples, and can be of use in many fields of research (vaccine development, epidemiology, auto-immune diseases, etc).

Acknowledgements

- Sanquin's Immunomonitoring team:
  - David Cobeta Lopez
  - Feresthe Rezai
  - Irma Rensink
  - Suzanne Lissenberg-Thunissen
  - Domenique Kivits
  - Sinéad Lougheed
  - Rianne Opstelten
  - Annelies Turksma
  - Anja ten Brinke
  - Annick de Vries

Special thanks to

- Healthy blood bank donors
- Ex-COVID donors