Development and Optimization of an IFN-γ ELISpot Assay to Monitor Immune Response Against SARS-CoV-2 Antigens

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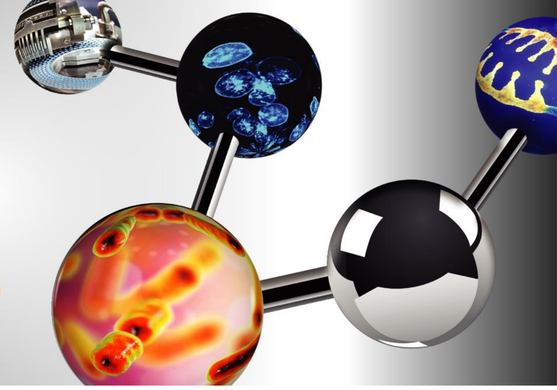
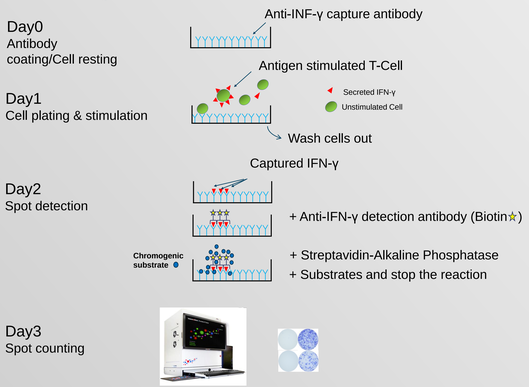


PURPOSE

The unprecedented and rapid spread of SARS-CoV2 into a global pandemic, combined with its high mutation rate, has motivated the need for robust vaccines that provide broad protective immunity. As a marker for vaccine activity, ELISpot assays permits detection of low frequency IFN-γ secreting T-cells in response to specific antigens. In addition, ELISpot assays may be used to monitor treatment-specific humoral and cellular immune response in large molecule drug development, which must be closely monitored for safety and efficacy in both clinical and nonclinical studies. Despite of the potential of ELISpot assays, implementation in regulated environments brings new and unique challenges, including a lack of regulatory guidance and gold standard reference materials. Here we present method development and optimization for an IFN-γ ELISpot assay in a regulated environment, demonstrating the value of the assay to support SARS-CoV-2 vaccine development.

METHOD

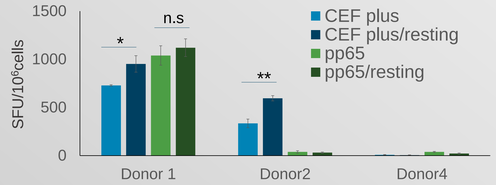
Figure 1. Schematic drawing of the principle of the ELISpot assay



IFN-γ ELISpot assays were performed using optimized human IFN-γ single color kit from ImmunoSpot. Cryopreserved PBMCs were thawed, rested overnight in CTL test media. Plate scanning and spot counting were performed using an ImmunoSpot 96 Universal analyzer. Spot counts were further analyzed as spot forming units (SFU) per well. Standard counting parameters were sensitivity of 180-210, background balance of 40, spot separation of 1-10, and spot size gating minimum of 0.0009 Sq.mm to maximum of 9.6296 Sq.mm and optimized for accurate enumeration of spots for each run.

RESULTS

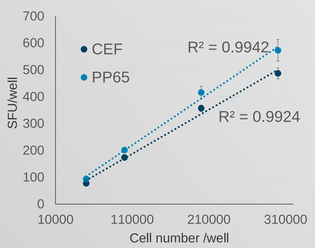
Figure 2. Resting effect evaluation



Comparison of the effect of no resting and overnight (20hr) resting periods for cells after thawing. Cell viability was measured using CTL-LDC, Live/Dead cell counting reagent before and after resting. Overnight cell resting yielded higher counts (SFU) upon both CEF plus (CMV, Epstein-Barr virus, Influenza virus) peptide pool and pp65 (human CMV) peptide pool stimulation. All subsequent data generated from overnight resting.

Figure 3. Linearity and proportionality of the IFN-γ response.

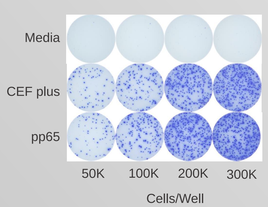
A



B

Cells/well	CEF plus		pp65	
	SFU	% of 200K	SFU	% of 200K
300K	487.0	90.9	572.7	91.8
200K	357.0	**100.0**	416.0	**100.0**
100K	173.7	97.3	201.3	96.8
50K	77.3	86.6	93.7	90.1

C



A. Linearity of the IFN-γ response. CEF plus and pp65 peptide pool stimulations tested with four cell densities from 50K to 300K per well. R^2 for both CEF plus and pp65 stimulation were ≥0.99. Intra-triplicate precision ranged from 3.4 to 11.8% CV, satisfying acceptance criteria of <30%CV for response ≥30 SFU/well.

B. Proportionality of IFN-γ response calculated by normalizing spot forming units (SFU) per 200K cells/well.

C. Representative well images showing dose response vs. media-only control.

Figure 4. Specificity of the IFN-γ response

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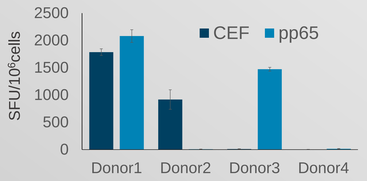

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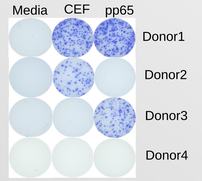
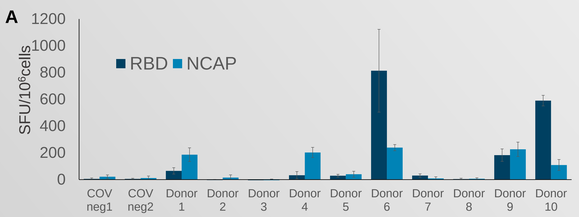


A) Specificity was evaluated via CEF plus and pp65 antigen stimulations on 4 different reference PBMCs from seropositive and seronegative donors. Negative response was defined as a mean spot count <10 SFU/well. SFU from reference PBMCs shows good correspondence with historical CTL results in all conditions within <10% variance, with %CV among seropositive PBMCs ranging from 2.4% to 19.5%. **B)** Representative well images for specificity evaluation.

Figure 5. Application of an IFN-γ ELISpot assay to measure immunogenicity against SARS-CoV-2 antigens

A



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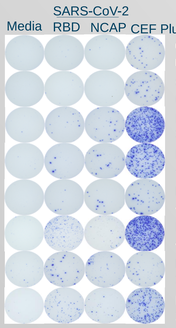


A. PBMCs from 10 convalescent COVID-19 nasopharyngeal RT-PCR positive patients evaluated. PBMCs were collected from 32 to 169 days after diagnosis and stimulated with SARS-CoV2 spike glycoprotein receptor binding domain of (RBD) and Nucleoprotein (NCAP) peptide pools. 6 out of 10 donors showed reactivity with SARS-CoV-2 antigen stimulation.

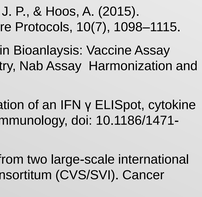
B. Examples of representative images of wells for stimulated or unstimulated PBMCs derived from COVID-19 positive donors. CEF plus pool was used for positive control.

Figure 6. Participation of 2021 ELISpot proficiency panel

A

	SFU/well		STD		%CV	
	PBMC1	PBMC2	PBMC1	PBMC2	PBMC1	PBMC2
Reagent1 (pp65)	0.3	**23.7**	0.6	**4.2**		**17.6**
Reagent2 (CEFX Ultra)	**461.3**	**161.3**	**9.9**	**9.7**	**2.1**	**6.0**
Reagent3 (Negative control)	0.7	6.3	0.6	2.9		

B



A. Annual T-cell ELISpot proficiency panel data was collected and submitted. Both PBMCs shows seropositive with CEFX ultra stimulation within <10% CV.

B. Representative well images for proficiency panel.

CONCLUSIONS

- We have optimized and qualified an IFN-γ ELISpot assay that can provide precise, specific, robust, and reproducible data on the antigen specific T-cell response in clinical specimens, and which can measure antigen-specific cellular immunity in a highly sensitive and high throughput manner.
- Each assay demonstrated performance consistent with ELISpot harmonization guidance and peer review articles.
- These data demonstrate robust ELISpot assay performance can be validated in regulated environment and can inform critical decision making in the drug development process.

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