Resolving variant-specific T cell immunity: wild-type vs. Omicron SARS-CoV-2 peptide pool stimulation by IFN-γ ELISpot

As SARS-CoV-2 continues to evolve, viral mutations alter T cell epitopes and complicate the tracking of variant-specific cellular immunity. This application note demonstrates that pairing wild-type and Omicron-specific peptide pools in an IFN-γ ELISpot assay effectively distinguishes variant-specific T cell immunity. By testing peripheral blood mononuclear cells (PBMCs) from donors with defined exposure histories, the method successfully resolves prior infection from variant-specific priming, offering a valuable tool for immune surveillance and vaccine monitoring.

INTRODUCTION

Since its emergence, SARS-CoV-2 has continued to evolve, with Omicron-lineage variants now carrying a significant number of mutations in spike and other proteins compared to the ancestral Wuhan-Hu-1 strain. These mutations alter the peptide sequences presented by MHC molecules, potentially modifying or eliminating pre-existing T cell epitopes and generating new variant-specific ones. Understanding whether an individual's cellular immunity is restricted to earlier variants or extends to Omicron-specific sequences has direct relevance for vaccine monitoring, clinical immune surveillance, and the study of correlates of protection.

Peptide pools allow variant-specific T cell immunity to be assessed without the biosafety constraints of live virus. This application note demonstrates how pairing a wild-type SARS-CoV-2 peptide pool with an Omicron-specific pool in a standard IFN-γ ELISpot assay discriminates variant-level T cell responses and resolves prior exposure history.

EXPERIMENTAL DESIGN

Two PBMC donors with defined SARS-CoV-2 exposure histories were selected to represent distinct immunological profiles. Donor selection was designed to allow direct discrimination between broad coronavirus T cell memory and Omicron-specific priming (Table1).

PBMCs from two donors with defined SARS-CoV-2 exposure histories were stimulated in parallel with a wild-type and an Omicron-specific SARS-CoV-2 peptide pool, with IFN-γ secretion measured by ELISpot (Figure 1).

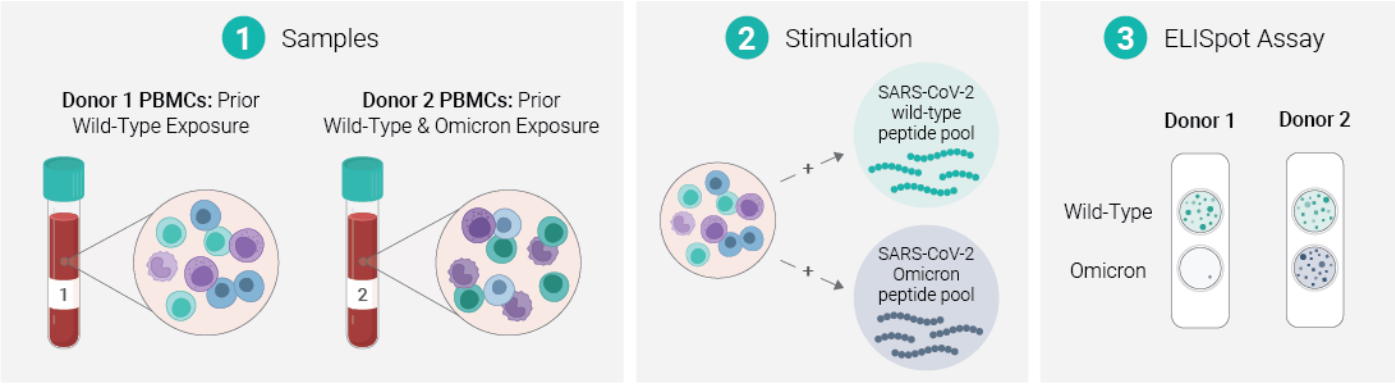

Figure 1. *Schematic overview of the experimental design. PBMCs from two donors with defined SARS-CoV-2 exposure histories were stimulated in parallel with a wild-type and an Omicron-specific SARS-CoV-2 peptide pool, with IFN-γ secretion measured by ELISpot.*

	DONOR 1	DONOR 2
Prior SARS-Cov-2 exposure	YES	YES
Known Omicron exposure	NO	YES

Table 1. *Donor exposure histories.*

MATERIALS

Human PBMCs were isolated from healthy in-house donors. The cells maintained viability of 90% or higher at the time of the assay. For the assay controls, unstimulated cell culture medium served as the negative control. Cells stimulated with PMA (phorbol 12-myristate 13-acetate) and ionomycin served as the positive control.

The ImmuneSelect reagents used in this study are listed in Table 2.

REAGENT	DETAILS	CAT #
SARS-CoV-2 Wild-Type Peptide Pool	ImmuneSelect, ancestral strain (Wuhan-Hu-1)	1081-07
SARS-CoV-2 Omicron Peptide Pool	ImmuneSelect, Omicron-specific sequences	1091-07
Human IFN-γ ELISpot Kit	ImmuneSelect, pre-coated plate	2091

Table 2. *ImmuneSelect reagents used in this study.*

METHODS

ELISpot assays were performed using ImmuneSelect Human IFN-γ ELISpot Kits with pre-coated plates, according to the standard instructions. PBMCs were seeded at a density of $2x10^5$ viable cells per well in complete medium. Cells were then stimulated with either the wild-type or Omicron peptide pools at a final concentration of 1.2 nmol/mL per peptide. Negative control wells received cell culture medium alone without stimulation. Positive control wells were stimulated with a combination of 25ng/mL PMA and 1µg/mL ionomycin. Six replicates were tested per stimulation condition.

Plates were incubated for 20 hours at 37°C, 5% CO_2. Following incubation, plates were processed and developed according to the kit protocol. Plates were read using an AID iSpot ELR08IFL Microplate Reader. Results are expressed as spot-forming units (SFU) per 10^6 PBMCs after subtraction of the negative control mean.

RESULTS

Donor 1 - Wild-type response without Omicron cross-recognition: PBMCs from Donor 1 produced a clear IFN-γ response when stimulated with the wild-type SARS-CoV-2 peptide pool (36 SFU/10^6 PBMCs), confirming the presence of T cell memory against ancestral strain sequences. In contrast, stimulation with the Omicron-specific pool produced no detectable response above background (1 SFU/10^6 PBMCs), indistinguishable from the negative control. The positive control confirmed PBMC viability and assay sensitivity (355 SFU/10^6 PBMCs).

Donor 2 - Responses to both wild-type and Omicron-specific pools: Donor 2 produced IFN-γ responses to both peptide pools. The wild-type pool generated the stronger overall response (73 SFU/10^6 PBMCs), consistent with broad T cell memory established across multiple SARS-CoV-2 exposures, including prior infection. The Omicron-specific pool also

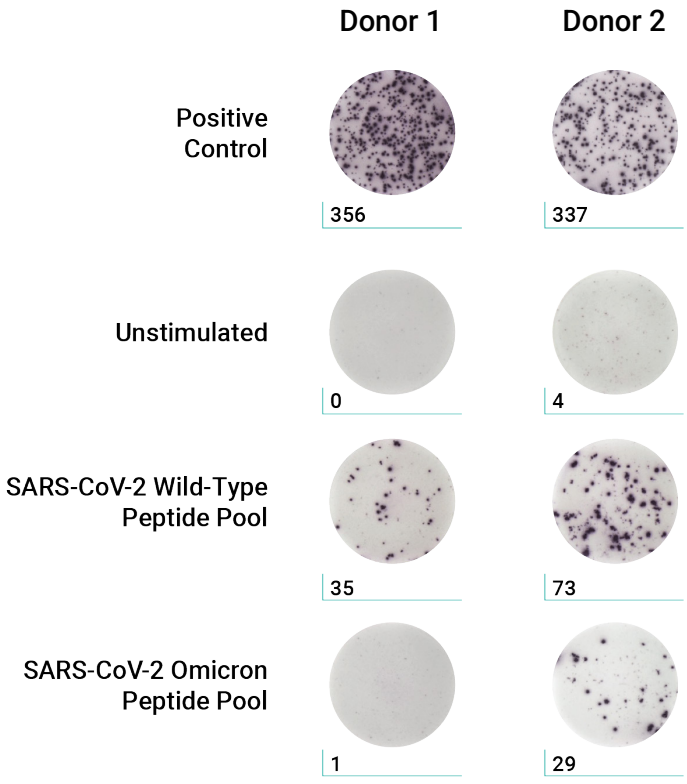

Figure 2. *Representative IFN-γ ELISpot well images for both donors across all four conditions. Images are displayed at equivalent contrast settings. Spot counts per well are indicated below each image. Images acquired using AID iSpot ELR08IFL Microplate Reader.*

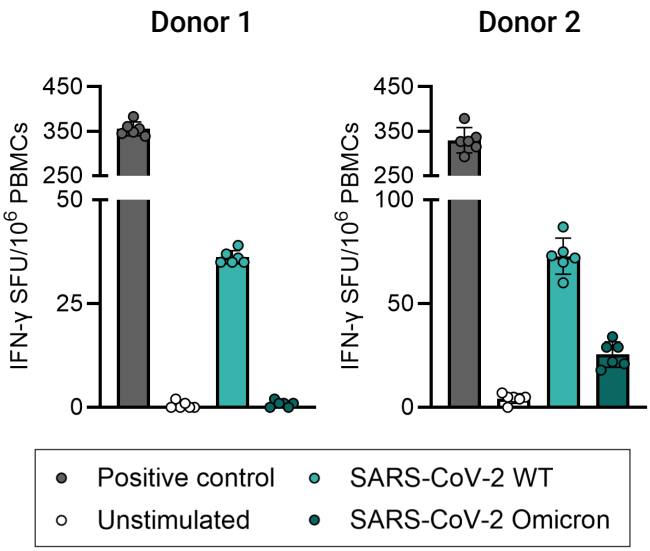

Figure 3. *Quantified IFN-γ ELISpot responses for Donor 1 (left) and Donor 2 (right). Bars represent mean SFU per 10⁶ PBMCs from duplicate wells; individual data points are shown.*

produced a clear and distinct response (26 SFU/10⁶ PBMCs), confirming that omicron exposure had primed T cells against variant-specific epitopes not present in the ancestral strain sequence.

The absence of any detectable Omicron-specific response in Donor 1, despite a robust wild-type response, demonstrates that T cell memory established against the ancestral wild-type SARS-CoV-2 strain does not cross-recognise Omicron-specific epitopes in this donor. This is consistent with reports of altered T cell epitope coverage in Omicron-lineage variants. The clear and specific Omicron response in Donor 2 validates that the Omicron-specific peptide pool selectively detects variant-primed immunity, rather than cross-reactive responses to sequences shared with the wild-type peptide pool.

KEY TAKEAWAYS

- Wild-type SARS-CoV-2 peptide pools reliably stimulate broad coronavirus T cell memory established by prior infection or vaccination, but cannot be used to identify Omicron-specific responses.
- Omicron-specific peptide pools resolve whether SARS-CoV-2 Omicron has primed T cells against variant-specific epitopes, providing a layer of discrimination not achievable with a single wild-type ancestral pool.
- Combining both pools in a standard IFN-γ ELISpot assay provides variant-level resolution of T cell immunity, directly applicable to vaccine monitoring, clinical immune surveillance, and SARS-CoV-2 variant immunity research.