Measurement of T cell responses using Immuno-T™ kits

Enhancing the standardisation and scale of T cell assays

By Martin Scurr, PhD

The COVID-19 pandemic drove the development of assays that measured immune responses to help identify individuals who were best protected or most at risk of infection. Whilst antibody tests had limited utility, multiple studies identified the T cell response as an effective correlate of protection against SARS-CoV-2 infection.[1-3] However, long-standing issues with the measurement of T cell responses precluded their widespread use, notably the lack of standardised approaches used by different laboratories, i.e. ELISpot and flow cytometric-based readouts of antigen-specific activation. Here, discover how your laboratory can utilise the Immuno-T™ test to detect antigen-specific T cell responses in a high-throughput, standardised, more physiologically relevant manner.

Background

To identify the presence and magnitude of an individual's T cell response to a particular antigen, the specific response must be recapitulated and identified *in vitro*. The effector cytokine interferon-γ (IFN-γ) is directly involved in coordinating anti-viral immunity, and is abundantly produced by T cells specific for the SARS-CoV-2 virus.[4-6] T cells stimulated with peptides spanning viral antigens produce IFN-γ, and this cytokine can be measured using ELISpot or flow cytometry. Whilst both assays have been the mainstay of T cell immunology labs for many years, both methods require isolation of peripheral blood mononuclear cell (PBMC) subsets for cytokine measurement, and neither approach is particularly conducive to high-throughput, large-scale studies (see Table 1).

What is Immuno-T™?

Given these limitations, human immunology studies are increasingly moving away from utilising fractionated PBMC samples as the source for T cell analyses.[7] Immuno-T™ is a whole blood-based test to identify antigen-specific T cells in a simple, robust and highly reproducible format. The Immuno-T™ laboratory test kit combines all necessary components to perform this test; the laboratory technician simply aliquots 1ml heparinised blood samples into negative / positive control tubes and a stimulation tube (containing overlapping peptides spanning entire antigens), incubates them for 20-26 hours at 37°C, before harvesting and storing the plasma. The amount of IFN-γ released in the plasma is then quantified using a validated IFN-γ ELISA, whereby the IFN-γ is calibrated against an international standard, to maximise standardisation. The same harvested plasma sample can also be used for multiple immunological assays, for example to measure antibodies or other cytokines. To further enhance test reproducibility across multiple laboratories and prolong kit longevity, blood stimulation tubes already contain exact quantities of lyophilised peptides. Correct blood sampling, handling and stimulation is crucial; as with all functional T cell assays, the success of the Immuno-T™ assay is dependent on the availability of fresh heparinised blood samples that are delivered to the laboratory within 48 hours of blood draw.

Table 1. Comparison of T cell assays

Flow Cytometry / ELISpot	Immuno-T
Specialist knowledge and equipment	Uses common laboratory equipment and techniques (ELISA)
Requires isolation of PBMCs (~2 hours)	Whole blood (~2 mins)
Laborious procedure	High throughput
Subjective readout	Absolute value (pg/ml) calibrated to an international standard
Difficult to standardise across multiple sites	Highly standardisable

Comparison with ELISpot

Given the current ubiquity of IFN-γ ELISpot for measuring T cell responses, ImmunoServ

performed a side-by-side comparison of SARS-CoV-2-specific T cell responses measured from the same blood samples using both the Immuno-T™ and ELISpot assays. When performed on fresh blood samples, there is a very strong correlation between the absolute value of plasma IFN-γ obtained from whole blood Immuno-T and the number of IFN-γ spot forming cells per million PBMC obtained by ELISpot (Figure 1a). However, when cryopreserved PBMC were tested by ELISpot, the correlation with results obtained by fresh whole blood Immuno-T™ was lost, primarily due to poor cell viability in certain samples post-freeze thaw (Figure 1b). Indeed, freezing and thawing PBMC has previously been described to reduce the detection of $CD4^+$ T cell responses.[8]

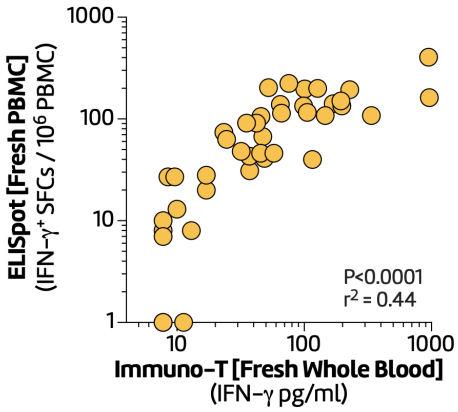

Figure 1a. Strong correlation between SARS-CoV-2-specific IFN-γ readouts obtained from whole blood Immuno-T™ with ELISpot. Fresh blood samples were obtained from 41 healthy donors; blood from the same tube was processed immediately and divided into sample for PBMC isolation prior to ELISpot assay and for whole blood Immuno-T assay.

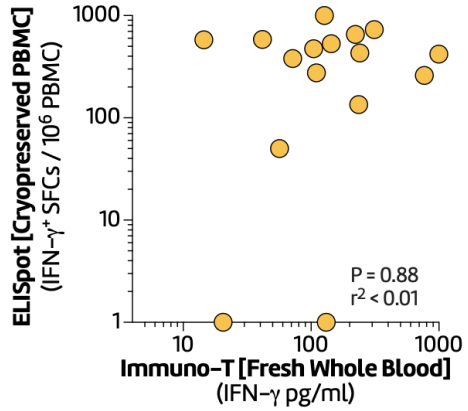

Figure 1b. No correlation between SARS-CoV-2-specific IFN-γ readouts obtained from whole blood Immuno-T™ with ELISpot on cryopreserved PBMC. Fresh blood samples were obtained from 15 healthy donors; blood from the same tube was processed immediately and divided into sample for PBMC cryopreservation, and for fresh whole blood Immuno-T™ assay. Cryopreserved PBMC were stored for up to 2 years prior to thawing and plating for assessment of IFN-γ responses by ELISpot.

Despite this, the Immuno-T™ assay is capable of recapitulating results obtained from cryopreserved PBMC when these are resuspended in media and placed in Immuno-T™ stimulation tubes.

Supernatants harvested for analysis of IFN-γ production correlated well with T cell responses obtained from fresh whole blood samples (Figure 1c) and even ELISpot on cryopreserved PBMCs (Figure 1d).

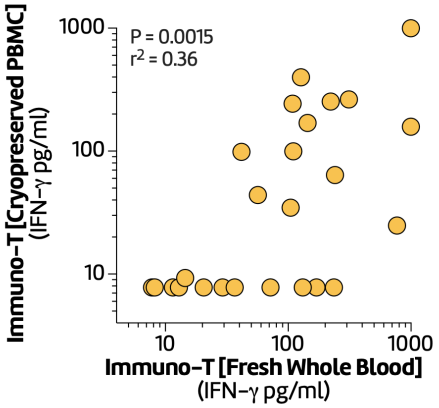

Figure 1c. Correlation between SARS-CoV-2-specific IFN-γ readouts obtained from Immuno-T using fresh whole blood or matched cryopreserved PBMC samples. Fresh blood samples were obtained from 26 healthy donors; blood from the same tube was processed immediately and divided into sample for PBMC cryopreservation and sample for whole blood Immuno-T™ assay. Cryopreserved PBMC were stored for up to 2 years prior to thawing and plating for assessment of IFN-γ responses by ELISpot and Immuno-T.

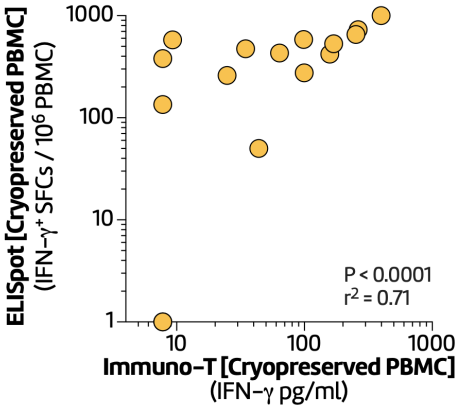

Figure 1d. Correlation between SARS-CoV-2-specific IFN-γ readouts obtained from ELISpot on cryopreserved PBMC with Immuno-T™ using matched cryopreserved PBMC samples. Cryopreserved PBMC samples from 15 healthy donors were thawed and divided into sample for ELISpot and for Immuno-T™ assay. Cryopreserved PBMC were stored for up to 2 years prior to thawing and plating for assessment of IFN-γ responses.

These results demonstrate that freshly isolated samples generate the most physiologically relevant data when performing ELISpot or Immuno-T. However, the isolation and enumeration of fresh PBMC for ELISpot is laborious and not conducive to large scale studies requiring high throughput

approach. Furthermore, subjective enumeration of spots, can introduce significant variability into the assay limiting comparability within labs and across labs. In contrast, the Immuno-T™ assay is ideally suited to large scale immunity studies on whole blood samples across multiple sites by any laboratory technician trained to perform ELISAs. PBMCs can also be used for Immuno-T™, but limit the scalability and physiological relevance if cryopreserved.

Venous or capillary blood

One of the main challenges with analysing T cell responses, particularly on larger scale immunity studies outside of healthcare settings, is obtaining and transporting large numbers of venous blood samples within a sufficient timescale. Currently, all other commercially available SARS-CoV-2 T cell tests require phlebotomists to obtain venous blood samples. In order to increase the accessibility of the Immuno-T™ assay, ImmunoServ developed an alternative capillary blood sampling technique, allowing participants to obtain 0.4ml blood samples at home from a finger prick using ImmunoServ's UKCA-marked At Home Blood Collection Kit. Once the sample is collected, it is shipped directly to a designated laboratory at ambient temperature using a domestic postal service. Whilst this limited blood volume precludes positive and negative controls, it allows researchers to generate far more comprehensive assessments of population immunity. This approach was utilised during the COVID-19 pandemic and demonstrated that the magnitude of IFNγ predicted immunity to COVID-19 (Figure 2).[1] These advancements have also enabled T cell testing to be made accessible to the general public without the need for a phlebotomist (www.Immuno-T.co.uk).

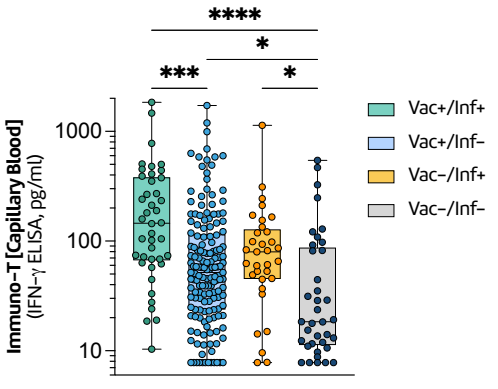

Figure 2. Magnitude of IFN-γ production by SARS-CoV-2-specific T cells, measured using Immuno-T™ on capillary blood samples. T cell responses were sub-divided based on participant vaccination and prior SARS-CoV-2 (PCR and/or lateral flow test confirmed) infection status. 'Vac+/Inf+' n = 42 (green), 'Vac+/Inf-' n = 158 (blue), 'Vac-/Inf +' n = 33 (yellow), 'Vac-/Inf-' n = 37 (grey). ****P < 0.0001, ***P < 0.001, *P<0.05.

Use of Immuno-T™ on clinical studies

Tests that measure T cell responses to SARS-CoV-2 aid in the identification of individuals at greater risk of severe COVID-19 with more certainty than just measuring antibodies alone. As such, the Immuno-T test is now being used clinically to determine whether sufficient immune responses are being generated to COVID-19 vaccines in immunocompromised patients, particularly those on B cell depleting therapies, for example Multiple Sclerosis (MS).[9,10] In addition, it has proven clinical utility in measuring the efficacy of treatment strategies aimed at clearing persistent SARS-CoV-2 infections.[11,12] Adoption of the test across the NHS and other healthcare systems will enable all clinicians and patients to benefit from this and enhance precision medicine.

Future utility

T cells play a fundamental role in the development and outcome of many diseases, including infectious diseases, autoimmunity and even cancer. The flexibility of the Immuno-T platform allows for simple alterations to the peptide stimulants for utility across any disease involving T cells. In our endeavours to be better prepared for future pandemics, Immuno-T is now being used for measuring T cell responses to influenza. In addition, ongoing studies are assessing the utility of Immuno-T to measure anti-tumour T cell responses as a prognostic indicator for post-surgical relapse in cancer patients, and also for measuring T cell responses to autoantigens as a diagnostic for MS.

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