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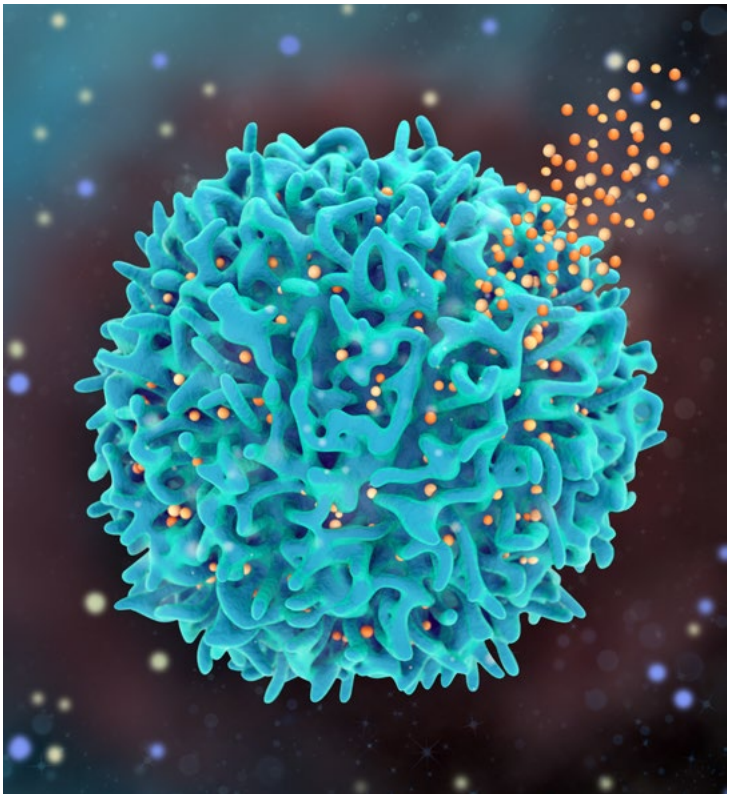
ACCURATE QUANTIFICATION OF INTERFERON GAMMA ACROSS IMMUNOASSAY PLATFORMS

APPLICATION NOTE



INTRODUCTION

Immunotherapy has revolutionized the way we treat conditions such as cancer. Some of the most striking outcomes have been observed in adoptive cell transfer (ACT). At the forefront of this fast-moving field is CD19 targeted chimeric antigen receptor (CAR) T cell therapy, which has been approved by the Food and Drug Administration for the treatment of refractory pre-B cell acute lymphoblastic leukemia and diffuse large B cell lymphoma among others[1].

In their seminal case study, Porter *et al*[2], documented the remission of chronic lymphocytic leukemia (CLL) in a patient that received a low dose second generation CART19-cell infusion. Analysis of the patient's serum evidenced delayed tumor lysis syndrome coincident with several cellular responses including decreased CLL, the expansion of CART19-cells, increased interferon gamma (IFN-γ) and IFN-γ responsive chemokines, peaking 17-23 days after the first CART19-cell infusion[2]. A follow-up study with 2 additional patients demonstrated similar findings[3]. Taken together it is clear that increased IFN-γ secretion is a useful correlate of CAR T expansion.

We know that IFN-γ is secreted by many cells[4] including macrophages, activated $CD8^+$ T cells, natural killer T cells and Th1 $CD4^+$ cells. More recently this pleiotropic molecule has been correlated with antiproliferative, proapoptotic and antitumor activities[5]. Although there is evidence that IFN-γ may also play a pro-tumorigenic role, it has been positively correlated with survival in cancer.

Given the central role of IFN-γ in cell therapy and immunology, consistent and reliable methods of quantifying IFN-γ levels are imperative. In order to meet this research and development workflow need, the 3rd generation Quantikine IFN-γ ELISA and the 3rd generation Simple Plex IFN-γ assay were evaluated using several criteria, including correlation, spike and recovery, and linearity of dilution or parallelism. Our data indicate that these next generation immunoassay platforms maintain the high standard for accuracy and sensitivity that you have come to expect from R&D Systems, a Bio-Techne brand. Whether you are looking for a traditional plate-based ELISA or an automated ELISA platform, both options utilize in-house monoclonal antibodies, thus guaranteeing a consistent assay for your cell therapy and immunology development needs.

METHODS

T Cell stimulation. Peripheral blood mononuclear cells (PBMCs) from 8 different donors were separated from whole blood by a density gradient centrifugation method using Ficoll-Paque Plus (GE Healthcare). $CD4^+$ T cells were further isolated from PBMCs using the MagCellect™ Human $CD4^+$ T cell Isolation Kit (Catalog # MAGH102), seeded at 5 x 10^5cells/mL and cultured using ExCellerate™ Human T Cell Expansion Media, Xeno-Free (Catalog # CCM030). T cells were left untreated or treated with 10 ng/mL GMP Recombinant Human IL-7 (Catalog # 207-GMP), 10 ng/mL GMP Recombinant Human IL-15 (Catalog # 247-GMP) and stimulated for 5 days. T Cell stimulation was mediated using either flask-immobilized Human CD3 epsilon Antibody (Cat# MAB100, coated at 1 ug/mL for 2-3 hours) followed by 5 ug/mL soluble Human CD28 Antibody (Cat# MAB342) or 25 µL Cloudz™ CD3/CD28 (Catalog # CLD001-GMP) per mL of culture media. CD4+ T cells were maintained in a 5% CO2 incubator at 37 °C. Cell conditioned media was collected by centrifugation and stored at -20°C until assayed.

Quantitation of IFN-γ. Conditioned media was analyzed on the 3rd generation Human IFN-γ Quantikine ELISA Kit (Catalog # DIF50C) and the 3rd generation Human IFN-γ Simple Plex Cartridge for Ella at an appropriate dilution factor according to manufacturer's instructions.

RESULTS

FIGURE 1A

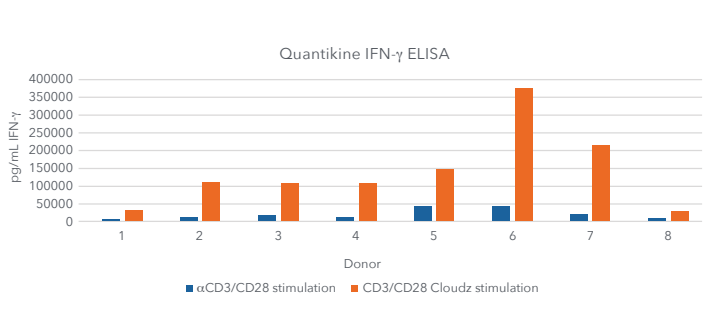


FIGURE 1B

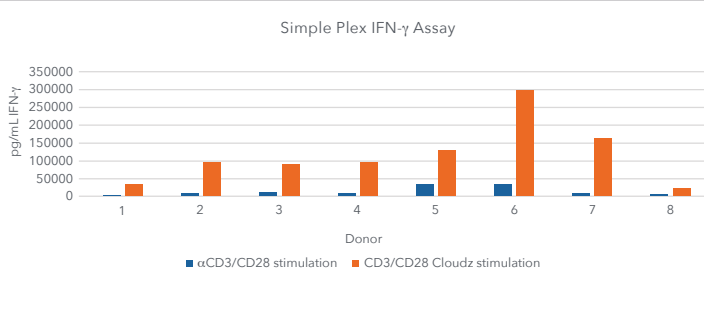


Figure 1. IFN-γ was quantified on the 3rd generation Quantikine ELISA and the 3rd generation Simple Plex assay. CD3/CD28 Cloudz stimulates IFN-γ secretion more robustly than immobilized anti-CD3/CD28. CD4+ T Cells were cultured with immobilized anti-CD3/CD28 (blue) or Cloudz CD3/CD28 (orange) for 5 days. Data from the new third generation Quantikine IFN-γ ELISA (**1A**) is concordant with that of the Simple Plex IFN-γ Assay (**1B**). Unstimulated CD4+ T cells were tested but levels were below the limit of detection on both assays (data not shown).

FIGURE 2

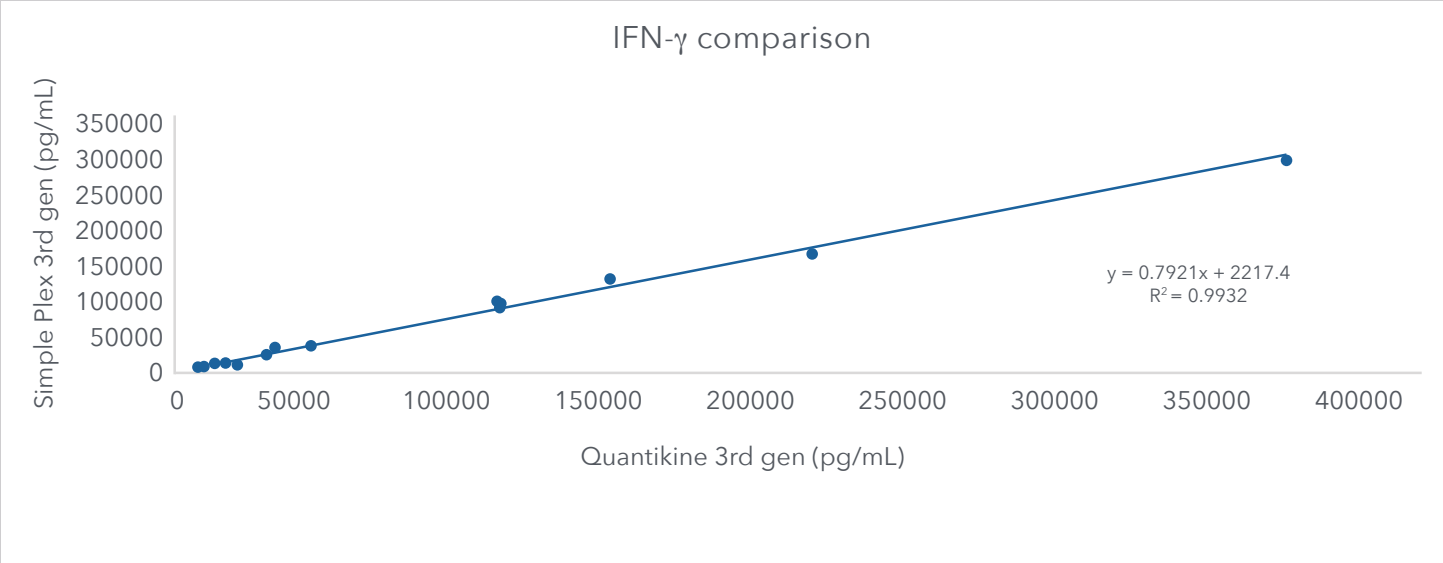


Figure 2. There is a nearly perfect correlation between the 3rd generation IFN-γ Quantikine ELISA data and 3rd generation Simple Plex IFN-γ assay data.

Analysis of conditioned media from 8 donors indicated that IFN-γ was secreted in all samples. Stimulated CD4+ T cells exhibited a 3 to 16-fold increase in IFN-γ secretion above non-treated cells as measured by both platforms (**Figure 1**) reinforcing the inter-platform consistency and broad dynamic range. IFN-γ levels from unstimulated CD4+ T cell supernates were below the limit of detection in the IFN-γ Quantikine ELISA and the Simple Plex IFN-γ assay (data not shown). We observed a nearly perfect correlation coefficient (R^2=0.9332) between assays in Cloudz and anti-CD3/CD28 treated cells (**Figure 2**).

Next, we evaluated the impact of matrix effect on IFN-gamma detection using different cell culture medias. Six brands of cell culture media were spiked with recombinant Human IFN-γ (Catalog #10067-IF) and tested for spike recovery and spike linearity. These tests are critical for assessing ELISA accuracy. They are also important for evaluating the degree to which the assay is optimized to measure target analytes in various complex matrices. Assay performance met R&D System's rigorous performance standard regardless of culture media using both platforms. The percent recovery for the Quantikine IFN-γ ELISA ranged from 87 to 125% across all the media (**Figure 3A**) and spike linearity ranged from 86% to 115% (**Figure 3B**). Natural linearity, also known as parallelism, is also an important performance measurement. It indicates whether an assay can accurately measure the natural analyte at all points along the standard curve. Natural linearity using six samples grown in ExCellerate media (Catalog #CCM030) were run in both platforms and ranged from 88 to 108% using the ELISA (**Figure 3C**) and 95 to 117% using the Simple Plex Assay (**Figure 3D**). Finally, we compared assays across several additional performance standards (**Table 1**) and our analyses indicate that the Quantikine IFN-γ ELISA and the Simple Plex IFN-γ Assay are highly concordant.

FIGURE 3A

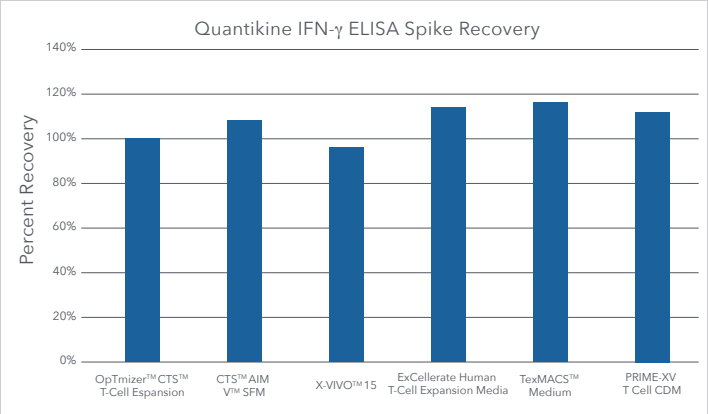


FIGURE 3B

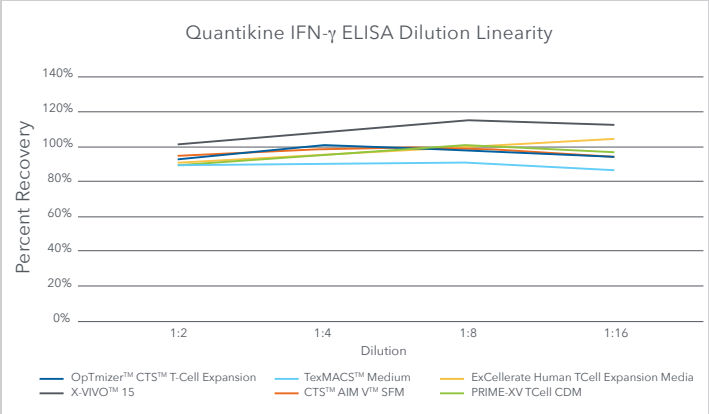


FIGURE 3C

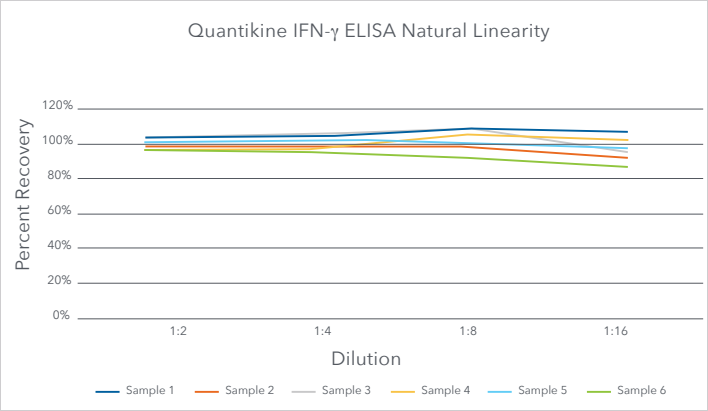


FIGURE 3D

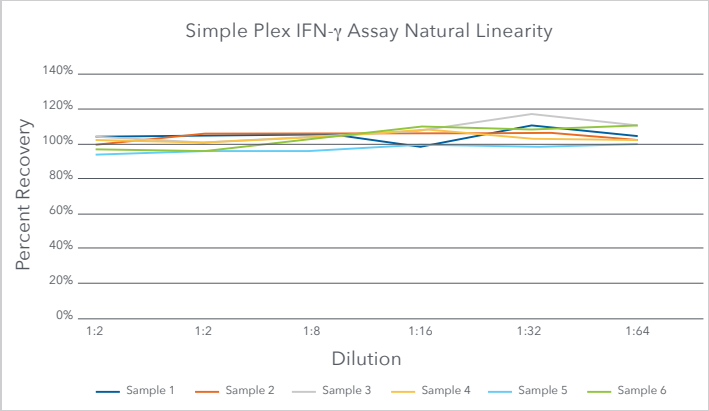


Figure 3. The Quantikine IFN-γ ELISA is accurate and does not suffer from matrix effects. Six different culture media bases were spiked with recombinant IFNγ and quantified on the Quantikine IFN-γ ELISA. This data indicates that components of these media do not interfere with IFN-γ detection. Representative data from one spike and recovery experiment (**3A**) Overall, ELISA spike recovery ranges from 87% to 125%. Dilution linearity in culture media ranged from 86-115% (**3B**). Natural linearity was evaluated on the Quantikine IFN-γ ELISA and the Simple Plex IFN-γ Assay. ELISA natural linearity ranged from 88 to 108% (**3C**). The Simple Plex natural linearity ranged from 95 to 117% (**3D**).

TABLE 1

ASSAY PERFORMANCE	Quantikine	Simple Plex
Limit of Quantitation	15.6 pg/mL	0.17 pg/mL
Limit of Detection	1.28 pg/mL	0.05 pg/mL
Intra-Assay Precision	low control 2.0%	7.0%
	high control 2.8%	2.8%
Inter-Assay Precision	low control 6.5%	10.0%
	high control 5.1%	8.3%

Table 1: Assay Performance Summary. The Quantikine IFN-γ ELISA is comparable to the Simple Plex IFN-γ assay in terms of detection and quantitation limits and precision.

CONCLUSIONS

It is apparent that IFN-γ's effects are context dependent as it has been shown to promote tumor immune surveillance and immune escape[2]. For these reasons and others, IFN-γ represents a popular target in the context of cell therapy and immunology. As such, it is critical that accurate and precise tools are available for measuring this pleiotropic cytokine.

Here, we provide novel data that quantifies IFN-γ secretion in response to TCR stimulation and CD28 co-stimulation. There are several take home messages from this data. First, both the 3rd generation IFN-γ Quantikine ELISA and Simple Plex assay represent a new gold standard for IFN-γ detection in cell culture supernates. They both utilize a pair of human monoclonal antibodies that are manufactured by R&D Systems, which provides a consistent supply for your long term needs. Second, the new 3rd generation Quantikine IFN-γ ELISA is highly concordant with the 3rd generation Simple Plex IFN-γ Assay. Both assays detected IFN-γ secreted in response to anti-CD3/CD28 and CD3/CD28 Cloudz with sample values within an average of less than 10% difference. They are also highly correlated with a correlation coefficient (R^2) of 0.99. The high correlation means that researchers can easily move between platforms without significant changes in their data. These samples also met natural linearity specification on both platforms. Both assays demonstrated superior performance when different cell culture medias were tested for spike recovery and spike linearity. Taken together, the aforementioned data indicates that the Quantikine ELISA and the automated Simple Plex platform accurately quantifies IFN-γ in a variety of matrices. Finally, it is important to note that all immunoassays undergo rigorous and extensive quality control processes prior to release. These processes ensure that each new lot of reagents will match prior lots so you can maintain long-term consistency and reproducibility in your studies.

WHICH ASSAY SHOULD YOU USE?

The Quantikine ELISA and Simple Plex assays are great options for IFN-γ expression and can fit into your workflow accordingly. Both platforms demonstrate consistency in measurement and high performance, making them ideal for your sample analysis. If you are familiar with running single-analyte ELISAs and wish to continue with plate-based ELISAs, then the Human IFN-γ Quantikine ELISA is a great option. In contrast, the Simple Plex IFN-γ assay streamlines the process in situations where IFN-γ is measured frequently and routinely. With the high correlation coefficient, you can easily move from the Quantikine to the Simple Plex for routine measurements. This automated assay reduces user error and is ideal for situations where comparisons across users and geographies is paramount. Finally, Simple Plex assays also save time (**Figure 4**) as they can be completed in less than 90 minutes.

FIGURE 4

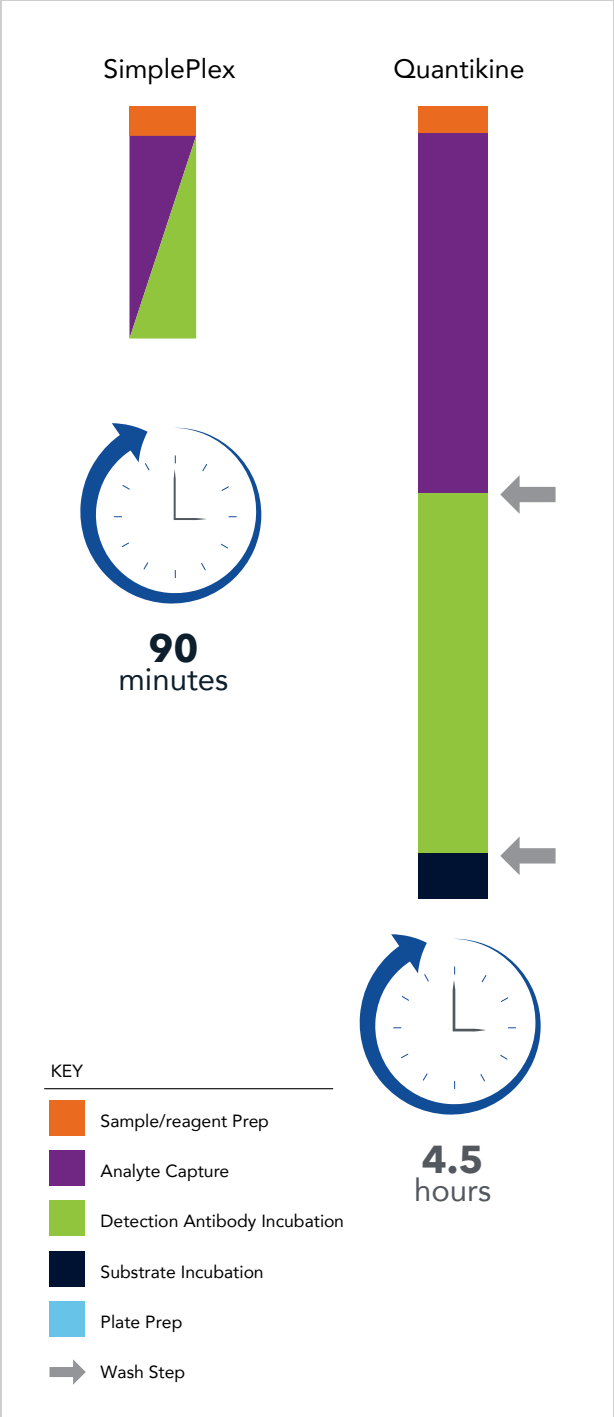


Figure 4. A comparison of the Simple Plex and Quantikine ELISA workflows.

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NOTES

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