Poster #112

Automated analysis of cytokine secretion using a novel microfluidic-based assay

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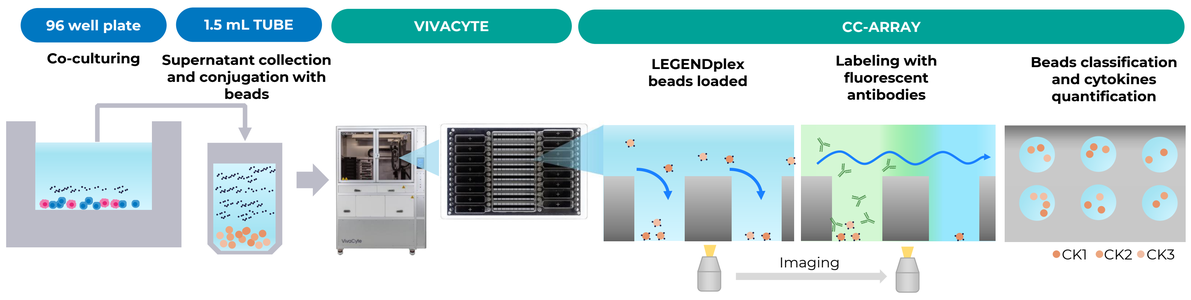
Abstract

Cytokines are key immunoregulatory proteins produced by various cell types. They play a crucial role in numerous disease states, including inflammation and oncogenesis. Understanding cytokine levels is essential in cell therapy development.

Here, we present **a novel microfluidic-based assay** running on the VivaCyte® cell analysis platform for detecting and quantifying cytokines associated with cell activation. VivaCyte is an analytical instrument which automates sample preparation, incubation and analysis through a fluorescence imaging readout. Protocol automation is enabled by a microfluidic device which a) delivers commercial microbeads in microwells and b) replaces the media in each microwell and provide the antibodies required to visualise the presence of cytokines on the microbeads.

This innovative technology enables **automation**, significantly **reducing hands-on time**. The assay is designed for multiplex analysis of cytokines from multiple co-culture supernatants, offering enhanced efficiency and precision.

We provide a comprehensive **validation** of the assay, demonstrating the potential of the assay in terms of **robustness** and **reproducibility.** Finally, automation avoids multiple user-dependent pipetting steps, **decreases hands-on time 2-fold, and speeds up data analysis 3-fold.**



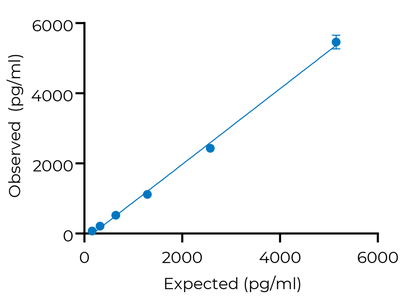
Methods

Supernatant samples were collected from a 24h co-culture of donor-derived NK cells, stimulated with IL-2 for 72h (100U/mL), and K562 cell line at different effector-target (E:T) ratios (from 0.25 to 10). Supernatants were loaded into VivaCyte platform tubes, where the bead conjugation step occurred. Each bead, conjugated with a specific antibody, captures its target analyte. After incubation and washing, a biotinylated detection antibody binds the analyte, followed by SA-PE, generating fluorescence proportional to analyte quantity (b).

The AI-powered VivaCyte analysis software classifies bead populations based on size and fluorescence intensity, quantifying delta TRITC signals between two imaging time points (scans) (b). Analyte concentrations are determined by interpolating the value using an internal calibration curve (c).

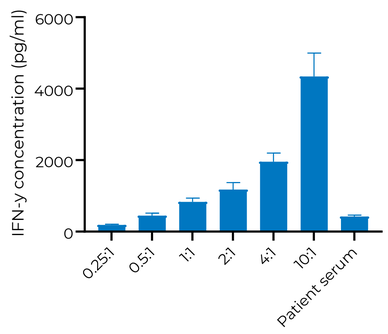
Results

1. Performance evaluation



Sample	Standard deviation	%CV
1:1	139.23	2.55%
1:2	48.08	1.98%
1:4	11.97	1.07%
1:8	26.93	5.18%
1:16	1.06	0.49%
1:32	1.61	2.18%

Figure 1a. IFN-γ intra-assay precision and linearity in co-cultures at different E:T ratios. The concentration of IFN-γ was measured in supernatant samples collected from a 24h co-culture of donor-derived NK cells, which were stimulated and incubated with K562 cell lines at 10 E:T ratio. To validate intra-assay precision and linearity, NK:K562 10:1 E:T supernatants were serially diluted six times and run in duplicate (n=2). Each duplicate demonstrated a %CV below 6% across dilutions. The assay's linearity was validated with an R^2 value of 0.9965.



Sample	Standard deviation	%CV
0.25:1	19.87	11%
0.5:1	61.28	14%
1:1	89.77	11%
2:1	168.17	14%
4:1	208.23	11%
10:1	567.40	13%
Patient Serum	35.67	8%

Figure 1b. IFN-γ inter-assay precision in co-cultures at different E:T ratios (n=4). The concentration of IFN-γ was measured in supernatant samples collected from a 24h co-culture of donor-derived NK cells, which were stimulated and incubated with K562 cell lines at different effector-to-target (E:T) ratios. Four assays have been conducted on different days. The mean values progressively increased with the E:T ratio, from 185.19 pg/ml at a 0.25 E:T to 4343.45 pg/ml at a 10 E:T. The %CVs ranged from 8% to 14%, demonstrating consistency across the experiments. This consistency was also confirmed with a patient serum sample, displaying a coefficient of variation (CV) of 8%.

2. Validation against gold standard assays

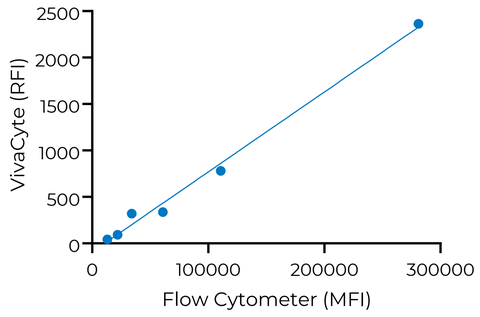


Figure 2. Flow cytometry comparison. IFN-γ quantification using VivaCyte was compared to traditional flow cytometry. IFN-γ concentrations were measured in the supernatants of NK:K562 co-cultures at six different effector-to-target (E:T) ratios (ranging from 0.25 to 10). A correlation between the two methods was observed (n=6, Pearson R = 0.9991, R^2 = 0.9981).

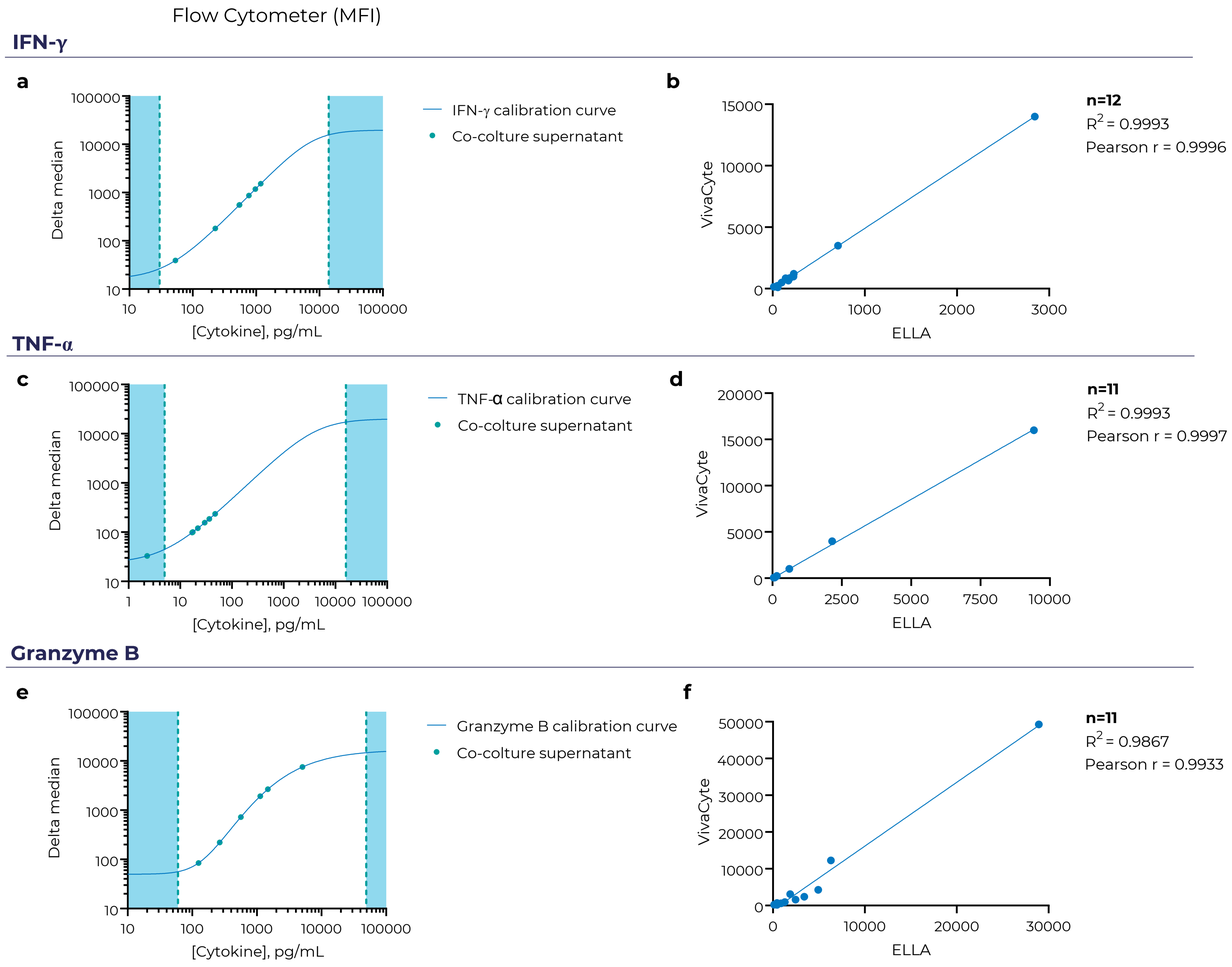


Figure 3. Multiparametric bulk quantification of IFN-γ, TNF-α, and Granzyme B from NK:K562 co-culture supernatant. IFN-γ (a-b,), TNF-α (c-d), and Granzyme B (e-f) concentrations were measured in supernatants from a 24-hour co-culture of donor-derived NK cells with K562 cells at six E:T ratios. An internal calibration curve was applied for each analyte. The Lower Limit of Quantification (LLOQ) and the Upper Limit of Quantification (ULOQ) are represented by the blue shaded area (a, c, e), with one sample falling outside the TNF-α range. Comparison of the quantification of analytes between the Ella® and Vivacyte platforms was performed to assess a correlation. Both assays were performed using the same sample types, supplemented with standard dilutions, to quantify three cytokines. A correlation was observed between the two platforms (b, d, f). For IFN-γ, an R^2 of 0.9993 was obtained, while TNF-α and Granzyme B showed R^2 values of 0.9993 and 0.9867, respectively.

Conclusions

This novel assay demonstrates significant advantages in terms of sample volume used, as well as robustness and reproducibility. A **correlation** between this presented method and the **two gold standards** was observed. Finally, automating the assay—including the conjugation, labeling, and detection steps—**eliminates multiple user-dependent pipetting steps**, **reduces hands-on time by half**, and **accelerates data analysis threefold**.

30% less sample volume

2x less hands-on time

3x data analysis speed-up

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