T CELL STIMULATION ASSAY AND IMMUNOASSAYS COMPARISON FOR SOLUBLE CYTOKINES PROFILING IN DRUG DISCOVERY

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Background T cell stimulation assays are commonly used in invitro pharmacological assays to understand immunomodulatory functions of drugs in development.1–3 Assays read outs are common activation markers measured by flow cytometry and cytokines secretion detected in culture supernatants. In addition to monitoring activation markers, cytokines and their expression are critical for biomarker discovery and essential in understanding immune biology in addition to being commonly used as a read out in T cell stimulation.

Methods In this work, we report CD25 CD69 expression after stimulation at 24, 48, 72h and IL-4, IL-2, TNFa, IFNg production from 33 healthy donors. We used a subset of samples to compare 4 technologies used in drug discovery; Meso Scale Discovery [MSD], Cytometric Bead Array [CBA] and Sartorius Q Beads Plexscreen. Through experimental data analysis, several assay features were compared, including sensitivity, dynamic range, and robustness.

Results Out of all parameters monitored, we identified that measurement of IL-2 at 48h was the best read out using these assay features to quantify T cell stimulation level. Our studies revealed normal range of cytokines production after CD3 CD28 stimulation of isolated T cells. Comparing cytokines measurement technologies, we showed that MSD has the best sensitivity in the low detection limit and the broadest dynamic range, while CBA and Luminex also demonstrate superior performance in the sensitivity and dynamic range. Additional aspects of these technologies, including assay principles, formats, throughputs, robustness, costs, and multiplexing capabilities, were also reviewed, and compared. MSD was the most sensitive technology for IL-4, while CBA was the most suitable one for cytokine high-throughput screening with multiplexing capability.

Conclusions This report aims to help readers understand analyte variance in healthy donors at baseline and upon stimulation, to select the proper cytokines secretion method for their specific applications.

REFERENCES