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iQue Qpanels®: Bead-Based, High Throughput Panels for T Helper Cell Cytokine Profiling in Human Peripheral Blood Mononuclear Cells

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Introduction

Upon T cell activation, naive CD4+ cells differentiate into various types of T helper (Th) cells. This differentiation is influenced by the cytokine milieu present during antigen engagement and is further driven by autocrine mechanisms. In physiological processes, Th subsets orchestrate adaptive immune responses to specific pathogens and dysregulation of Th cells are major contributors to autoimmune and allergic diseases. Th subsets can be identified, in part, by their unique cytokine secretion profile.

The Sartorius iQue Qpanels® T Helper Kits enable measuring cytokine secretion from Th cells. The iQue Qpanels® T Helper Kits are pre-configured multiplexed bead-based kits used to quantitate Th1 and Th2 (4-, 6-, or 9-plex) or Th1, Th2, and Th17 (7-plex) associated cytokines in cell supernatants. These miniaturized assays require only 10 µL for each sample, and all reagents are pre-mixed for ease of use. Data acquisition is performed using 96-, 384-, or 1536-well plates on the iQue® Advanced Flow Cytometry Platform. Pre-mixed reagents and cytokine standards, and templated data analysis, including event gates, gating strategy, 4 or 5 parameter logistic standard curves and sample quantitation are all auto-generated to allow for fast enumeration of Th cell cytokine profiles. The multiplexed assay detects all of the proteins simultaneously in an easy to perform, one-wash protocol that provides actionable results in a little over three hours from sample addition to quantitated cytokine profiles.

Assay Principles

iQue Qpanels® T Helper Kits contain pre-mixed multiplexed cytokine capture beads that are coated with antibodies directed against the Th cytokines, with each bead binding only one of the specific Th analytes. Each bead is also encoded with various intensities of two separate fluorescent colors, allowing the different beads to be multiplexed for simultaneous binding and detection followed by quantitation on the iQue® platform, which determines the cytokines captured by each specific bead. Each kit also comes with pre-mixed lyophilized standards for quick setup of the standard curve. iQue Forecyt® software calculates 4 or 5 parameter logistic curves to quantitate your sample's Th cytokine secretion levels in picograms per milliliter (pg/mL).

Assay Workflow

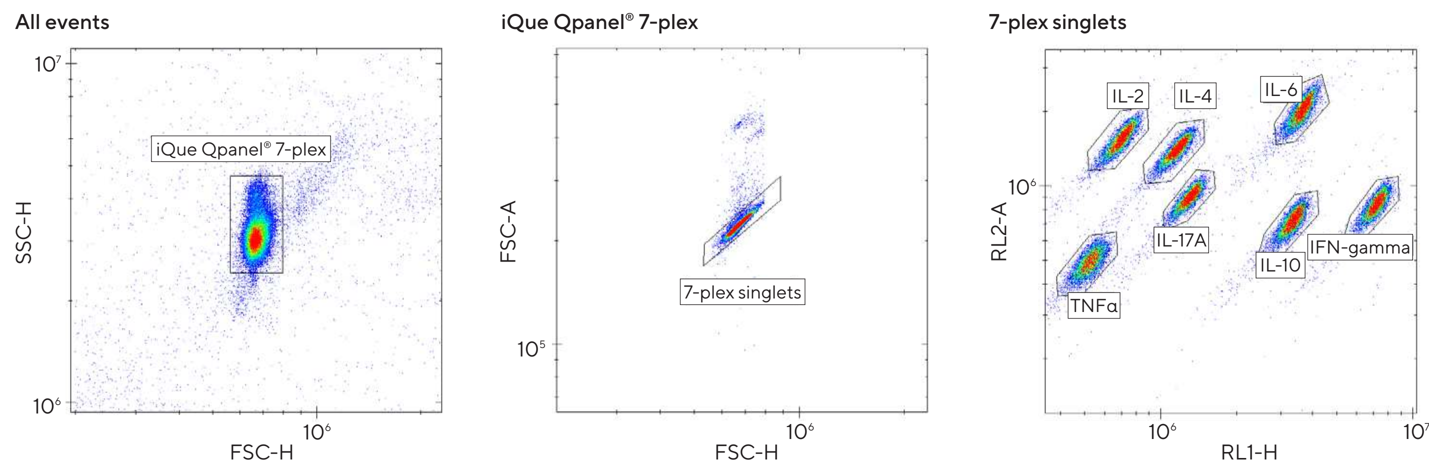
For ease of use, all of the components of the kit are pre-mixed. You only have to dissolve the cytokine standards and dilute the capture beads before starting your assay. First, dissolve the pre-mixed lyophilized standards, prepare a standard curve, and dilute the pre-mixed capture beads with included assay buffer. Next, combine 10 µL of your samples or standards with 10 µL of the pre-mixed capture beads and incubate for 1 hour. Then add 10 µL of the pre-mixed detection reagent and incubate for 2 hours. Perform one wash at the end of incubation, resuspend the beads, and read on the iQue® Advanced Flow Cytometry Platform. The entire protocol requires approximately 3.5 hours, with plate reads of less than 20 minutes for 384-well or 10 minutes for 96-well plates, regardless of which iQue Qpanel® (4-, 6-, 7-, or 9-plex) kit is used.

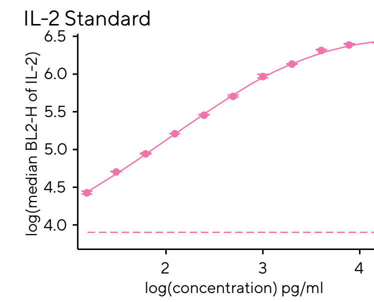
Methods

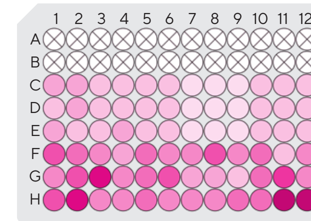
Cell culture: For these studies, peripheral blood mononuclear cells (PBMCs) from 3 donors were cultured in media containing human sera and IL-2. Cells were stimulated with anti-CD3 | CD28 beads; control cells received no stimulation beads. Supernatants were collected 1, 2, and 3 days post-stimulation and kept frozen at -80° C until assayed.

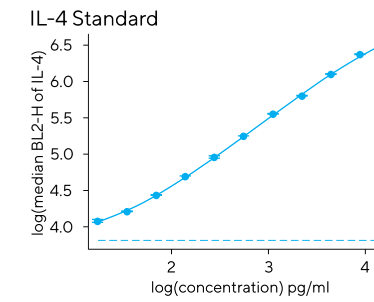
Cytokine quantitation: The 7-plex Th1 | Th2 | Th17 iQue Qpanel® kit (IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ, and TNFα) was performed on the cell culture supernatants as follows. The lyophilized standard was dissolved and serially diluted 1:2 to prepare a 12 point dilution series. 10 µL of each dilution of the standard curve was transferred to a new 96-well assay plate. Supernatants were thawed and 10 µL of each was transferred to the assay plate in triplicate for each sample. The pre-mixed capture beads were diluted to working volume and 10 µL was delivered to each well containing supernatant or standard, mixed, and then incubated for 1 hour at room temperature. Next, 10 µL of pre-mixed detection reagent was added to each well, mixed, and incubated for 2 hours at room temperature. Subsequently, 50 µL of buffer was added to each well as a wash, the plate centrifuged for 5 minutes at 1100 xg, then aspirated with a BioTek ELx405 plate washer, and 10 µL buffer added to resuspend the beads. Finally, the 96-well plate was read on an iQue® Screener PLUS (VBR configuration) in under 10 minutes.

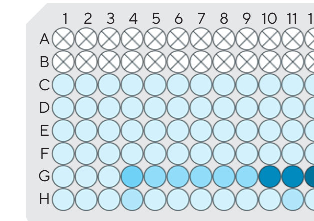
iQue Forecyt® software determined curve fits for the cytokine standards and used the fits to quantitate the amount of cytokines secreted by the PBMCs, converting the median fluorescence intensity (MFI) associated with each bead's captured analyte to actual secreted concentrations.

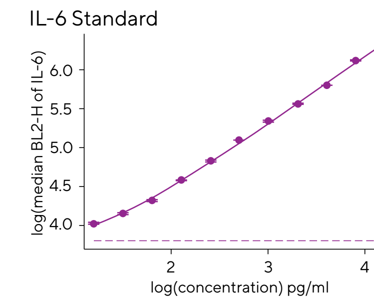


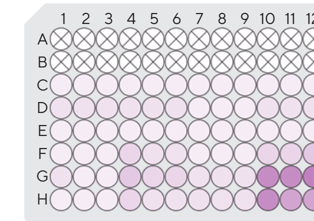


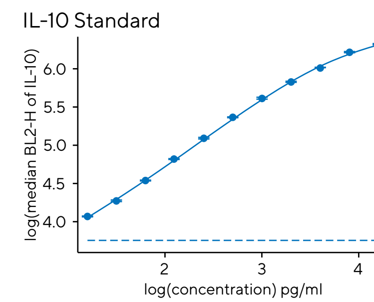


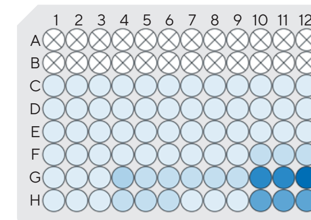


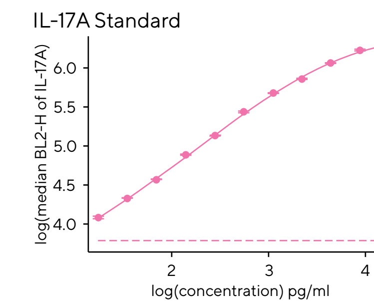


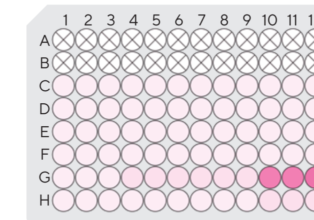


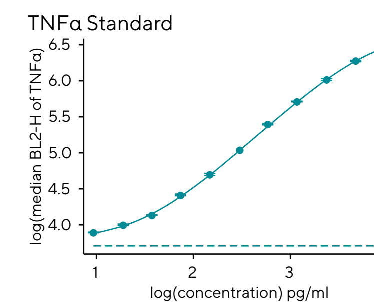


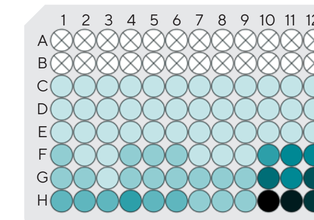


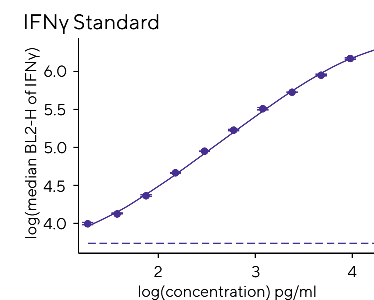


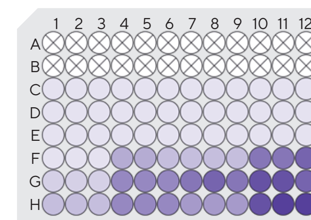












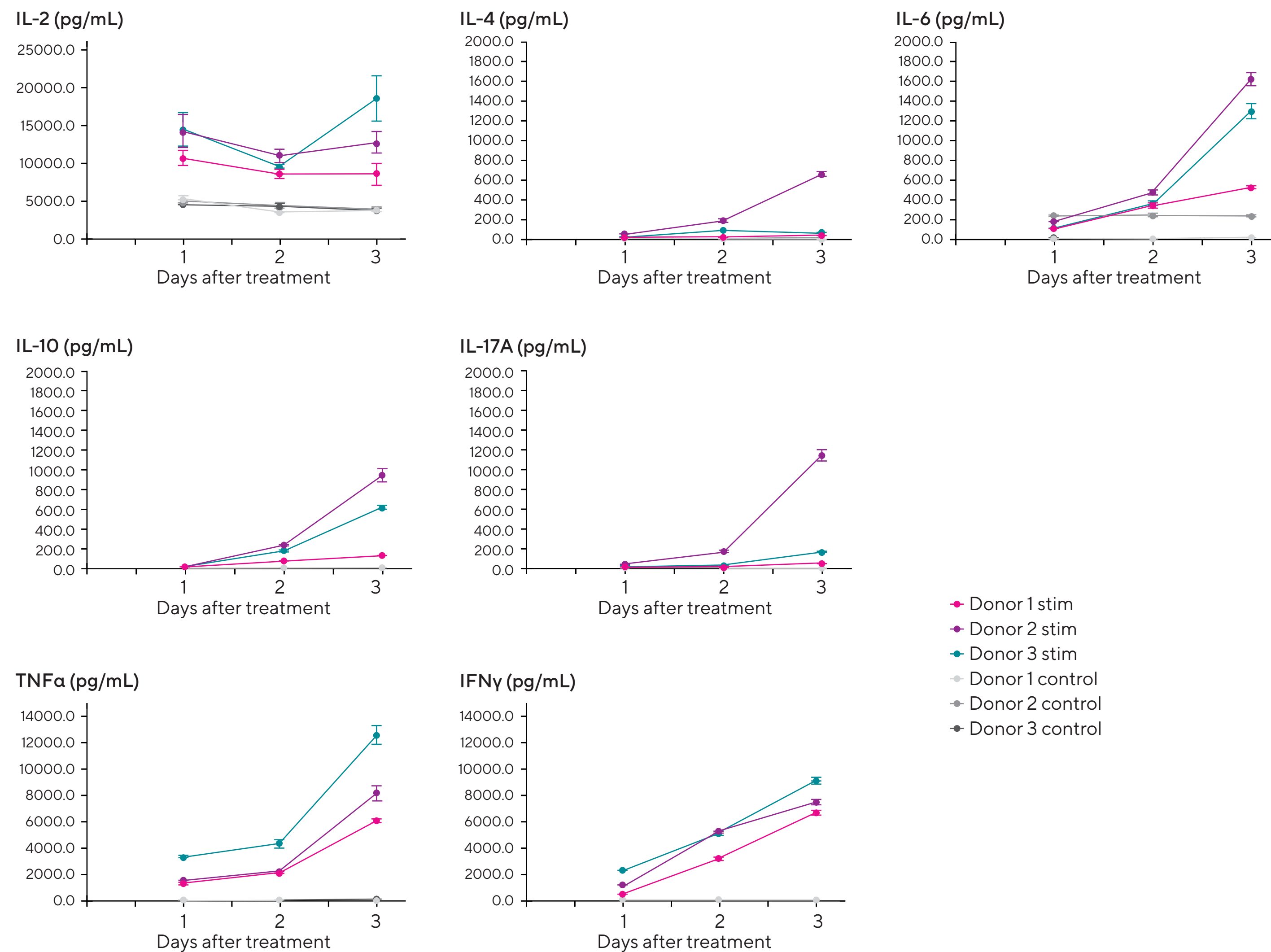
Results

Donor to donor variation and temporal differences in secreted cytokine levels were observed. Exogenous IL-2 was added to the media to encourage T cell growth and viability, and was detectable in control wells, but increased IL-2 production (approximately 2–3 fold over control) was observed in stimulated cultures from all three donors. All donor PBMCs demonstrated a Th1-type response, secreting IFNγ and TNFα post-stimulation, with secretion increasing in a time-dependent manner on days 2 and 3. Notably, one of the donors (donor 2) showed more robust Th2 (IL-4, IL-6, and IL-10) and Th17 (IL-17A) responses compared to other donors.

Discussion

iQue Qpanels® provide easy to use kits for profiling Th cell subsets, efficiently screening secreted proteins from T cells to quantitate multiple cytokines in a simple, pre-mixed format that requires minimal hands-on time while maximizing meaningful data. These data highlight the use of iQue Qpanels® in T cell screening, which allows easy assessment of effector function and the identification of Th subsets, showing donor and time-dependent cytokine secretion:

- Streamlined, pre-mixed Th panels
  - 4-plex Th1 | Th2 panel: IL-4, IL-6, IFNγ, TNFα
  - 6-plex Th2 | Th2 panel: IL-2, IL-4, IL-6, IL-10, IFNγ, TNFα
  - 7-plex Th1 | Th2 | Th17 panel: IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ, TNFα
  - 9-plex TH1 | Th2 panel: IL-2, IL-4, IL-6, IL-10, IL-12(p70) IL-13, IFNγ, TNFα, GM-CSF
- Standards, capture beads, and detection reagent are all pre-mixed for quick and easy assay setup
- iQue Forecyt® acquisition and analysis templates for rapid data analysis and quantitation



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