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TruCulture® Case Study: Standardized immune insights with flow cytometry

An integrated whole blood workflow for simultaneous cytokine and cellular analysis

Abstract

TruCulture® tubes offer a closed whole blood culture system that enables simultaneous analysis of soluble protein biomarkers and immune cell phenotypes from the same sample. TruCulture can be utilized for flow cytometry panels tailored to the specific needs of each clinical project. This adaptable approach supports exploratory immune profiling, pharmacodynamic assessments and precision-driven clinical research.

Background

Conventional PBMC methods require specialized equipment and introduce variability across sites and technicians.[1] TruCulture offers an alternative approach that combines blood collection and culture in a single tube, preserving all non-mononuclear cells and circulating soluble factors that can impact immune responses. This case study describes a protocol for analyzing TruCulture cell samples for surface marker expression by multi-color flow cytometry immediately after supernatant separation.

Methods

The following study assessed feasibility across donors and stimulants. Whole blood from 10 healthy donors was collected into TruCulture tubes preloaded with a single stimulant (null [unstimulated], lipopolysaccharide [LPS], staphylococcal enterotoxin B [SEB], or anti-CD3/CD28). For each stimulant, two tubes were collected per subject. Samples were incubated for either 24 or 48 hours at 37°C. Supernatants were analyzed for cytokine expression using Luminex-based multi-analyte profiles (MAPs) developed by Rules-Based Medicine (RBM). Cells were assessed by flow cytometry using a nine-color antibody panel.

TruCulture collection instructions are available using this QR code or online at rbm.iqvia.com.

Flow cytometry staining protocol for TruCulture samples

The flow cytometry protocol was optimized for the Beckman Coulter CytoFLEX LX using a nine-color panel. Panel composition may vary by project, and adjustments should be made based on specific instrumentation and study goals. This guidance serves as a foundational workflow that can be customized for diverse applications.

1. Following incubation, the supernatant was separated from the cell layer using the Seraplas valve.
2. The supernatant was removed from the TruCulture tube.
3. Then, the Seraplas valve was removed.
4. Entire cell layer was collected and transferred to a 15 mL conical tube containing 5mL of ACK buffer (Quality Biological: 118-156-101) and incubated in an ice bucket filled with chilled Lab Armor® beads and two ice packs (1-8˚C) for 20 minutes.
5. Samples were washed with 5mL 1xDPBS (Quality Biological: 119-068-131).
6. Samples were stained for viability using 1µL/sample Zombie Red in 1xDPBS (100µL/sample) at room temperature for 15 minutes.
    - FITC anti-human CD45 Antibody (368508)
    - Pacific Blue™ anti-human CD3 Antibody (300431)
    - APC/Fire™ 750 anti-human CD4 Antibody (300560)
    - PerCP/Cyanine5.5 anti-human CD8 Antibody (344710)
    - Brilliant Violet 785™ anti-human CD19 Antibody785TM (302240)
    - Brilliant Violet 650™ anti-human CD11b Antibody (301336)
    - APC anti-human CD66b Antibody (305118)
    - PE anti-human CD161 Antibody (339904)
    - PE/Cyanine7 anti-human HLA-DR Antibody (361612)
7. Samples were washed with 1mL/sample 1xDPBS with 1% BSA from heat shock fraction, protease free, fatty acid free, essentially globulin free (Sigma: A7030).
8. Samples were blocked with 5µL/sample Human TruStain FcX™ (Fc Receptor Blocking Solution) (BioLegend: 422302).
9. Samples were stained with 2.5µL/sample of the following antibodies (BioLegend) on cold Lab Armor® beads for 30 minutes. Alternatively: Incubate at room temperature for 15 minutes.
10. Samples were washed (1mL/sample 1xDPBS with 1% BSA) and fixed with 4% paraformaldehyde (250µL/sample) for 15 minutes at 1-8˚C.
11. Samples were stored in 1xDPBS with 1% BSA (500µL/sample) at 4˚C until analysis.

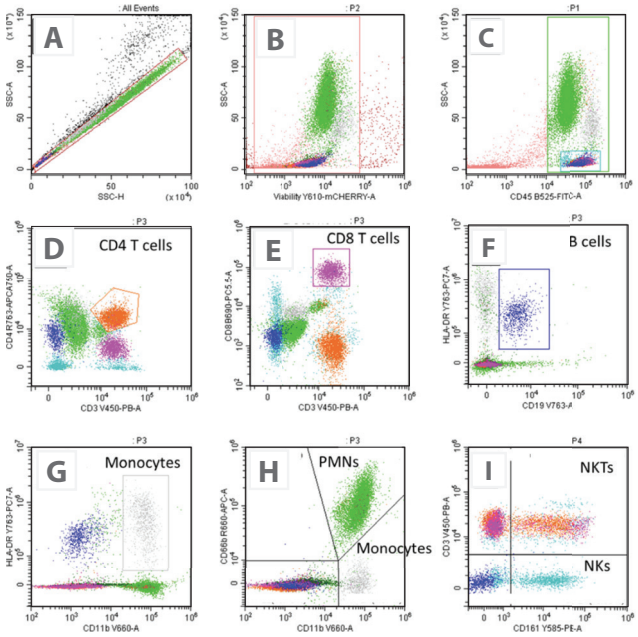
Results

Sample analysis by flow cytometry analysis provided a clear view of immune cell dynamics in response to stimulation. As shown in **Figure 1**, the gating strategy enabled distinct separation of lymphocytes, monocytes and granulocytes.

Flow gating strategy for TruCulture cell samples

Samples were read on the Beckman Coulter CytoFLEX LX flow cytometer. P2 is the single cell gate (A), P1 is the viability gate (B), P3 is CD45+ cells (C), and P4 is gated on lymphocytes (C). Figure D to H were all gated on CD45+ cells. Figure 1 was gated on lymphocytes.

Figure 1



The case study demonstrated that leukocytes exhibit unique immune responses to different stimulants.

- **Null (unstimulated)**
  CD45+ cells showed normal side scatter (SSC) distribution, serving as a baseline.
- **LPS stimulation**
  Activated monocytes, which express TLR-4, demonstrating a noticeable shift in the monocyte population.
- **SEB stimulation**
  Stimulated both monocytes and T cells through antigen presentation and TCR engagement.
- **Anti-CD3/CD28 stimulation**
  Anti-CD3/CD28 antibodies strongly activate T cells by triggering two key signals. In this case study, half were responders and half were non-responders due to a FcγR1 polymorphism, as identified in a 2018 study.[2] In the responders, T cells showed clear changes in shape and behavior, and monocytes were likely activated indirectly. In the non-responders, cell patterns resembled those from null tube samples.

The shifts in immune cell populations found using flow cytometry correlated to cytokine levels assessed in TruCulture supernatants using RBM multi-analyte profile (MAP) assays.

In the unstimulated (null) condition, cytokine levels were predictably low, with most markers below the assay's detection threshold, with only IL-8 and ENA-78—two neutrophil-associated chemokines—showing measurable levels, consistent with prior studies.[3,4] In samples stimulated with LPS, SEB, or anti-CD3/CD28, strong cytokine responses were observed. Notably, donors who didn't respond to anti-CD3/CD28 stimulation also showed minimal cytokine release.

Importantly, TruCulture tubes utilize suspended anti-CD3/CD28 antibodies to activate all T-cell subsets by engaging both primary and co-stimulatory signals.[5] Unlike immobilized formats, this approach preserves natural immune variability, enabling detection of non-responder phenotypes linked to FcγR1 polymorphisms.[2] These findings underscore TruCulture's value in immune profiling, biomarker discovery, and safety assessment.

Advantages

This case study highlighted several strengths of using a TruCulture-based workflow:

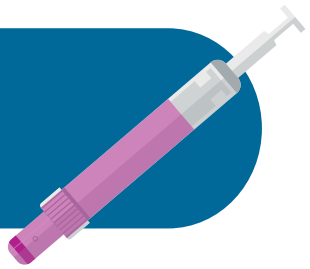
- Whole blood culture preserves immune complexity
- Closed system reduces contamination and variability
- Compatible with standard flow cytometry workflows
- Enables simultaneous cytokine and cellular analysis
- No centrifugation or complex cell isolation required

Conclusion

This proof-of-concept study illustrates that TruCulture cell samples can be analyzed using standardized flow cytometry methods. The use of TruCulture stimulations in clinical trials for pharmacodynamic monitoring and other applications can be accomplished through a variety of analytical methods and can be optimized based on the choice of stimulant.

TruCulture tubes, combined with downstream flow cytometric analysis, offer a powerful approach for assessing immune response — capturing shifts in immune cell populations and changes in surface marker expression that reflect cellular function, viability and activation status.

Rather than a one-size-fits-all approach, TruCulture supports precision-driven immune profiling by integrating into a variety of platforms and clinical projects.



References

1. Duffy D, Rouilly V, Braudeau C, et al. Standardized whole blood stimulation improves immunomonitoring of induced immune responses in multi-center study. Clin Immunol. 2017;183:325–335. doi:10.1016/j.clim.2017.09.019
2. Stein MM, Hrusch CL, Sperling AI et al. Effects of an FcγRIIA polymorphism on leukocyte gene expression and cytokine responses to anti-CD3 and anti-CD28 antibodies. Genes Immun. 2019; 20: 462–472. doi.org/10.1038/s41435-018-0038-8
3. Han Z-J, Li Y-B, Yang L-X, et al. Roles of the CXCL8-CXCR1/2 Axis in the Tumor Microenvironment and Immunotherapy. Molecules. 2022; 27(1):137. doi.org/10.3390/molecules27010137
4. Gardella, B., Dominoni, M., Gritti, A. et al. Endometriosis pain and epithelial neutrophil activating peptide-78 levels. Sci Rep. 2022; 12, 3227. doi.org/10.1038/s41598-022-07349-3
5. Shah K, Al-Haidari A, Sun J, Kazi JU. T cell receptor (TCR) signaling in health and disease. Signal Transduct Target Ther. 2021;6(1):412. Published 2021 Dec 13. doi:10.1038/s41392-021-00823-w

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