

ABSTRACT

TruCulture® is a whole blood collection and culture system designed for use at clinical sites. TruCulture outperforms traditional peripheral blood mononuclear cell (PBMC) culturing methods, with improved reproducibility and higher levels of stimulation (Duffy et. al. Clinical Immunology 2017). The TruCulture proprietary culture medium ensures minimal non-specific immune activation, delivering a steady baseline and decreased variation across study populations. OptiMAP is a multiplex immunoassay panel that measures 13 cytokines of the major immune response pathways (T_H1 , T_H2 , T_H17 , and monocyte/macrophage). Using TruCulture and OptiMAP, we analyzed healthy donor whole blood responses to common stimulants: T cell activators, TLR agonists, and superantigens. The subjects ranged from 24 to 70 years old and consisted of 48% males and 52% females. The results indicated that healthy donors responded similarly to stimulation with anti-CD3/CD28 and staph enterotoxin type B (SEB) in production of T cell cytokines (IFN- γ , IL-2, and IL-17). The response to SEB is positively correlated to the monocyte population in whole blood. T cell responses to SEB and CytoStim™ (an antibody based alternative to SEB) are similar in production of IFN- γ and IL-17, but CytoStim™ had a reduced stimulation of IL-2. As expected, all healthy donors responded to a TLR-4 agonist (LPS) by producing high levels of TNF- α , IL-6, and IL-1 β . LPS stimulated production of IFN- γ was similar to SEB, most likely due to bystander activation. The TLR-7 agonist (gardiquimod, GDQ) stimulated IL-10 levels at a level comparable to LPS. These data illustrate the utility of combining TruCulture and OptiMAP to investigate human immune stimulation pathways for clinical research.

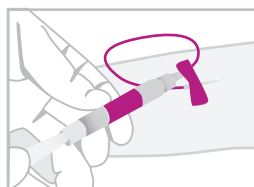
MATERIALS AND METHODS

TruCulture Tubes

- Null – 782-001086
- *S.aureus* enterotoxin type B (SEB) – 782-001124
- Lipopolysaccharide (LPS) – 782-001087
- Anti-CD3 and Anti-CD28 – 782-001125
- CytoStim™ – 782-001333
- Gardiquimod (GDQ) – 782-001269

OptiMAP – 13 Analyte Multiplex Assay

| | | | |
|--------|---------------|----------|---------------|
| ENA-78 | IFN- γ | IL-12p70 | TNF- α |
| IL-8 | IL-2 | IL-10 | IL-6 |
| | IL-17 | IL-23 | IL-1 β |
| | IL-13 | GM-CSF | |



01. COLLECT

Draw 1 mL of blood directly into the TruCulture Tube and break off the plunger.



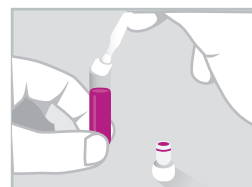
02. MIX

Gently invert tube to mix 3 to 5 times



03. INCUBATE

Place tube in 37°C heat block for up to 24 or 48 hours.

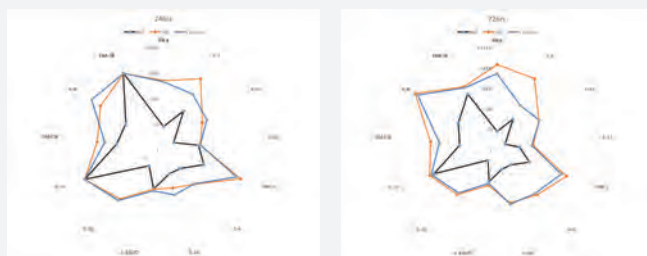


04. SEPARATE

Manually insert valve to separate supernatant from the cells. Collect supernatant and cell layer for downstream analysis.

OPTIMAP PROFILE OF TRUCULTURE STIMULANTS

SEB and CytoStim



Stimulation of Human Whole Blood by SEB and CytoStim: Whole blood from 10 healthy adults were collected in Null, SEB, or CytoStim TruCulture tubes (in duplicate). Samples were incubated for 24 or 48 hours at 37°C on a heatblock. Supernatant were removed and submitted for OptiMAP analysis. Concentration is in pg/1mL of whole blood. CytoStim is comparable to SEB in inducing the analytes measured by OptiMAP. Only exception is IL-2. CytoStim does not induce high production of IL-2.

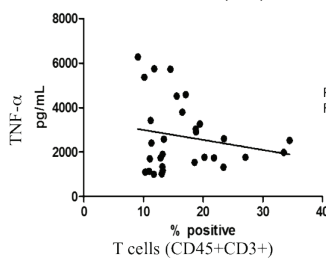
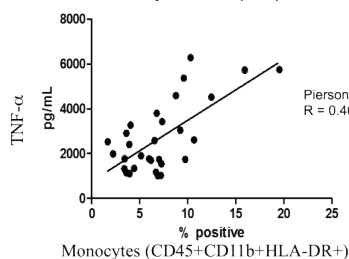
LPS and GDQ



Comparing TLR-4 and TLR-7 Agonists: Whole blood from 7 healthy adults were collected in Null, LPS, or GDQ TruCulture tubes (in duplicate). Samples were incubated for 24 hours at 37°C on a heatblock. Supernatant were removed and submitted for OptiMAP analysis. Concentration is in pg/1mL of whole blood. As expected, LPS is a strong inducer of all innate inflammatory mediators.

CORRELATION OF IMMUNE CELL POPULATIONS WITH PROTEIN PRODUCTION

SEB TruCulture Tubes



SEB Stimulated TNF α Production Positivity Correlated to the Monocyte Population:

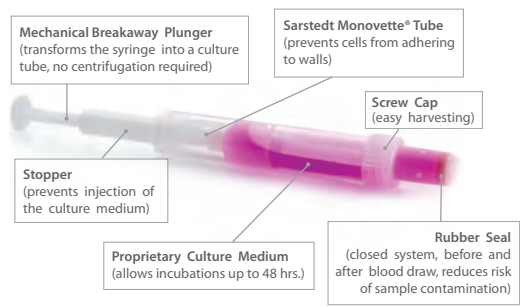
Whole blood from 16 healthy adults were collected in SEB TruCulture tubes. Samples were incubated for 24 hours at 37°C on a heatblock. Supernatant were removed and submitted for OptiMAP analysis. Concentration is in pg/1mL of whole blood. Cell layer was collected for flow analysis. Each TruCulture cell sample was added to 5mL ACK buffer (VWR) to lyse RBCs on ice for 20 minutes. Cell samples were stained with Zombie Red in 1xPBS at room temperature for 15 minutes. Cells were then blocked using Human TruStain FX (BioLegend) in 1xPBS/1%BSA on ice for 15 minutes. Samples were stained with CD45-FITC, CD66b-APC, CD4-APC/FIRE750, CD11b-BV650, CD19-BV785, CD3-PB, CD161-PE, HLADR-PE/Cy7, CD8-PerCP/Cy5.5 (BioLegend) in 1xPBS/1%BSA on ice for 30 minutes. Samples fixed with 4% paraformaldehyde on ice for 15 minutes. Samples stored in 1xPBS/1%BSA at 4°C until analysis on Beckman Coulter CytoFLEX.

TruCulture whole blood culture

- Integrated closed sterile whole blood collection and culture system
- Retains all blood components, including all leukocytes, platelets, soluble factors, and Fc receptor expressing cells
- Easy to use, eliminates the need for cell manipulation
- Standardized to ensure consistent performance across multiple users and clinical sites

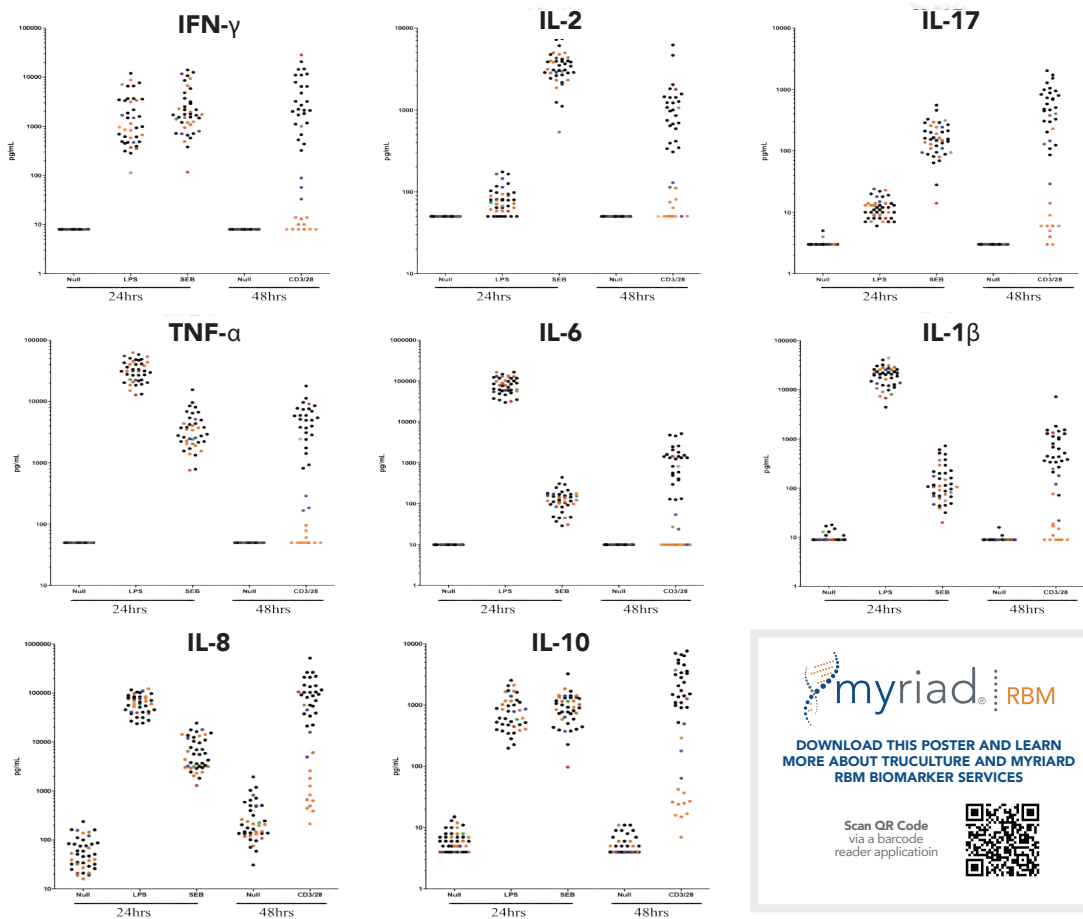
PBMC (Peripheral blood mononuclear cells) culture

- Separate blood collection and specialized cell culture procedures
- Does not retain all blood components, including granulocytes (ex. neutrophils which makes up >50% of all circulating leukocytes), soluble factors, etc.
- Extensive manipulation, processing, and often freezing/shipping prior to culturing
- Poor reproducibility



RESULTS

Immune Responses to Common TruCulture Stimulants



Profile of Healthy Adult Responses to TruCulture Stimulants: Whole blood from 41 healthy adults were collected in Null, LPS, SEB, or CD3/CD28 TruCulture tubes (in duplicate). Samples were incubated for 24 or 48 hours at 37°C on a heatblock. Supernatant were removed and submitted for OptiMAP analysis. Orange = subjects who did not respond to CD3/CD28. Blue = subjects who were low responders to CD3/CD28. Red = Subject who was a low responder to SEB. Green = Subject was a low responder to LPS. There are 14 out of 41 subjects who did not respond to anti-CD3/anti-CD28. No correlation observed between CD3/CD28 non-responders in their responses to LPS or SEB stimulation.

SUMMARY

- Combining TruCulture and OptiMAP is a simple and reliable workflow to investigate target specific immune stimulation pathways for clinical research
- Flow cytometry analyses demonstrate correlation of monocyte levels with inflammatory cytokine production in response to SEB stimulation.