TruCulture®: A simple whole blood collection and culture system for quantifying physiological interactions of the human immune system in the clinic

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Abstract

Accurate measurement of immune activity in relation to therapeutic dosing is an important part of many clinical trials, yet is poorly addressed by traditional techniques. Typical immune activity monitoring is performed using isolated leukocytes or even sorted sub-population culturing methods followed by an analytical measurement.

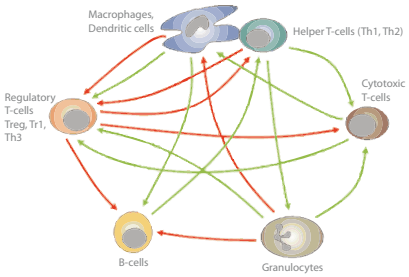
These methods require the shipment of intact blood samples to a cell culture lab plus extensive manipulation that leads to alteration of the *in vivo* physiological environment as well as cell stress and subsequent poor utility and reproducibility.

TruCulture is a simple, reproducible method that eliminates such extensive manipulation and provides ease of use for sample collection and downstream analysis. Every feature of the TruCulture system is designed to give researchers the ability to reproducibly and accurately capture *ex vivo* immune cell activity to better characterize the efficacy and safety of a new drug, explore disease states, or develop new diagnostic tests.

MYRIAD RBM®

Introduction

The immune system is not only intricate, but extremely variable in the elicited response to external stimuli. The network of signals responsible for the inflammatory response is highly complex, involving the cells of the immune system as well as those of healthy and diseased organs. This makes it difficult to model such regulatory processes *in vitro*.



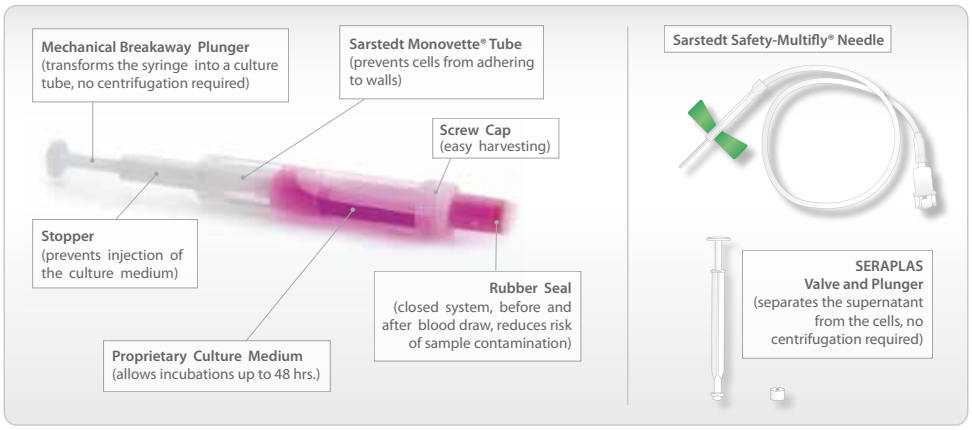
The complexity of the immune system is a major obstacle to testing immune cell function *in vitro* and *ex vitro*

The traditional in vitro method used to measure immune activity and response to stimuli is peripheral blood mononuclear cell (PBMC) cultures, where lymphocytes, monocytes, or macrophages are grown separately. However, in addition to being a poor model due to the incomplete collection of immune cell types, PBMC cultures require expensive equipment, specialized training, and multiple handling steps to establish the culture. These inherent factors of PBMC cultures provide ample opportunities for extensive variability to be introduced.

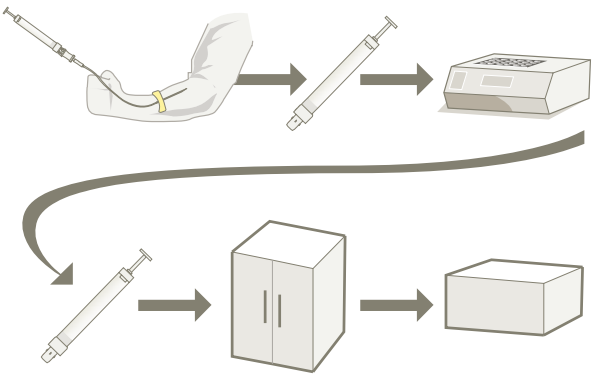
Researchers require an in vitro model that preserves physiological cellular interactions, and allows for easy measurement(s) of the immune system with and without stimulation. In order to ensure reliable data, the system must also be reproducible.

Why TruCulture®?

TruCulture Blood Collection and Whole Blood Culture System is a more relevant model of human leukocyte function than the traditional method of cultured PBMCs. TruCulture creates a more complete *in vivo*-like culture environment containing multiple cell types including all types of leukocytes known to circulate in the peripheral blood, particularly the different subsets of T and B lymphocytes, but also NK cells, monocytes, granulocytes, and platelets. TruCulture also eliminates time consuming and delicate processing steps by standardizing the collection, preparation and processing of whole blood cultures, within a single tube, completed at the collection site.



TruCulture tubes are blood collection tubes that can be used by anyone trained in drawing blood. There is no need for centrifugation or to transport the tube to a specialized cell culture lab. Instead, once the blood is drawn into the tube and mixed by gentle inversion, the plunger handle is simply removed; the tube is then inverted and placed in a dry heat block onsite at 37°C for up to 48 hours. After incubation, the valve separator component is inserted in the tube in order to separate cells from the culture supernatant. The tubes are then recapped, and stored at -20°C until needed for the downstream application.



This process requires less than 5 minutes hands-on-time, no specialized laboratory equipment or personnel, and provides the investigator with a system that more closely resembles the *in vivo* processes than the complicated, laborious PBMC assays.

In addition, by providing sufficient nutrients for up to 48 hour incubation times, TruCulture enables one to investigate longer duration immune cell activation, including *de novo* cytokine and chemokine synthesis and release. In contrast, whole blood experiments are generally short in duration (2-6 hrs) as a consequence of poor culture conditions leading to premature termination of the normal physiological immune response. When looking for pharmacodynamic markers of immune regulation, a longer more robust response usually provides results of higher sensitivity and greater relevance than short duration incubation that may only lead to the release of stored pre-synthesized mediators.

TruCulture Blood Collection and Whole Blood Culture System Work Flow

TruCulture tubes are provided with or without a stimulant. The table to the right describes the stimulants that are currently available, and the cells that are targeted by those stimulants. Custom stimulant TruCulture tubes are also available. Please inquire.

Null Tubes do not contain any stimulants and can be used to determine an individual's baseline immune system status or used as a control.

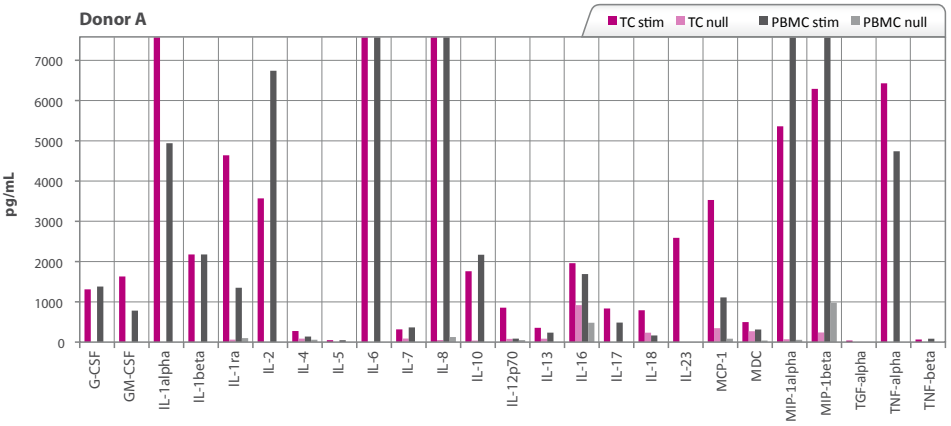
Stimulant	Major Target Cells
Lipopolysaccharide (LPS)	Monocytes, T-cells
Zymosan	Granulocytes, monocytes
Anti-CD-3 antibody	Th1>Th2
Anti-CD3 antibody + Anti-CD28 antibody	Th1, Th2, regulatory T cells
Staphylococcal Enterotoxin type B (SE-B)	Th1>>Th2
LPS + SE-B	Monocytes, T-cells and Th1>>Th2

Utilizing TruCulture Blood Collection and Whole Blood Culture System in Research and Clinical Studies

TruCulture provides a more complete *in vivo*-like testing environment that closely mimics the conditions in the human body upon treatment. This is important when studying responses of immune cells to different therapy and stimulus during research and clinical studies.

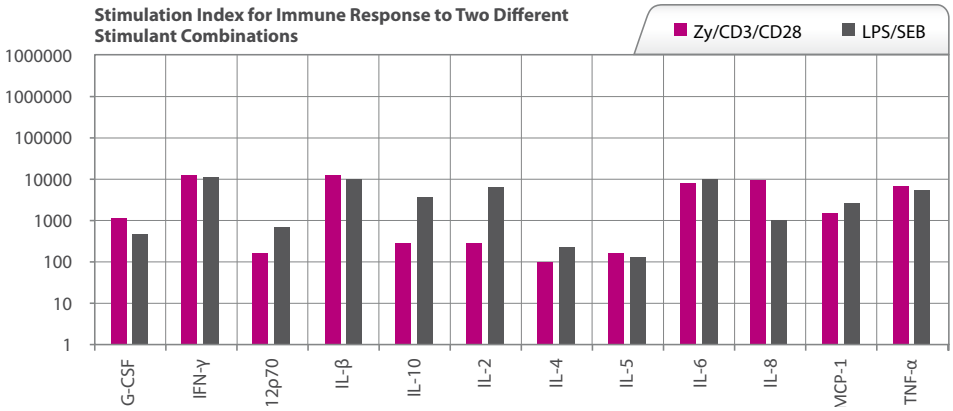
A. TruCulture versus PBMC in Pre-Clinical Studies

The graph to the right represents a comparison of immune cell response(s) with and without stimulation of an individual donor for TruCulture System versus PBMC cultures. In general, the response of immune cells cultivated in TruCulture mirrors that of PBMC for the majority of the cytokines measured; however, measurable stimulation is observed with TruCulture for cytokines such as IL-12p70 and IL-23 , whereas no or negligible stimulation is observed with PBMC cultures. By providing a more inclusive set of cellular components, TruCulture is able to show a more complete picture of cytokine activation versus PBMC.



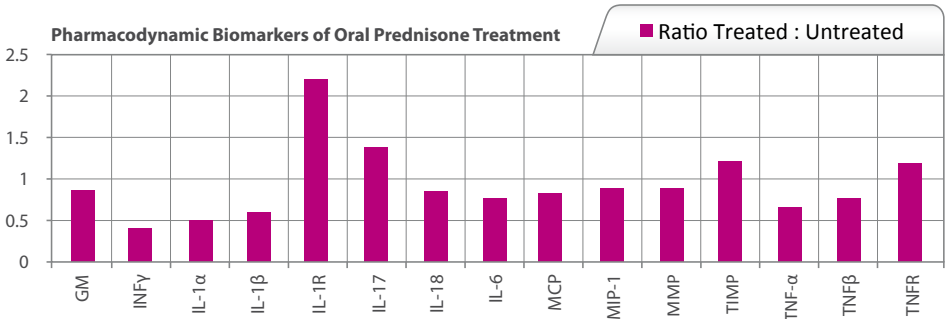
B. Typical Cytokine Measurements using TruCulture

By utilizing the proper combination of stimulants, one can ensure a physiological type of immune cell activation which will result in a wide spectrum of easily measurable endpoints. This helps to identify relevant immune regulatory activities of drugs or other substances, or the current state of leukocyte activities of a given subject.



For example, the data described in the table to the right represents twelve cytokine measurements, which had the highest levels of stimulation of the 46 analytes measured in the TruCultureMAP Service offered by Myriad RBM. In this experiment, two different combined stimuli; a) Zymosan (Zy) + anti-CD3 antibodies (CD3) + anti-CD28 antibodies (CD28), and b) Lipopolysaccharide (LPS) + Staphylococcal enterotoxin B (SEB) are measured. The cytokine concentrations from the stimulated TruCulture tubes are reported in pg/ml. The cells of the immune system show a physiological response to these stimulants during the 24-hour incubation. The pattern of stimulation is distinct for each condition, with Zy/CD3/CD28 showing increase stimulation of Il-8 and LPS/SEB cultures showing increased stimulation of IL-2 and IL-10.

C. Clinical Study Applications for TruCulture

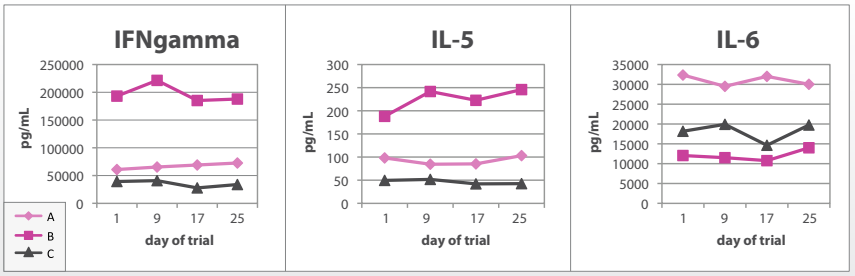


From early Proof of Concept and Phase I studies, the discovery of a pattern of pharmacodynamic biomarkers can potentially translate into stratification, safety or efficacy biomarkers that will be useful in subsequent studies. A common application for TruCulture is characterizing the immune response in clinical trial subjects taking a candidate drug. The subject's immune system is tested *ex vivo* with immune cell stimulation that mimics *in vivo* inflammatory conditions. The data below shows immune stimulation using TruCulture (LPS+SEB stimulant) from a subject before and after 24 hr oral Prednisone treatment of 0.36mg/kg. The TruCulture supernatants were tested on the 46 analyte InflammationMAP, and the graph shows the subset of 15 immune markers that exhibited a significant quantitative change in concentration before and after treatment. As expected, the mainly immunosuppressive characteristics of Prednisone are easily discernable from the data, with GM-CSF, INFgamma, and other inflammatory cytokines showing reduced levels and the anti-inflammatory IL-1RA showing increased levels.

D. Reproducibility of the TruCulture System

One of the most difficult aspects of accurately measuring leukocyte response is the variability of results due to the complexity inherent in the immune system. Therefore, it is imperative that the in vitro system is consistent, and does not introduce artifacts that would result in incorrect conclusions. The graph below shows immune system response at four different time points up to 25 days, for three different donors (A, B and C) measured in pg/mL for three different cytokines (IFNγ, IL-5 and IL-6). As expected, the immune response is variable between individuals. However, the data shows very consistent measurements within an individual with whole blood collected and cultured using TruCulture. In summary, the TruCulture tubes show consistent and reliable results, with CVs ranging from 5 to 14% for each data point.

	IFNg			IL-5			IL-6		
DAY	A	B	C	A	B	C	A	B	C
1	60844	193119	39273	98	188	50	32347	12048	18167
9	65366	221443	40830	84	242	52	29497	11506	19914
17	69013	185170	27752	85	223	42	32008	10760	14638
25	72600	187840	33815	103	246	42	30015	14006	19741
MEAN	66956	196893	35417	93	225	46	30967	12080	18115
SD	5032	16697	5930	9	26	4906	1421	1389	2447
CV%	8%	8%	17%	10%	12%	11%	5%	11%	14%



Conclusions

The TruCulture Whole Blood Collection and Culture System provides minimization of *ex vivo* handling artifacts, ease of use in any lab, quick sample collection, clinical trial standardization, and robust data that more closely resembles *in vivo* immune conditions. Every feature of the system is designed to give researchers the ability to reproducibly and accurately capture *in vivo* immune cell activity that better characterizes their drug, disease state, discovery or diagnostic development project.