

# *RULES* *BASED MEDICINE*

*a Q<sup>2</sup> Solutions Company*

 **tru**culture®



For Research Use Only  
Not Intended for Use in Diagnostic Procedures.

[RBM.Q2labsolutions.com/TruCulture](http://RBM.Q2labsolutions.com/TruCulture)

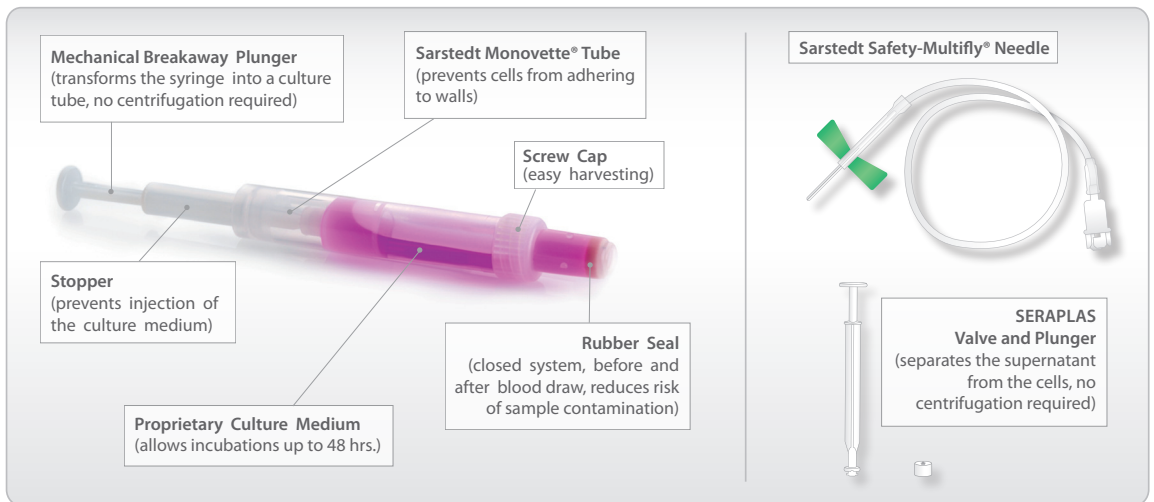
## TruCulture®: Whole Blood Culture System for Clinical Studies

*Ex vivo* assessment of pharmacodynamics (PD), dosing, and safety of potential therapeutic drug candidates on the immune system during early stage clinical trials is essential yet poorly addressed by the inherently variable traditional methods of using whole blood or peripheral blood mononuclear cells (PBMC) isolation and culture that:

- Require a specialized cell culture facility and personnel.
- Exhibit high cellular viability & variability due to delays in processing (shipment) and extensive manipulation of the sample.

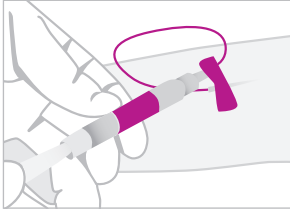
TruCulture® addresses these shortcomings through the use of a closed system for whole blood collection and culturing that is performed at the site of sample collection with no more than a phlebotomist and a heat block. The TruCulture whole blood culture system:

- Starts the **instant** a 1mL blood sample is drawn directly into the TruCulture tube without delay or manipulation which substantially improves reproducibility.
- Nourishes the leukocytes with 2 mL optimized media with immune stimulants or drug candidates for up to 48 hours of culture.
- Uses a simple valve separator to separate the cells from the culture supernatant after incubation for downstream measurement of secreted cytokines or cell analysis.



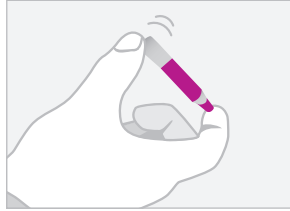
## TruCulture Procedure

TruCulture tubes are pre-loaded with cell culture media and immune stimulant(s) or drug candidates. Blood is drawn directly into the TruCulture tube and incubated in a dry heat block. Supernatants are collected by simply inserting a valve separator to separate cells from the culture supernatant.



### 01. COLLECT

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



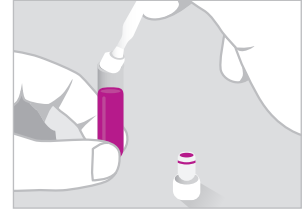
### 02. MIX

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.

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## Advantages of TruCulture

- Integrated closed sterile instant whole blood collection and culture system.
- Standardized to ensure consistent performance across multiple users and clinical sites.
- Reliable, easy to use, and reproducible - eliminates the need for cell manipulation.
- Retains all blood components, granulocytes, platelets, red blood cells, soluble factors and Fc receptor expressing cells.
- Only inexpensive heat block needed, no lab equipment nor centrifugation steps.
- Has been successfully deployed in hundreds of clinical drug trials.

## Disadvantages of Traditional PBMC

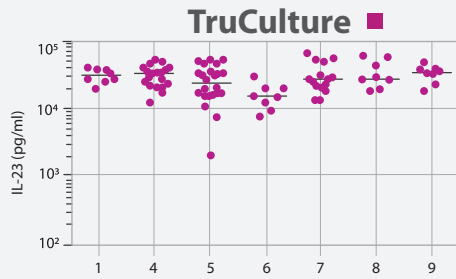
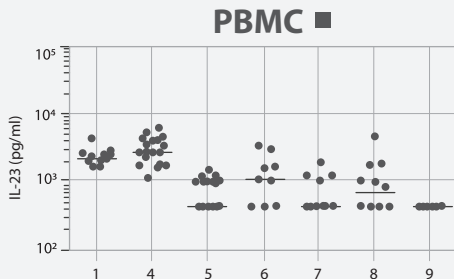
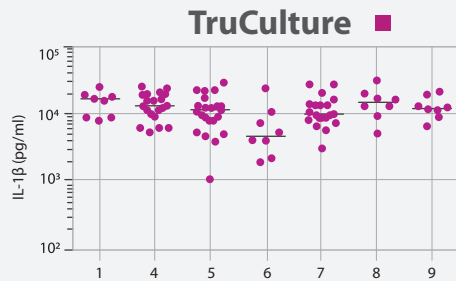
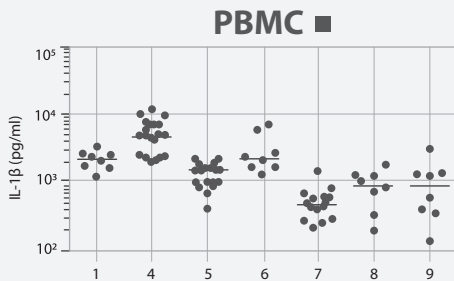
- Separate blood collection and specialized cell culture procedures.
- Extensive manipulation, processing, and often freezing/shipping prior to culturing.
- Requires technical expertise with increased variability across users and clinical sites.
- Requires CO<sup>2</sup> incubator, biosafety cabinet, centrifuge, media, and cell culture plastics.
- Culture procedures/conditions are difficult to standardize for clinical trial applications.
- Open, less sterile, artificial system.
- Poor reproducibility.

Traditional pharmacodynamic whole blood experiments are generally short in duration (2-6 hours) as a consequence of poor culture conditions leading to premature termination of the normal physiological immune response. A longer more robust response usually provides higher sensitivity and greater relevance than short incubation times that may only lead to the release of stored pre-synthesized mediators.

## Reproducibility of the TruCulture System

Standardized whole blood stimulation improves immunomonitoring of induced immune responses in multi-center study. (2017) Duffy D, et al. *Clinical Immunology* 2017 Volume 183, Pages 325-350

TruCulture reduces assay variability by eliminating the need for sample processing prior to culturing. In a multi-center trial coordinated by the Institut Pasteur, TruCulture consistently demonstrated superior reproducibility and consistency of data compared to PBMC cultures. In 7 different testing centers, donor samples were split into PBMC or TruCulture groups and stimulated with LPS for 22 hours. Cytokines from supernatants were analyzed by Rules-Based Medicine (RBM), a Q<sup>2</sup> Solutions Company. For the 35 proteins measured, 29 showed variance between centers in the PBMC group while only 4 proteins were found to be variable between centers with TruCulture. TruCulture demonstrated superior reproducibility across users and sites compared to traditional PBMC cultures and demonstrates higher levels of secreted immune factors in response to stimuli.

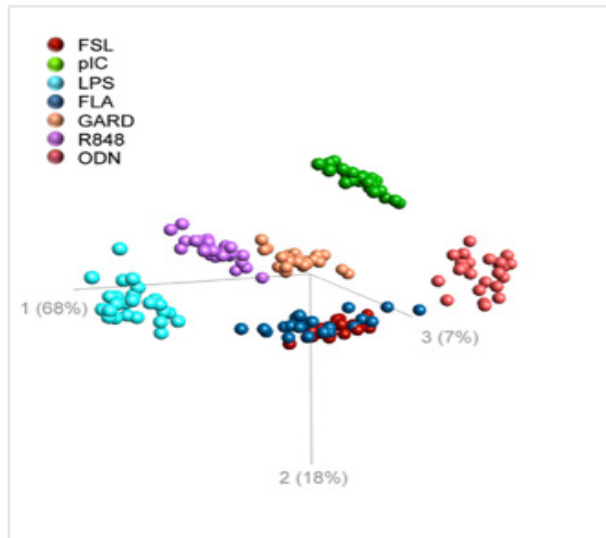


### TruCulture Custom Development Service: Meeting the Needs of Immunology/Oncology Pharmacodynamic Studies

TruCulture can be customized to contain almost any (soluble) substance to target the immune pathway of choice. Our team of scientists are experts in developing and validating TruCulture systems with proprietary substances or biologics to support clinical studies. We have a well-established process for making customized TruCulture tubes. The general development process includes: (1) solubility testing, (2) biological activity testing, and (3) real-time stability testing of the custom TruCulture tube while it is actively used in a clinical trial. The versatility of the TruCulture system allows customization to meet customer specific applications in clinical research environment.

## TLR Agonist Responses can be Segregated Using TruCulture

Duffy et al., *Functional Analysis via Standardized Whole-Blood Stimulation Systems Defines the Boundaries of a Healthy Immune Response to Complex Stimuli* (2014) *Immunity* 2014 Volume 40, Issue 3, Pages 436-450



As a whole blood culturing system, TruCulture is able to examine the comprehensive response of all immune cells and circulating factors to specific stimulation parameter(s). By being able to stimulate all components of whole blood, TruCulture can generate data that accurately models the *in vivo* response. Protein secretion of 11 cytokines were analyzed after culturing whole blood in TruCulture tubes supplemented with the following toll-like receptor (TLR) agonists: **(1)** FSL-1 (synthetic diacylated lipoprotein against TLR-2), **(2)** pIC (poly IC against TLR-3), **(3)** LPS (E.coli O111:B4 lipopolysaccharide against TLR-4,

**(4)** FLA (flagellin against TLR-5, **(5)** GARD (Gardiquimod against TLR-7), **(6)** R848 (Resiquimod against TLR-7 and TL-8), and **(7)** ODN (class A CpG-2216 oligonucleotide against TLR-9). As shown here by principal component analysis, the individual TLR agonists can be clustered by the distinct secreted levels of the 11 cytokines measured, with the exception of FSL and FLA.

## RBM, A Total Solution for Clinical Research

RBM is the world's leader in multiplexed biomarker testing. Our CLIA certified immunoassay testing facility services, for TruCulture supernatants or standard serum/plasma samples, can offer support directly to clinical trial sponsors or indirectly via central lab partners.

OptiMAP is a single cost-effective multiplex assay optimized for TruCulture consisting of 13 analytes that covers the immune response to a variety of TruCulture immune stimulants. For situations (antigen recall) when the analysis of cytokines at low concentrations are needed, the RBM ultrasensitive Simoa™ IL-2 & IFN $\gamma$  service is available. TruCulture separated cells are compatible with flow cytometry and gene expression (NanoString, RT-PCR, and RNAseq) analysis. RBM scientists are available for protocol development and consultation.

The TruCulture system works seamlessly with our protein biomarker assays, offering a simple, one-stop solution for PD studies.

## Ordering TruCulture

TruCulture tubes are available with or without stimulant(s). Custom TruCulture tubes can be developed to meet almost any customer request.

Part #	Tube Name	Stimulant Type
782-001086	Null (no stimulant)	Control
782-001291	NegCo	Control
782-001295	TNF-alpha ( TNFa 10ng/ml)	Cytokine
782-001277	Interferon beta (IFN-beta)	Cytokine
782-001278	Interleukin-1beta + tumor necrosis factor-alpha (IL-1b+TNFa)	Cytokine
782-001272	Adenosine Triphosphate + Lipopolysaccharide, high concentration (ATP+LPS-EB)	NLRP3 Inflammasome / TLR4 Ligand
782-001273	Lauroyl-γ-D-glutamyl-meso-diaminopimelic acid (C12-iE-DAP)	NOD Ligand
782-001411	SARS-CoV-2 Spike Protein (2019 nCoV)	Spike Protein
TBD	SARS-CoV-2 Spike Protein (Delta variant)	Spike Protein
782-001125	Anti-CD3 + Anti-CD-28 (CD3+CD28)	T-Cell
782-001202	Anti-CD3 (CD3)	T-Cell
782-001416	TStim TruCulture Tubes	T-Cell
782-001259	Zymosan (ZYM)	TLR2 Ligand
782-001274	Fibroblast-stimulating Lipopeptide (FSL-1)	TLR2 Ligand
782-001282	Polyinosinic:polycytidylic acid (Poly I:C)	TLR3 Ligand
782-001087	Lipopolysaccharide (LPS)	TLR4 Ligand
782-001264	Resiquimod R848 (R848)	TLR7/8 Ligand
782-001269	Gardiquimod (GDQ)	TLR7/8 Ligand

If you are interested in placing an order for TruCulture or inquire about our custom TruCulture products, please contact your local sales representative, or email us at [rbm.info@q2labsolutions.com](mailto:rbm.info@q2labsolutions.com).