SLiNGSHOT

Application Note

A workflow to demonstrate the use of TruCytes™ TBNK, a synthetic cell-based TBNK control reagent

Introduction

The TBNK assay, in flow cytometry parlance, is an enumeration of key immune cell populations from patient blood. TBNK screening panels generally include T-cells (total, helper, and cytotoxic), B-cells, and natural killer (NK) cells. These distinct lymphocyte subsets are differentiated by surface markers, where CD3/4 and CD3/8 indicate T cells, CD19 identifies B cells, and NK cells are recognized by the presence of CD16/56.

TBNK assays can be used clinically to monitor a variety of health conditions. For example, they are used to track B-cell populations in patients undergoing treatment with immunosuppressive drugs or monoclonal antibodies, to monitor T cell counts in patients with HIV, and in the diagnosis of suspected immunodeficiencies such as severe combined immunodeficiency (SCID).

Sourcing biologically relevant controls for TBNK assays can present unique challenges for reasons such as donor to donor heterogeneity and consistency of supply. Currently, researchers have three primary options for sourcing blood controls. While the currently available blood controls usually provide an adequate distribution of each lymphocyte population of interest, there are significant drawbacks associated with each option.

First, researchers may use fresh donor blood as experimental controls. A significant shortcoming with this option is the limited shelf stability and utilization window. Another option is to use preserved human blood reagents, which consist of donor blood that is treated with additives to extend its shelf-life. While preserved blood reagents offer the user more flexibility, both fresh and preserved donor-derived controls are subject to high variation from sample to sample and limited to fixed number of "thermal cycles" of cold storage to bench usage instances. A third option is to use mixed blood controls, which derive components from multiple organisms. Generating mixed blood controls endeavors to simulate the necessary components of a blood control without depending on the fluctuating availability of human donor blood, but this results in controls that are more far-removed from the human experimental samples to which they are compared.

Irrespective of class, all three types of blood controls are naturally subject to lot-to-lot variation. Biological-derived controls necessitate extensive quality control and quality assurance. This is especially crucial when blood controls are used in bridging or crossover studies, in which there must be consistency and continuity in the processing and analysis of samples across different times and treatment groups. Furthermore, the steep cost of purchasing these natural blood reagents can be a limiting factor for many labs. Lastly, the ability to control population ratios and markers present in donor blood populations is entirely limited, and controls for rare diseases are practically non-existent.

To address the need for more flexible, consistent, and cost-effective whole blood controls, TruCytes™ were created. TruCytes™ products are first-of-their-kind synthetic whole blood cell mimics. They contain biologically relevant, heterogeneous mix of synthetic cell types, and therefore are a complete blood control without the drawbacks of using donor-derived biologics. TruCytes™ are non-biohazardous and do not require special handling, nor are they subject to inconsistencies of the blood supply. This synthetic control option can also be customized to suit the user's unique needs or to create meaningful controls for rare diseases that would otherwise be unavailable.

In this workflow, we present TruCytes™ TBNK control by Slingshot Biosciences. Using the Cytek® Northern Lights 3-color cytometer and its accompanying software, SpectroFlow®, the performance of this TBNK control reagent was tested under several conditions to ensure its capacity to generate consistent scatter

profiles and biomarker detection in a variety of clinically relevant environments. TruCytes™ TBNK were tested across multiple experiment sites without controlling for site to site process and sample handling variation. TruCytes™ TBNK remained assay stable up to 14 days post reconstitution (data not shown) and performed as expected under various wash conditions. Mean fluorescence intensity was reported for all of the lineage specific CD antigens in proportion to their expected densities in healthy donor blood. Overall, TruCytes™ TBNK outperformed traditional blood controls by exhibiting minimal lot-to-lot variation and remarkable consistency across testing conditions.

Results

TBNK assays evaluate several distinct subpopulations in blood simultaneously. Used in a variety of clinical applications, controls for the TBNK assay must faithfully represent the key cell populations being evaluated. To ensure that TruCytes™ can perform in proxy of biologically relevant whole blood control, this reagent was tested side by side to whole donor-derived blood. To ensure that TruCytes™ achieve the reproducibility in performance necessary for long-term and large-scale clinical applications, the consistency of fluorescent signal was evaluated across multiple lots of TruCytes™ TBNK controls.

Figure 1 demonstrates the ability of TruCytes™ to replicate distinct populations of cells seen in whole blood: granulocytes, monocytes, and lymphocytes. As in its biological counterpart, the lymphocyte population of TruCytes™ can further be divided into subpopulations representative of B cells, NK cells, Helper T cells, and Cytotoxic T cells.

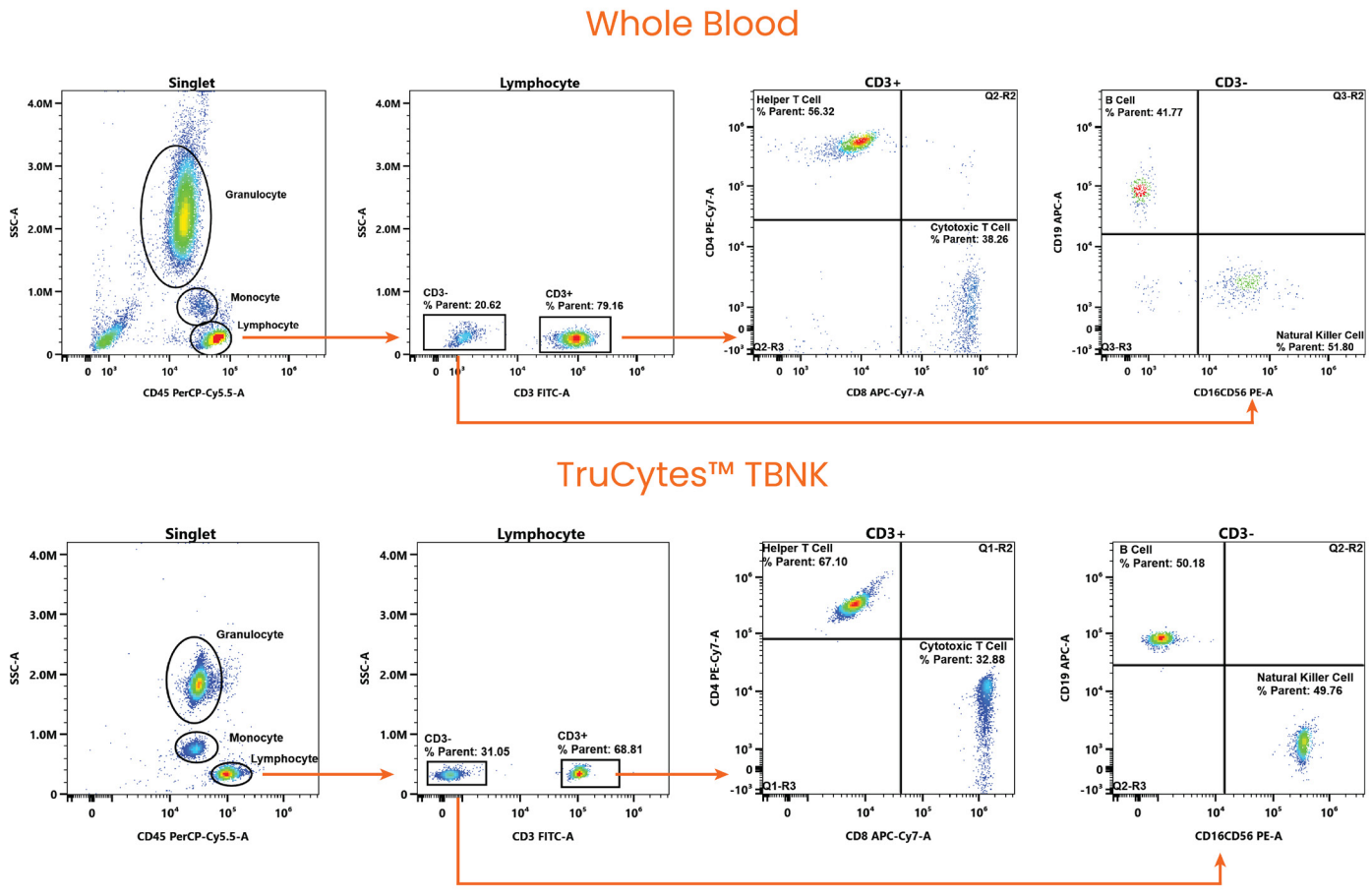

Figure 1: TruCytes™ match the scatter and biomarker profile of genuine TBNK subsets of whole blood.

TruCytes™ Individual Positive Population

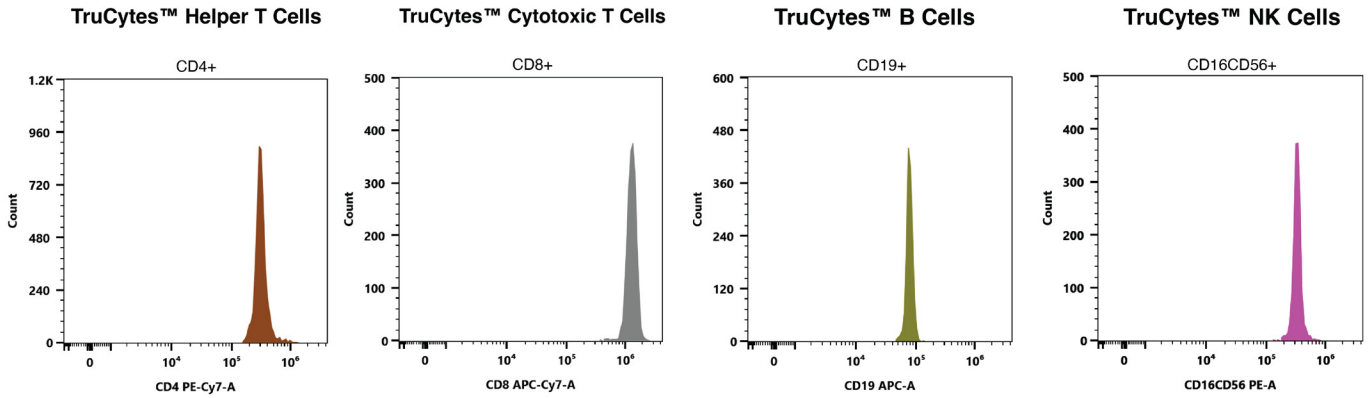

Figure 2: TruCytes™ achieve broad separation of negative and positive signals for their individual lymphocyte populations.

In Figure 2, the clean separation of signal for each individual lymphocyte mimic within TruCytes™ is shown. These synthetic lymphocytes yield consistent and bright intensities, allowing for unambiguous delineation of each immune subpopulation.

TruCytes™ TBNK Lot-to-Lot Consistency

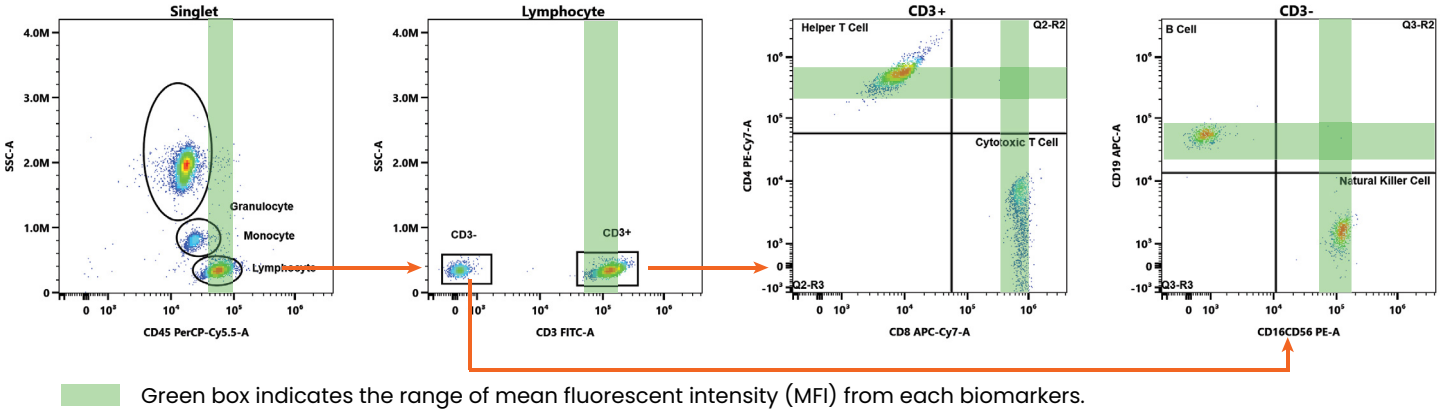

Figure 3: TruCytes™ TBNK controls exhibit minimal lot-to-lot variation in fluorescence signal

TruCytes™ TBNK controls showed remarkable consistency in mean fluorescent intensity (MFI) across lots and between multiple samples taken from the same lot. For MFI measurements taken for individual synthetic cell type populations across different lots, the CV remained below 20 percent. As shown in Table 1, for measurements taken for individual cell populations from different samples, the CV did not exceed 6 percent.

	"Granulocyte CD45"	Monocyte CD45+	Lymphocyte CD45+	T cells CD3+	Helper T CD4+	Cytotoxic T CD8+	B cell CD19+	NK cell CD16/CD56+
MFI	1.54E+04	2.18E+04	6.39E+04	1.17E+05	3.75E+05	6.30E+05	5.19E+04	9.16E+04
%CV	2.54%	1.62%	1.89%	2.42%	3.88%	2.07%	4.34%	5.03%

Table 1: TruCytes™ achieved high consistency in MFI across multiple samples from the same lot.

As shown in Figure 3, a highly reproducible fluorescent signal was achieved across multiple lots of TruCytes™ TBNK controls. For each biomarker shown, MFI remained within a tight target range. Hence, TruCytes™ allow reliable and consistent identification of desired subpopulations.

Summary

TruCytes™ are a unique alternative to traditional biological blood controls, containing synthetic granulocytes, monocytes, and lymphocytes. This reagent matches both the scatter signature and biomarker staining profile recapitulating data from genuine whole blood with distinct advantages over existing blood controls. TruCytes™ are non-biohazardous and avoid the many caveats of using donor-derived biologics. They experience lower lot-to-lot variation and facilitate compliance with both quality and regulatory programs and agencies. When tested at multiple testing sites under varying process workflows, TruCytes™ continued to perform as expected.

For either clinical testing or research studies, TruCytes™ TBNK control can be synthesized to include different ratios of each cellular sub-population, while quantitatively tuning both the internal and surface presentation of cellular markers. In order to facilitate clinical validation, one may require a greater number of a specific immune subpopulation. Biological cell enrichment procurement strategies may be both costly and time consuming, whereas generating a synthetic population is simple, cost-effective, and rapid using TruCytes™. Reporting antigen densities carries significant value in understanding the etiology of disease, developmental biology trajectories and treatment efficacies or outcomes. If sample archetypes are hard to source, perhaps the only solution would be to create one.

In short, TruCytes™ provide a cost-effective, stable, and convenient solution to sourcing whole blood cell (WBC) controls and open the doors to unprecedented avenues of clinical and research testing and discovery.

Founded in 2012, Slingshot Biosciences is a fast growing life sciences company with a platform technology and paradigm-shifting mission to make synthetic cells the gold standard for all cell-based applications. Our industry-disrupting synthetic cells erase the limitations of bio-based reference cells for diagnostics, adoptive cell therapy development and instrument calibration. Beginning with breakthrough cellular mimics called FlowCytes®, our products now include SpectraComp® for nextgen spectral compensation, TruCytes™ for biomarker mimics and ViaComp® for first-of-its-kind viability controls. Slingshot Bio is headquartered in Emeryville, California. Visit us at www.slingshotbio.com to learn more.