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Validation of a Flow Cytometric Method for Immune Cell Populations Analysis in Miniature Swine Whole Blood with 71-Hour Pre-Stain Stability

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INTRODUCTION

The increased utilization of miniature swine models as an alternative to large animals in biomedical research has underscored their relevance in drug toxicity testing, necessitating assessment of immunomodulation. Assessment of the test article's impact on immune cells via flow cytometry is important, and only limited data is available in Sinclair miniature swine.

OBJECTIVE

This study aims to develop and validate a flow cytometry method to assess immune cell populations. Subsequently, to increase the swine whole blood pre-analysis stability.

METHODS

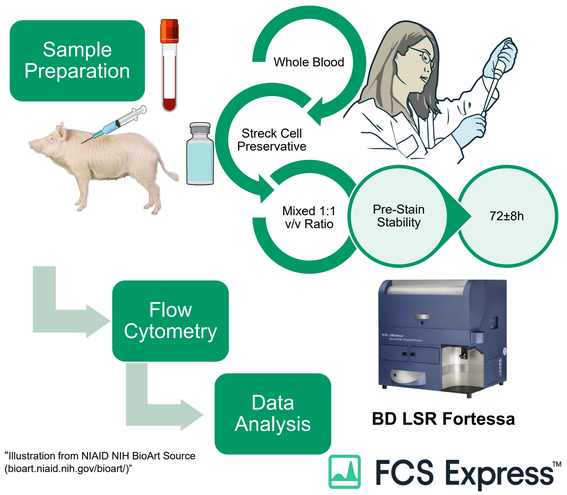
Whole blood samples were stabilized with 1:1 Streck Cell preservative and processed according to established protocols. Total, Helper, Cytotoxic, γδ T cells, B cells, and NK cells were evaluated using a BD LSR Fortessa Flow cytometer. Data analysis was performed using FCS Express software. Quantification of absolute counts was performed using a dual-platform method with the Advia 120 hematology system.

Validation following Good Laboratory Practices (GLP) focused on the following assay performance:

Table 1: Validation Parameters

Parameter Tested	Testing Information
Sensitivity (FMO/FMX)	3 animals, all Individual antibodies (FMO) FMX without CD3,CD21,CD335
Intra-Assay Precision	11 animals/3 replicates
Inter-Assay Precision	11 animals/ 3 replicates/ 2runs
Pre-Stain Stability	9 animals, Baseline, Timepoint 72±8h
Post-Stain Stability	9 animals, Baseline, Timepoint 24±4h

Flow Cytometry Analysis Workflow



"Illustration from NIAID NIH BioArt Source (bioart.niaid.nih.gov/bioart/)"

Figure 1: Pictorial representation of workflow in Flowcytometry analysis of the TBNK Panel

RESULTS

- Absolute Count (x10^3 cells/µl) at each Cell Population

Table 2: Reporting Population and Reportable Measures

Cell Population	Markers	Reportable %
$CD3^+$ T cells (Total T cells)	$CD45^+$ $CD14^-$ $CD3^+$	Relative percentage from lymphocytes
$CD4^+$ T cells (Helper T cells)	$CD45^+$ $CD14^-$ $CD3^+$ $\gamma\delta$ TCR^- $CD4^+$	Relative percentage from CD3+T cells
$CD8^+$ T cells (Cytotoxic T cells)	$CD45^+$ $CD14^-$ $CD3^+$ $\gamma\delta$ TCR^- $CD8^+$	Relative percentage from CD3+T cells
$CD21^+$ B cells (B cells)	$CD45^+$ $CD14^-$ $CD21^+$	Relative percentage from lymphocytes
$CD335^+$ NK cells (NK cells)	$CD45^+$ $CD14^-$ $CD3^-$ $CD21^-$ $CD335^+$	Relative percentage from lymphocytes
gdTCR $^+$ T cells (γδ T cells)	$CD45^+$ $CD14^-$ $CD3^+$ $\gamma\delta$ TCR^+	Relative percentage from CD3+T cells

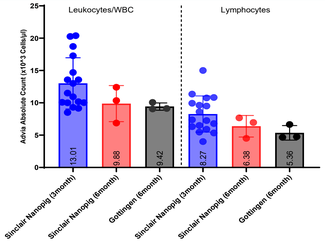


Figure 2: Comparison of Advia count between Sinclair Nanopig® vs. Gottingen; *Data Expressed as mean±SD*

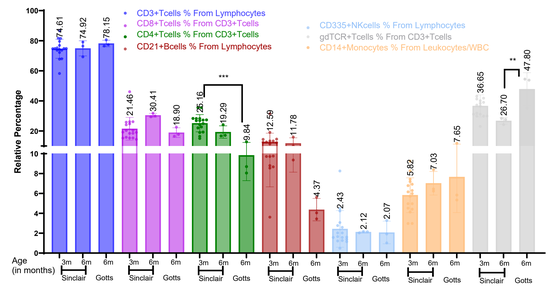


Figure 3: Relative Percentage: Sinclair Nanopig® vs. Gottingen; *Data Expressed as mean±SD One-Way ANOVA*

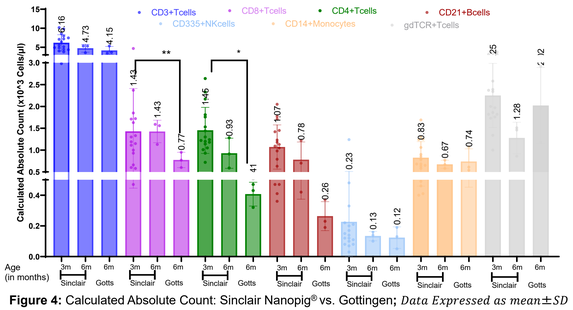


Figure 4: Calculated Absolute Count: Sinclair Nanopig® vs. Gottingen; *Data Expressed as mean±SD One-Way ANOVA*

Validation

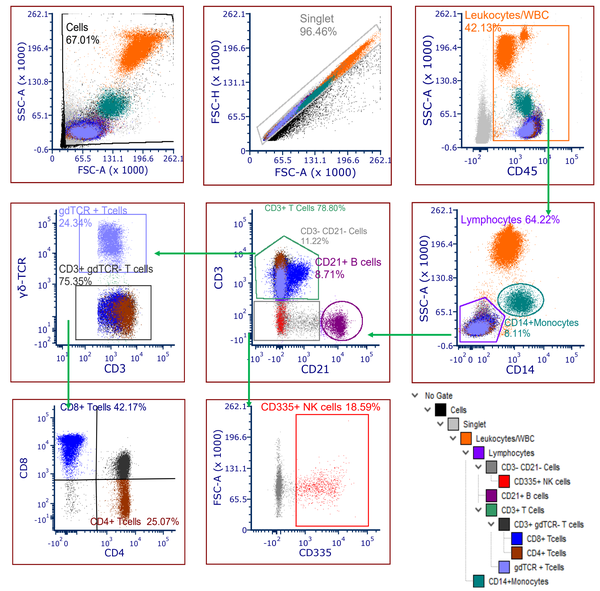


Figure 5: Gating Strategy used for analysis in FCS Express; CD14 monocytes are not reportable (NR) and only used for gating purposes

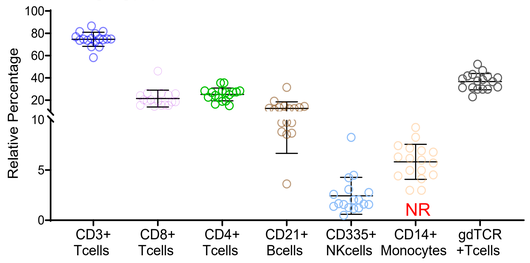


Figure 6: Inter-individual variability with respect to the Relative percentage in Sinclair Nanopig®

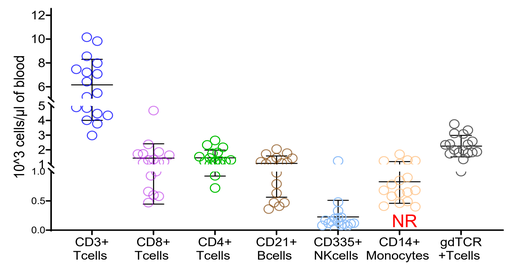


Figure 7: Inter-Individual variability with respective to calculated absolute count in Sinclair Nanopig®

Validation Summary

Table 3: Validation Summary of All Parameter Evaluated

Validation Parameter	Acceptance Criteria	Results
Species/Strain	-	Sus Scrofa/ Sinclair Nanopig®
Stabilized Sample Reception	-	Matrix: Whole blood collected in K2EDTA tubes. Stabilized Sample: Whole blood matrix mixed with Streck Cell preservative (SCP) (1:1 Ratio, v/v). Mixed within **1 hour and 30 minutes** from the time of collection.
Sensitivity (FMO/FMX)	-	Range: 0.01-0.67 % and 0.01-0.06 x10^3 cells/µl for all populations.
Intra-Assay Precision	<25% CV, 66.7% of animals	Met the % CV in the entire population, and 100% animals met the acceptance criteria.
Inter-Assay Precision	<20% (% Difference)	Met the % difference in the entire population, and 100% animals met the acceptance criteria.
Pre-Stain Stability	<20% (% Difference)	Met acceptance criteria, stability was established for all reporting cell populations until 71 hours **in a refrigerator** set to maintain 4 °C.
Post-Stain Stability	<20% (% Difference)	Met acceptance criteria, Stability was established for **24 hours, with all reporting cell populations, except CD14 monocytes,** stored in a refrigerator set to maintain 4 °C.

CONCLUSION

This validated panel is suitable to support GLP studies for reporting relative frequency and absolute counts of CD3+ T cells (Total T cells), CD4+ T cells (Helper T cells), CD8+ T cells (Cytotoxic T cells), CD21+ B Cells (B cells), and CD335+ NK Cells (NK Cells) and γδ TCR+ T cells (γδ T cells) in stabilized samples prepared from porcine whole blood which has a 71-hour stability using BD LSR Fortessa flow cytometer.

This study provides a reliable immunophenotyping method for toxicological testing in Sinclair miniature swine models and validated the extended stability of samples, which ultimately facilitates batch processing of samples, optimizing resource utilization, and reducing costs for both GLP and non-GLP studies.