VALIDATION OF FLOW CYTOMETRY METHODS FOR USE WITH ENGRAFTED HUMANIZED MOUSE SPECIMENS

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ABSTRACT

Background: Mice engrafted with functional human cells, i.e. humanized mice, are useful for addressing critical questions that are not possible to examine in patients and otherwise limited to ex vivo or non-invasive characterization. Humanized mice provide a robust platform for *in vivo* investigation of the effectiveness of potential new drugs in modulating the immune system. However, there are limitations that are continuously being addressed, such as, a standardized method for engraftment, the optimal recipient strain and potential limited access to humanized mice samples by test sites performing assay qualification/validation. In this study, we used mouse whole blood that has been mixed with CD-Chex Plus® as a surrogate for humanized mouse specimens for validation of the flow cytometry methods. Using this validated method, we then compared the expression of several immune cell populations in engrafted humanized mouse whole blood samples using flow cytometry. **Methods:** Whole blood specimens from three humanized mice were obtained from The Jackson Laboratory. Whole blood specimens from three normal mice were also obtained from commercial vendors which were mixed with equal volume of CD-Chex Plus® (Streck) human control cells. Specimens were added to fluorochrome-conjugated monoclonal surface antibodies and prepared for flow cytometry staining. The following markers were tested: anti-human CD3, anti-human CD4, anti-human CD8, anti-human CD16, anti-human CD45 and anti-mouse CD45. Samples were analyzed using a BD FACSCanto™II flow cytometer equipped with BD FACSDiva™ software. To test stability, the samples were stored at 2-8°C. Flow cytometry staining was repeated after 24 and 48 hours. **Results:** The results show that both models were able to differentiate human and mouse immune cell expression. These results suggest that this strategy may be used for qualification/validation of many flow cytometry assay parameters prior to assessment of engrafted humanized mouse study specimens.
Conclusion: Using flow cytometry, humanized mice samples can be carefully examined and human immune cells can be effectively quantified. Additionally, investigations that require validation such as antibody titration, sample stability or optimization of flow cytometry staining protocols can benefit from using mouse blood "spiked" with human control cells such as CD-Chex Plus® to mimic engrafted humanized mouse specimens.

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INTRODUCTION

A "humanized" mouse is defined as a mouse engrafted with functioning human genes, cells, tissues, and/or organs. Immunodeficient mice are often used as recipients for these human cells because they can be manipulated to accept heterologous cells due to severely compromised host immunity. Thus, humanized mice are a robust small animal model for testing human therapeutics and represent a potent tool in both basic, preclinical and clinical research.

Flow cytometry is often used as a technology for the characterization of the presence of humanized cells in a mouse background. In order to be confident in the fit for purpose application on actual study specimens, flow cytometry assays often require qualification or validation. However, the specialized production of humanized mice limits the broad commercial availability of humanized blood and tissues for the purposes of analytical assay development.

The purpose of this study was to evaluate mouse whole blood that was mixed with human whole blood (either from normal donors or CD-Chex Plus, a commercially available stabilized blood control) as a surrogate for humanized mouse specimens for assay development and validation. Using the same flow cytometry method, comparison of the expression of the same immune cell populations in engrafted humanized mouse whole blood samples was also conducted.

MATERIALS AND METHODS

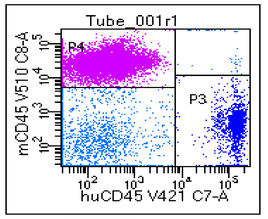
Whole blood specimens from three humanized mice were obtained from The Jackson Laboratory. Whole blood specimens from three normal mice were also obtained (Bioreclamation) which were mixed with equal volume of CD-Chex Plus® (Streck) human control cells or human whole blood from normal donors. Specimens were added to fluorochrome-conjugated monoclonal surface antibodies and prepared for flow cytometry staining. The following markers were tested; anti-human CD3, anti-human CD4, anti-human CD8, anti-human CD16, anti-human CD45 and anti-mouse CD45. Samples were analyzed using BD FACSCanto™II flow cytometer equipped with BD FACSDiva™ software. To test stability, the samples were stored at 2-8°C. Flow cytometry staining was repeated after 24 and 48 hours.

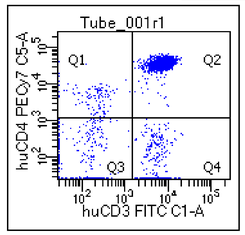
RESULTS

Expression of hCD45, hCD3, hCD4 and hCD8 Markers by flow cytometry

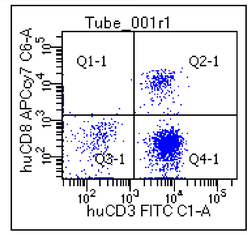
Normal mouse whole blood "spiked" with human cells

Mouse and Human Total Lymphocytes





Human T Helper Cells

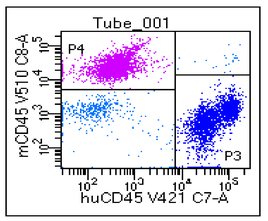


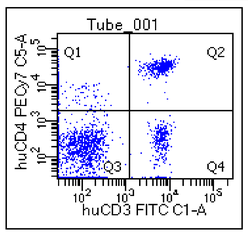
Human Cytotoxic T Cells

Figure 1. Evaluation of the human lymphocytes and human T-cells in mouse whole blood "spiked" with human cells

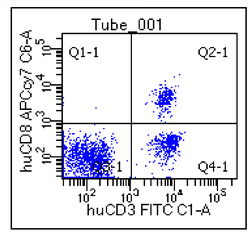
Humanized mouse whole blood

Mouse and Human Total Lymphocytes





Human T Helper Cells



Human Cytotoxic T Cells

Figure 2. Evaluation of the human lymphocytes and human T-cells in humanized mouse whole blood

RESULTS (cont.)

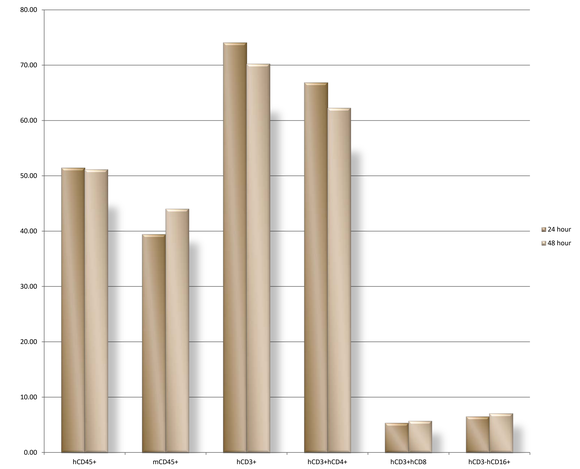


Table 1. Relative percent stability of mouse whole blood "spiked" with human cells 24 hours and 48 hours post draw.

CONCLUSIONS

- Flow cytometry assay development/validation can benefit from using mouse blood "spiked" with human cells to mimic engrafted humanized mouse specimens.
- Although we demonstrated comparable results with highly expressed and well characterized cell populations, each cell type marker of interest will require testing to determine if this type of control is appropriate.
- Mouse blood "spiked" with human cells showed similar lymphocyte, T-cell and NK cell relative percentages up to 48 hours post draw.

REFERENCES

Shultz, LD, Brehm, MA, Garcia-Martinez, JV, and Greiner, DL (2012). Humanized mice for immune system investigation: progress, promise and challenges. Nature Reviews Immunology 12, 786-798.

ACKNOWLEDGEMENTS

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