Poster Number: Day2-P21

METHOD QUALIFICATION FOR IMMUNOPHENOTYPING OF CD137+ T CELL SUBSETS IN HUMAN WHOLE BLOOD BY FLOW CYTOMETRY USING CONTRIVED SAMPLES

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NOVEL ASPECTS

- Contrived sample mimics the intend-use sample and are crucial for bioanalytical method validation/qualification in supporting clinical study.
- A contrived sample, prepared by spiking in-vitro stimulated PMBCs into whole blood samples, was used to qualify an Immunophenotyping FACS Method.
- This assay allows detection of CD137+ T cell subsets with high precision, accuracy and sensitivity, and offers up to 72 hours of contrived sample stability and post-processing stability.

INTRODUCTION

Validation of bioanalytical methods must be conducted in the intended-use samples. Contrived or surrogate material must be used when true samples are not available when assay performance is established. CD137 is a marker of T cell activation, commonly used to identify tumor-reactive T cells following cancer immunotherapies. Since CD137+ cells are absent in normal donors, a contrived sample must be engineered for assay qualification, with its fit-for-purpose performance demonstrated before clinical sample analysis. In this poster, we present a qualified flow cytometry method utilizing a contrived sample, created by spiking in-vitro stimulated PBMCs into blood, for measuring CD137+ cells in whole blood, supporting future immuno-oncology drug development efforts.

METHODS

This Method is to determine the CD4+ and CD8+ of CD3+ cell percentage and CD137+ of CD4+ amd CD8+ cell percentage in human whole blood. Fresh blood samples and frozen PBMC samples are obtained from healthy donors. PBMC are stimulated by overnight incubation in R10 medium with anti-human CD3 and CD28 antibodies to induce CD137 expression on CD4+ and CD8+ T cells. Activated PBMCs are then mixed with whole blood.

Samples are stained with antibody cocktail, treated with FACS lysing solution, washed multiple times and resuspendend in FACS staning buffer before being analysed on a BD LSR Fortessa X20. Raw Data is captured by FACS Divand analyzed using FlowJo.

Figure 1. Assay procedure, contrived sample preparation and analysis

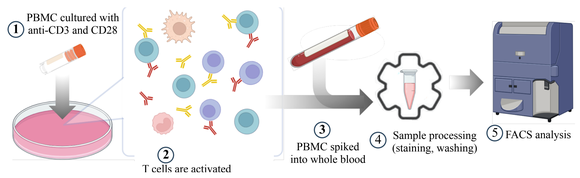


Figure 2: FACS staning panel for CD137+ T cell subset quantification

Population	CD45	CD3	CD4	CD8	CD137
T cell	+	+	+	+	-
Helper T cell	+	+	+	-	-
Cytotoxic T cell	+	+	-	+	-
Activated Helper T cell	+	+	+	-	+
Activated Cytotoxic T cell	+	+	-	+	+

ASSAY QUALIFICATION OVERVIEW

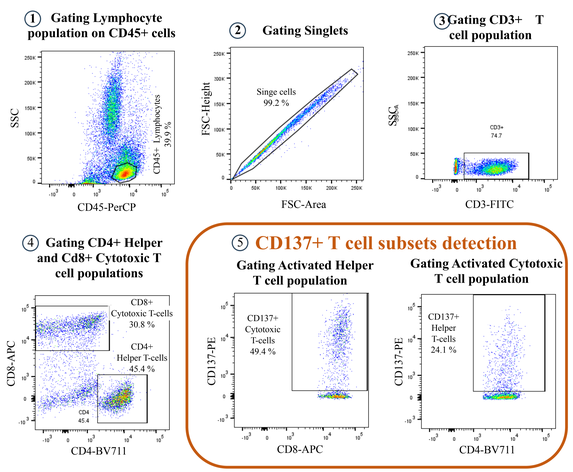
Figure 3: Assay development and qualification overview

Following panel setup, the following parameters of the Method for FACS quantification of CD137+ T cell subsets were evaluated:

- Intra-assay precision
- Limit of detection
- Contrived sample stability
- Post procesing stability
- Limit of quantification
- Accuracy

RESULTS

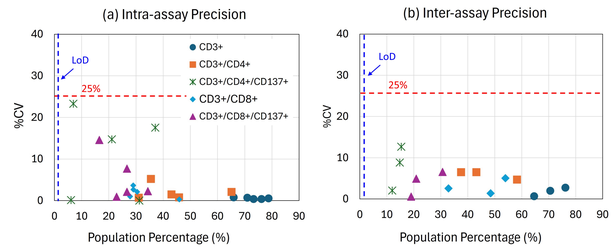
Gating strategy: identification of CD137+ T cell subsets



Assay precision and limit of detection

Intra assay precision was assessed for all population readouts in whole blood from 5 heathy donors, unspiked or spiked with activated PBMCs. Intra-assay precision, inter-assay precision and inter-operator precision passed %CV ≤ 25% criteria for all samples and readouts, with a limit of detection established at 1.10% and 1.28% for the CD4+/CD137+ and CD8+/CD137+ population, respectively. The LoD were calculated using 10 determinations total from 5 donors over 2 analytical runs, as Mean + 3*SD, ensuring that 99% of negative samples will generate results ≤ LoD.

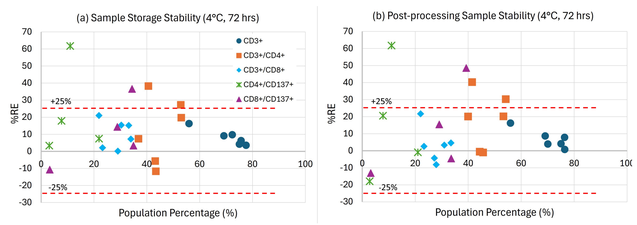
Figure 4: Intra/Inter-assay precision and LoD



Sample stability and post-processing stability

Sample stability and post-processing stability was assessed for all populations in whole blood at 4 oC from 3 heathy donors, unspiked or spiked with activated PBMCs to reach CD137+ at low (~3%) and high (~34%) levels. Criteria for %change from fresh ≤ 25% met in 2/3 donors, for all samples and readouts above limit of qualification for 72 hours. Sample stability will be further reassessed when clinical study samples are available.

Figure 6: Sample storage and post-processing stability at 4 °C for 72 hours.

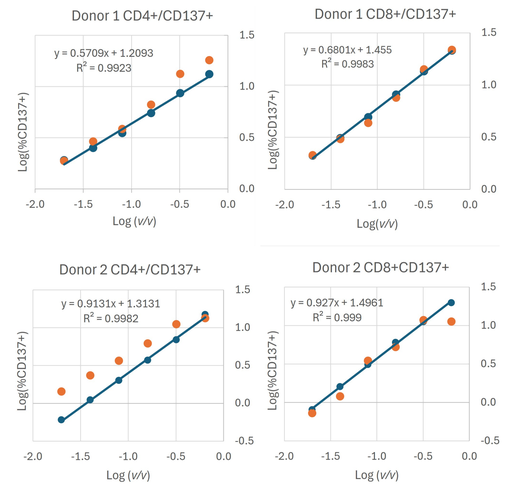


Lower limit of quatification and accuracy

LLoQ was determined by spiking known amount of serially diluted activated PBMC in whole blood from 2 healty donors. At the highest dilution where the lowest CD137+ percentage meets precision %CV ≤ 25% was reported as the LLoQ. The LLoQ was established at 2.4% for the CD4+/CD137+ population and 2.5% for the CD8+/CD137+ population.

Accuracy was demonstrated by the CD137+ in CD3+ subets in a range of 1.9% to 15.7% with %RE ≤ 30% using the contrived samples from 2 healthy donnors, where the activated PBMC spiked percentages were calculated and used s the nominal value. Same data for the assay LLoQ were used.

Figure 5: Assay LLoQ and accuracy



CONCLUSION

This study successfully qualified a flow cytometry assay for detecting CD137+ T-cells subsets in human whole blood using a contrived sample approach. By engineering a biologically relevant surrogate sample, we demonstrated the method's accuracy, precision, sensitivity, and sample stability, ensuring its suitability for future clinical sample analysis.

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