Optimizing Cell Viability in Cell Therapy: A Comparative Study of Whole Blood Sample Stabilization Techniques

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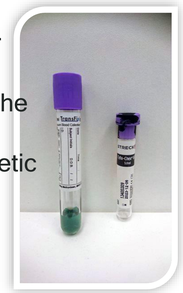
INTRODUCTION



Flow cytometry is a cornerstone in bioanalytical research, enabling detailed cell analysis crucial for immunophenotyping, disease diagnosis, and monitoring. With the emergence of cell therapies, the role of sample stability has become increasingly critical, as these cells are integral for pharmacokinetic and cell-kinetic measurements in clinical trials. There's a heightened demand for effective sample preservation methods which is essential for maintaining the precision of flow cytometry results across clinical sites and phases.

Flow Cytometry's Role: Essential for immunophenotyping and understanding cell functions, morphology, and cellular kinetics.

Challenge of Sample Stability: Maintaining cell marker integrity is crucial for accurate analysis but is threatened by factors like time and storage conditions.

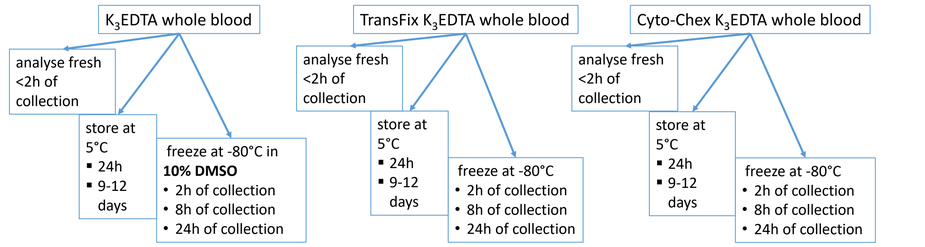
Importance in Cell Therapies: As cell therapies emerge, particularly CAR-T and CAR-NK treatments, precise cell analysis becomes crucial for therapy development and clinical outcomes.

Clinical Relevance: Samples now serve as critical endpoints in clinical trials, making their stability paramount for the reliable evaluation of therapeutic efficacy.

The intersection of flow cytometry and cell therapy development highlights a growing demand for improved sample stability measures, guiding our investigation into effective preservation strategies.

METHOD AND RESULTS

BD™ Multi-Check Control and CD4 Low Control with BD Multitest™ BD 6-color TBNK kit reagent in BD Trucount™ tubes and analyzed on two BD LSRFortessa™ flow cytometer high throughput sampler (HTS) systems.



	Relative Accuracy to Fresh Non-Stabilized Samples	Freeze-Thaw Stability	Ease of Gating	Suitability for Clinical Collection
10% DMSO	All cell types pass when frozen within 2-8h of collection	All cell types pass at 2xFT when frozen within 2-8h of collection	Similar to fresh non-stabilized	• 10% DMSO has to be manually added to a defined volume of whole blood (or vice versa) • **Speed of freezing not critical within ~2h of DMSO addition** • Cheapest option
TransFix	Baseline NK enumeration not accurate	• NK cells fail at 2xFT when frozen within 2h of collection • All cell types fail at 2xFT when frozen within 8h of collection	NK gating more challenging	• Direct collection • Total volume needs to be estimated to calculate the correct dilution factor • Underfilling can lead to inaccurate results
Cyto-Chex	• All cell types pass when frozen within 2h of collection • NK cells fail when frozen within 8h of collection	NK cells fail at 2xFT when frozen within 2-8h of collection, other cell types borderline pass	NK gating very challenging	• Direct collection • Underfilling leads to inaccurate results?
Best Choice	**10% DMSO**	**10% DMSO**	**10% DMSO**	**10% DMSO**

ASSAY VALIDATION SUMMARY

Parameter	Assessment	Acceptance criteria	Comments	Result
Overall run acceptance criteria	2 sets of 2 commercials QCs per run	% lymphocytes: 100% must be within the accepted ranges cells/µL: 50% per cell type and 80% overall must be within the accepted ranges	QC accepted ranges: • Assess in 4 precision runs over 2 days • If mean results outside the vendor ranges, calculate new ranges based on experimental mean + 3xSD	All QC precision results within vendor ranges
Intra-assay and inter-assay precision	At least 5 healthy donors analysed in triplicate in 4 runs by 2 analysts over at least 2 days	≤20% CV for all results within and overall runs	Add-on validation of precision and stability in the respective diseased samples should be performed prior to analysing diseased samples	<12% CV **pass**
Instrument carryover	Sequential analysis of validation samples or QCs and «blank» buffer samples Data from the blank samples evaluated in the same gating template as the validation/QC samples.	<20% of the $CD3^+CD4^+$ cells/µL of the BD Multi-Check CD4 Low Control		0.6 cells/µL **pass**
Long-term stability	At least 5 healthy donors analysed at baseline (within 2h of collection without stabilizer) and at various timepoints (1 month, 3 months, 6 months) in triplicate	80% of the results per cell type are within ±20% change of the baseline	Freshly collected baseline samples processed in an independent run	Assessment ongoing **1 month pass**
Short-term & freeze-thaw stability	At least 5 healthy donors analysed at baseline (within 2h of collection without stabilizer) and at least 2x freeze-thaw cycles in triplicate	80% of the results per cell type are within ±20% change of the baseline	Same baseline samples as for the long-term stability	3x FT **pass**
Post-staining/ processed sample stability	At least 5 healthy donors and 2 QCs analyzed immediately after processing (within 1h of staining) and at various timepoints after storage at 5°C (e.g. 6h, 16h, 24h)	80% of the results per cell type are within ±20% change of the baseline	Precision runs used as the baseline	up to 24h **pass**

CONCLUSION

Our investigation into sample stability for flow cytometry assays, focusing on immunophenotyping across various stabilizers and conditions, reveals significant insights and practical guidelines for both research and clinical applications. The findings are particularly relevant in the realm of advanced cell therapies, such as CAR-T and CAR-NK, where accurate cell characterization is critical. Here are the key conclusions and critical considerations:

1. **Effective Stabilization**: 10% DMSO has shown to be an effective stabilizer across diverse conditions, ensuring robust sample integrity for accurate flow cytometry analysis. This finding is pivotal for the reliable evaluation of therapeutic cell products.
2. **Importance in Cell Therapies**: The emergence of cell therapies accentuates the need for sample stability assessments. Our results directly contribute to improving the quality control of such therapies, where cells are not only therapeutic agents but also critical endpoints in pharmacokinetic/cell-kinetic studies.
3. **Impact on Clinical Studies**: The integrity of samples in flow cytometry has gained unprecedented importance with the samples now serving as pharmacokinetic and cell-kinetic endpoints in clinical studies of cell therapies. Ensuring stability is paramount for the accurate interpretation of clinical outcomes and the efficacy of therapies.
4. **Adaptation of Gating Strategies**: The influence of stabilizers on cell marker integrity necessitates the adaptation of gating strategies in flow cytometry. This is crucial for maintaining accuracy in cell enumeration and characterization, especially in complex therapeutic contexts.
5. **Context-Specific Selection**: The choice of stabilizer must be tailored to the specific needs of the sample and the context of its use. Factors like cell type, expected storage duration, and temperature conditions play a crucial role in this decision-making process.
6. **Broadening Clinical Applications**: These findings not only bolster the reliability of flow cytometry in research but also enhance its application in clinical settings, providing a solid foundation for the development and validation of cell-based therapies.

This study not only advances our understanding of sample stability in flow cytometry but also underscores the critical role of precise sample preparation in the success of innovative cell therapies. As the field of cell therapy continues to evolve, the insights gained here will be invaluable for ensuring the accuracy and reliability of cell-based assays, ultimately contributing to better patient outcomes.