Validation of a Flow Cytometry Method for CAR■ Cell Frequency in CAR-T Drug Product

VR-QC-0207 · v1.0 (DRAFT) · Method MOA-FLOW-014 · CAR-T Drug Product · ICH Q2(R2); GMP / 21 CFR 211; data integrity per ALCOA+

1. Scope

Validation of a flow cytometry method for determining %CAR■ (of viable CD3■) in CAR-T drug product, to support in-process and lot-release testing. Covers accuracy, precision, linearity, range, specificity, and robustness.

2. Method Summary

Cells are stained for viability (7-AAD), CD3, and CAR, acquired on a qualified flow cytometer, and gated (Live → CD3■ → CAR■) using a locked template. %CAR■ of viable CD3■ is the reportable result.

3. Validation Results vs. Acceptance Criteria

Parameter	Acceptance Criterion	Result	Verdict
Accuracy (% recovery)	90–110%	98.4% (mean, n=9)	Pass
Repeatability (intra-assay %CV)	≤ 10%	4.2%	Pass
Intermediate precision (%CV)	≤ 15%	7.8%	Pass
Linearity (R^2)	≥ 0.98	0.996	Pass
Range	10–60% CAR■	Confirmed 12–58%	Pass
Specificity	No signal in CAR-negative control	0.14% (below LOD)	Pass
LOD / LOQ	LOQ ≤ 5% CAR■	LOQ 3.1% CAR■	Pass
Robustness (stain time ±5 min)	≤ 10% change	2.9% change	Pass

4. Conclusion

The method meets all predefined acceptance criteria across the validated parameters and is considered suitable for its intended use in in-process and lot-release testing of %CAR■, pending QA approval. *(final suitability determination pending QA approval)*

5. References

1. Validation Protocol [protocol number]
2. Method MOA-FLOW-014 [current version]
3. Instrument Qualification Record [IQ/OQ/PQ ref]
4. Data Integrity Policy [policy number]

QC Review Record

#	Category	Review Item	Status
1	Completeness	All planned validation parameters from the protocol are reported.	PASS
2	Traceability	Report references the approved protocol, method version, and instrument qualification.	REVIEW
3	Results Integrity	Each result is compared to its predefined acceptance criterion with an explicit verdict.	PASS
4	Human Decision Required	Confirm raw data, calculations, and statistics against source (independent verification).	HUMAN DECISION
5	Human Decision Required	Confirm acceptance criteria match the approved validation protocol exactly.	HUMAN DECISION
6	Human Decision Required	Approve the overall suitability conclusion (validated / not validated).	HUMAN DECISION
7	Regulatory	Confirm alignment with the applicable guideline (e.g., ICH Q2(R2)) for the claimed parameters.	HUMAN DECISION

Status: DRAFT — NOT approved. Human-decision items outstanding.
Reviewed by: Kalif Shear **Date:** _____

Drafted by an AI-assisted, human-in-the-loop workflow (ValiForge). Not a valid validation record until results are verified against source data and the report is completed and approved by qualified QA/QC. The validated/not-validated conclusion is a human determination.