

Standardizing Flow Cytometry QC to Enable Global Expansion

Synthetic Cell Mimics Enable Site-Independent Analytical Comparability

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Background & Rationale

As cell therapy manufacturing expands across geographically distributed facilities, analytical comparability becomes a prerequisite for scale. Flow cytometry is a foundational QC method for cell therapy characterization, yet the industry continues to rely on donor-derived PBMCs as reference controls. These materials inherently vary in cell subset frequencies, marker expression, and population distributions from donor to donor.

This biological variability makes it difficult to distinguish true method or instrument performance from donor-driven noise, complicating site-to-site comparisons, extending validation timelines, and increasing operator dependency during method transfer.

Biological QC controls introduce variability that slows technology transfer and increases regulatory risk in multi-site manufacturing

As Cellares expands from its California operations to a global network of Smart Factories in New Jersey, Europe, and Asia, analytical methods must transfer rapidly and reproducibly between sites. This requires QC reference materials that:

- Are independent of donor availability and biology
- Enable direct site-to-site comparison
- Support regulator expectations for analytical similarity

Without standardized controls, QC becomes a bottleneck that limits the speed and reliability of global manufacturing expansion.

Global Smart Factory deployment requires standardized, reliable, site-independent QC controls

TruCytes™ Lymphocyte Subsets (synthetic controls developed by Slingshot Biosciences) are polymer-based cell mimics engineered to provide defined scatter properties, controlled antigen density, and stable subset ratios. Unlike donor PBMCs, these materials are manufactured to defined specifications with certified acceptance criteria and lot-to-lot consistency.

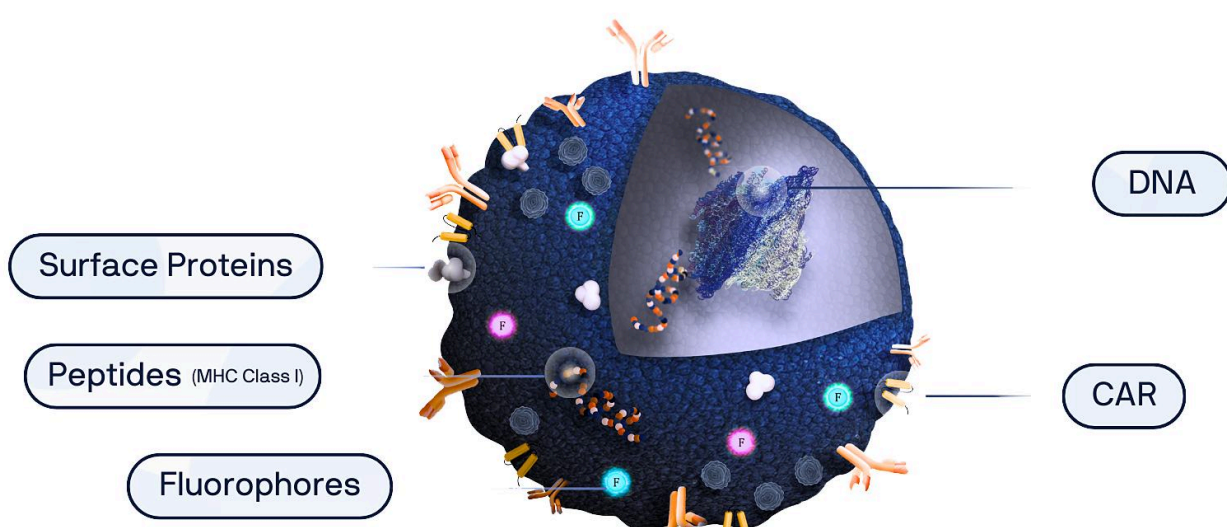


Figure 1. Anatomy of a TruCyte Cell Mimic

By design, cell mimics eliminate biological heterogeneity, allowing QC workflows to focus on what truly matters: instrument performance, method robustness, and operator consistency.

Engineered cell mimics replace biological variability with manufacturer-certified, reproducible QC standards

Objectives & Study Design

Establish a site-ready framework for cross-site analytical comparability in flow cytometry QC by evaluating polymer-based cell mimics (Slingshot Biosciences' TruCytes™ Lymphocyte Subset) as standardized controls across reagent lots, operators, days, and instruments, supporting technology transfer for multi-site cell therapy manufacturing.

Variable Tested	Instrument		Experimental Day		New TruCytes Lot		Laboratory Site	
Instrument	Cytek 1	Cytek 2	Cytek 2	Cytek 2	Cytek 1	Cytek 2	Cytek 1	Cytek 3
Material	TruCytes Lot 1	TruCytes Lot 1	TruCytes Lot 1	TruCytes Lot 1	TruCytes Lot 2	TruCytes Lot 2	TruCytes Lot 1	TruCytes Lot 1
Experimental Day	Day 1	Day 1	Day 125	Day 140	Day 1	Day 7	Day 140	Day 141
Scientists	Scientist 1	Scientist 1	Scientist 3	Scientist 3	Scientist 2	Scientist 2	Scientist 3	Scientist 3

Figure 2. Study design matrix for multi-factor comparability testing. Slingshot Biosciences' TruCytes™ Lymphocyte Subset cell mimics were tested across 2 reagent lots, 3 operators, multiple experiment days, and 3 Cytek Northern Lights flow cytometers in different locations to evaluate lot-to-lot consistency and inter-operator/inter-instrument reproducibility.

Results

Cell mimics reduce donor PBMC-driven noise from up to 62% to single-digit

Subset	TruCyte Lymphocyte Panel			Subset	Donor PBMC	
	Reported % Population	Avg. Measured Population	% Difference		Historical %CV (published)	Measured %CV (in-house)
Granulocyte	5%	4.99%	0.2%	CD3+CD8+ (T Cytotoxic)	22%	42%
Monocyte	14%	14.79%	5.5%	CD3+CD4+ (T Helper)	14%	52%
Lymphocyte	80%	77.88%	2.7%	CD45+CD19+ (B Cell)	39%	13%
CD3+CD8+ (T Cytotoxic)	32%	32.17%	0.5%	CD45+CD14+ (Classical Mono)	18%	21%
CD3+CD4+ (T Helper)	68%	66.90%	1.6%	CD16+CD56+ (NK Cell)	42%	62%
CD45+CD19+ (B Cell)	14%	15.20%	8.2%			
CD45+CD14+ (Classical Mono)	100%	99.11%	0.9%			
CD16+CD56+ (NK Cell)	99%	99.11%	0.1%			

Across two certified TruCytes reagent lots, measured differences across TBMNK lymphocyte populations (i.e., CD3 and CD4/8 T cells, CD19 B cells, CD14 Monocytes, CD16/CD56 NK cells) compared to manufacturer reported values were less than 8.2%. In contrast, healthy donor PBMC controls showed substantial deviations from each other, with variation as high as 62%.

This reduction in variability removes a primary confounding factor in analytical comparability studies and enables meaningful performance comparisons across sites and time.

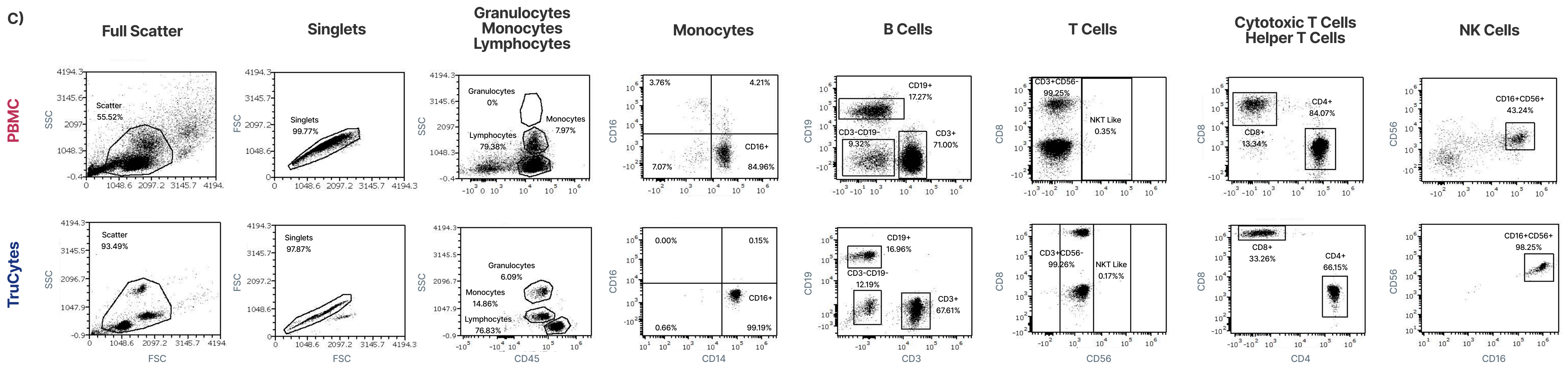


Figure 3. (A) Percent (%) difference between reported vs. measured TruCytes Lymphocyte Subset populations for 2 reagent lots. Color coding indicates performance levels: green ($\leq 5\%$ difference), yellow (5–10% difference), and red ($>10\%$ difference). (B) Coefficient of variation (%CV) of TBMNK sub-populations in cryopreserved PBMC compared to published data. (C) Representative flow plots for TruCytes Lymphocyte Subsets and PBMC.

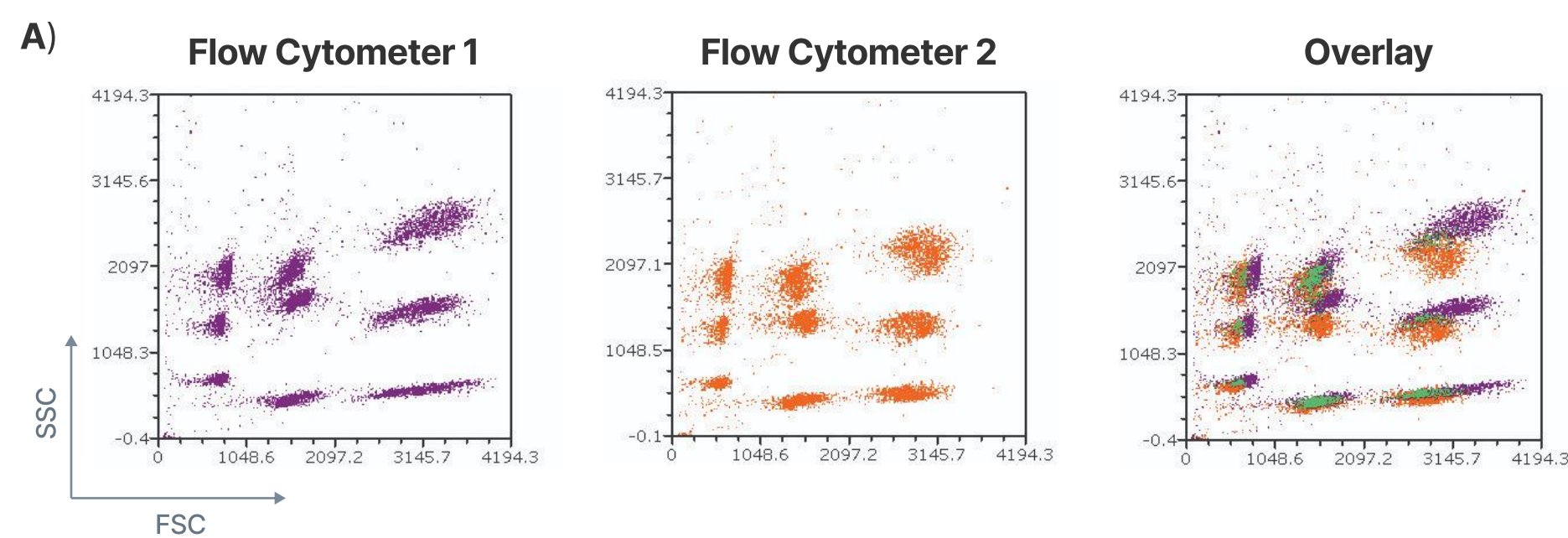
Cell mimics deliver operator-, instrument-, and laboratory-independent accuracy aligned to manufacturer specifications

Intermediate precision testing of two TruCyte Lymphocyte Panel lots across 3 instruments achieved coefficients of variation ranging from 0% to 10% for all lymphocyte subsets.

Inter-laboratory comparison between Quality Control and Analytical Development laboratories showed differences ranging from 0.2% to 15.2% across all lymphocyte populations.

This level of reproducibility confirms that synthetic controls enable standardized gating strategies and minimize operator subjectivity, a critical requirement for scalable QC operations.

Figure 5. (A) ScatterBridge™ calibration reagent confirms instrument-to-instrument equivalence. (B) Intermediate precision and inter-laboratory reproducibility of TruCyte Lymphocyte Panels. Coefficient of variation (%CV) for intermediate precision testing across two TruCyte reagent lots, alongside percentage differences between Quality Control (QC) and Analytical Development (AD) laboratory measurements. Color coding indicates performance levels: green ($\leq 5\%$ difference), yellow (5–10% difference), and red ($>10\%$ difference).



Subset	Intermediate Precision %CV		% Difference
	TruCyte Lot 1	TruCyte Lot 2	QC vs. AD
Granulocyte	10%	3%	3.5%
Monocyte	6%	1%	1.2%
Lymphocyte	2%	0%	1.6%
CD3+CD8+ (T Cytotoxic)	10%	1%	15.2%
CD3+CD4+ (T Helper)	5%	0%	6.9%
CD45+CD19+ (B Cell)	5%	3%	6.4%
CD45+CD14+ (Classical Mono)	1%	0%	0.8%
CD16+CD56+ (NK Cell)	0%	0%	0.2%

Conclusions

Synthetic cell mimics developed by Slingshot Biosciences replace donor-driven variability with standardized, traceable QC controls, enabling reliable site-to-site comparability at scale.

Across multiple laboratories, operators, instruments, and days, cell mimics delivered single-digit variability and consistent accuracy to certified specifications. By removing biological variability as a confounding factor, this approach enables operator-independent, instrument-independent and site-independent analytical performance, directly supporting global technology transfer and regulatory comparability expectations.

Acknowledgements

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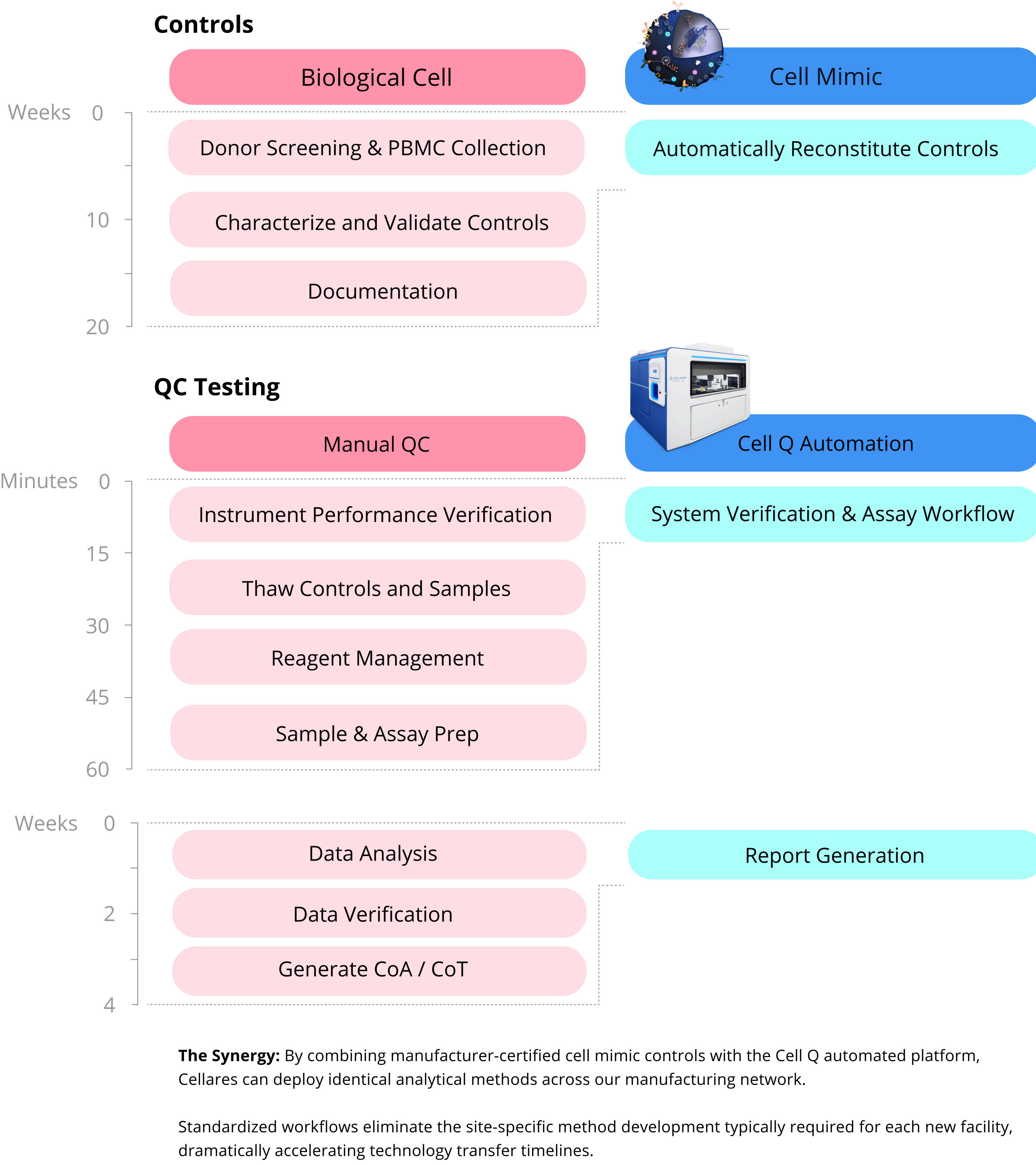
Future Directions

Paradigm Shift in Quality Control Standardization

This work validates a fundamental shift from variable biological controls to standardized synthetic alternatives for flow cytometry quality control in cell therapy manufacturing. Slingshot Biosciences' TruCytes cell mimics have demonstrated superiority as reference controls that are precise, highly reproducible, and scalable, addressing the industry's most significant analytical comparability challenge.

By combining manufacturer-certified cell mimic controls with the Cell Q™ automated platform, Cellares can deploy identical QC methods across its Smart Factory network without site-specific revalidation. This enables:

- Faster technology transfer between sites
- True site-to-site analytical comparability
- Reduced regulatory and operational risk
- Lower training and execution burden
- Daily QC checks prior to run execution
- Real-time QC monitoring
- Predictive maintenance that detect instrument drift



The Synergy: By combining manufacturer-certified cell mimic controls with the Cell Q automated platform, Cellares can deploy identical analytical methods across our manufacturing network.

Standardized workflows eliminate the site-specific method development typically required for each new facility, dramatically accelerating technology transfer timelines.

