SCE 0608 Public Evidence Appendix

EV characterization

Literature review date: September 9, 2026

Controlling principle. EV characterization is multidimensional; no single assay establishes EV identity, purity, exosome biogenesis, potency, safety, or clinical effectiveness.

Characterization map

Characterization domain	What it measures	Interpretive boundary
Particle concentration	Number of detectable particles per defined volume	Particle count does not establish how many particles are EVs or carry active cargo.
Particle size	Physical dimensions or size distribution	Size overlaps with lipoproteins, aggregates, viruses, and other nanoparticles.
Electron microscopy	Particle morphology and membrane-associated structure	Images sample a limited field and morphology is not biologically specific.
Flow cytometry	Optical/fluorescent signals and selected surface markers	Small-particle detection requires calibration and controls; marker-negative populations may be missed.
Western blotting	Selected proteins in the bulk preparation	Marker presence does not establish particle-level distribution or purity.
Proteomics	Bulk protein composition	Detected proteins may derive from EVs or co-isolated material.
Lipidomics	Bulk lipid composition	Bulk profiles do not localize lipids to individual vesicles or prove function.
RNA analysis	RNA species associated with the preparation	Association does not prove intravesicular localization, delivery, or biological activity.
Single-vesicle analysis	Properties of individual detectable particles	Reveals heterogeneity but may sample only particles within the platform's detection range.

Key evidence conclusions

Orthogonal characterization is stronger. Independent assays measuring different properties reduce dependence on the limitations of any one platform.

Bulk and single-vesicle data answer different questions. Bulk assays can hide subpopulations; single-vesicle methods reveal heterogeneity but have detection limits.

Controls are part of the evidence. Blanks, reagent controls, calibration, reference particles, dilution series, and assay-specific controls are required for reproducible interpretation.

Clinical claims require additional evidence. Characterization supports product definition and quality assessment but does not itself establish clinical effectiveness or safety.

Principal references

1. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). J Extracell Vesicles. 2018;7:1535750.

2. Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles. 2024;13:e12404.

3. van der Pol E, Coumans FAW, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, et al. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. J Thromb Haemost. 2014;12:1182-1192.

4. Welsh JA, van der Pol E, Arkesteijn GJA, et al. MIFlowCyt-EV: a framework for standardized reporting of extracellular vesicle flow cytometry experiments. J Extracell Vesicles. 2020;9:1713526.

5. International Society for Extracellular Vesicles guidance relevant to EV characterization, assay controls, reporting, and clinical translation.