SCE 0609 Public Evidence Appendix

Purity and contaminants

Literature review date: September 9, 2026

Controlling principle. EV purity is defined by both the intended vesicles and the non-EV material that remains. Particle count, size, or positive markers alone cannot establish purity.

Contaminant map

Contaminant	Why it matters	Control/interpretive issue
Protein aggregates	Can overlap EVs in size, density, sedimentation, and light scattering and can inflate particle/protein signals.	Assess with orthogonal methods and consider source material, cell death, freeze-thaw, agitation, pH, and processing.
Lipoproteins	Chylomicrons, VLDL, LDL, and HDL overlap EV physical properties and can contribute abundant proteins and lipids.	Biofluid-derived preparations require methods capable of addressing relevant lipoprotein backgrounds.
Cell debris	Membrane fragments, organelle fragments, apoptotic material, and intracellular molecules can enter preparations after cell injury or death.	Cell health, clarification, and direct contaminant assessment are required.
Media-derived particles	Culture media, supplements, proteins, micelles, and other background particles can co-isolate.	Use media-only/process-matched controls where feasible.
Serum-derived EVs	Serum contains endogenous EVs that can be difficult to distinguish from cell-derived EVs.	EV-depleted serum reduces but may not eliminate background; serum-free or chemically defined media alter the background differently.
Viruses	Enveloped and non-enveloped viruses or virus-like particles can overlap EV properties.	Use source- and process-appropriate adventitious-agent testing rather than assuming separation removes viral risk.
Residual processing materials	PEG, gradient media, beads, filter/membrane material, extractables, and leachables may remain after processing.	Control raw materials, washing, process blanks, in-process testing, and release specifications.

Key evidence conclusions

Purity is multidimensional. Relevant contaminants must be measured directly; absence of one contaminant marker does not establish comprehensive purity.

Particle-to-protein ratio is contextual, not definitive. Both the numerator and denominator can contain non-EV material.

Process-matched controls matter. Media-only controls and process blanks help identify background introduced by culture media, reagents, equipment, and workflow.

Functional signals can be confounded. Soluble proteins, lipoproteins, residual reagents, cytokines, viruses, or other co-isolated material may contribute to measured biological activity.

Principal references

1. Théry C, et al. MISEV2018. J Extracell Vesicles. 2018;7:1535750.

2. Welsh JA, et al. MISEV2023. J Extracell Vesicles. 2024;13:e12404.

3. Karimi N, et al. Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins. Cell Mol Life Sci. 2018;75:2873-2886.

4. Jeppesen DK, et al. Reassessment of exosome composition. Cell. 2019;177:428-445.e18.

5. International Society for Extracellular Vesicles guidance relevant to purity, contaminants, controls, reporting, and clinical translation.