MISEV2018 Checklist


1-Nomenclature

Mandatory

O Generic term extracellular vesicle (EV): With demonstration of extracellular (no intact cells) and vesicular nature per these characterization (Section 4) and function (Section 5) guidelines OR
O Generic term, e.g., extracellular particle (EP): no intact cells but MISEV guidelines not satisfied Encouraged (choose one)
O Generic term extracellular vesicle(EV) + specification (size, density, other)
O Specific term for subcellular origin: e.g., ectosome, microparticle, microvesicle (from plasma membrane), exosome (from endosomes), with demonstration of the subcellular origin
O Other specific term: with definition of specific criteria

2-Collection and pre-processing

Tissue Culture Conditioned medium (CCM, Section 2-a) O General cell characterization (identity, passage, myco- plasma check…)
O Medium used before and during collection (additives, serum, other)
O exact protocol for depletion of EVs/EPs from additives in collection medium
O Nature and size of culture vessels, and volume of medium during conditioning
O Specific culture conditions (treatment, % O2, coating, polarization…) before and during collection
O Number of cells/ml or /surface area and % of live/ dead cells at time of collection (or at time of seeding with estimation at time of collection)
O Frequency and interval of CM harvest

Biofluids or Tissues (Sections 2-b and -c)
O Donor status if available (age, sex, food/water intake, collection time, disease, medication, other)
O Volume of biofluid or volume/mass of tissue sample collected per donor
O Total volume/mass used for EV isolation (if pooled from several donors)
O All known collection conditions, including additives, at time of collection
O Pre-treatment to separate major fluid-specific contaminants before EV isolation
O Temperature and time of biofluid/tissue handling before and during pre-treatment
O For cultured tissue explants: volume, nature of medium; and time of culture before collecting conditioned medium
O For direct tissue EV extraction: treatment of tissue to release vesicles without disrupting cells

3-EV separation and concentration

Experimental details of the method

O Centrifugation: reference number of tube(s), rotor(s), adjusted k factor(s) of each centrifugation step (= time+ speed+ rotor, volume/density of centrifugation conditions), temperature, brake settings
O Density gradient: nature of matrix, method of generating gradient, reference (and size) of tubes, bottom- up (sample at bottom, high density) or top-bottom (sample on top, low density), centrifugation speed and time (with brake specified), method and volume of fraction recovery
O Chromatography: matrix (nature, pore size,…), loaded sample volume, fraction volume, number
O Precipitation: reference of polymer, ratio vol/vol or weight/vol polymer/fluid, time/temperature of incubation, time/speed/temperature of centrifugation
O Filtration: reference of filter type (=nature of membrane, pore size…), time and speed of centrifugation, volume before/after (in case of concentration)
O Antibody-based : reference of antibodies, mass Ab/ amount of EVs, nature of Ab carrier (bead, surface) and amount of Ab/carrier surface
O Other…. all necessary details to allow replication
O Additional step(s) to concentrate, if any
O Additional step(s) to wash matrix and/or sample, if any

Specify category of the chosen EV separation/concentra- tion method (Table 1):

O High recovery, low specificity = mixed EVs and non- EV components OR
O Intermediate recovery, intermediate specificity = mixed EVs with limited non-EV components OR
O Low recovery, high specificity = subtype(s) of EVs with as little non-EV as possible OR
O High recovery, high specificity = subtype(s) of EVs with as little non-EV as possible

4-EV characterization

Quantification (Table 2a, Section 4-a)

O Volume of fluid, and/or cell number, and/or tissue mass used to isolate EVs
O Global quantification by at least 2 methods: protein amount, particle number, lipid amount, expressed per volume of initial fluid or number of producing cells/ mass of tissue
O Ratio of the 2 quantification figures

Global characterization (Section 4-b, Table 3)

O Transmembrane or GPI-anchored protein localized in cells at plasma membrane or endosomes
O Cytosolic protein with membrane-binding or - association capacity
Assessment of presence/absence of expected contaminants (At least one each of the three categories above)

- Presence of proteins associated with compartments other than plasma membrane or endosomes
- Presence of soluble secreted proteins and their likely transmembrane ligands
- Topology of the relevant functional components (Section 4-d)

Single EV characterization (Section 4-c)

- Images of single EVs by wide-field and close-up: e.g. electron microscopy, scanning probe microscopy, super-resolution fluorescence microscopy
- Non-image-based method analysing large numbers of single EVs: NTA, TRPS, FCS, high-resolution flow cytometry, multi-angle light-scattering, Raman spectroscopy, etc.

5-Functional studies

- Dose-response assessment
- Negative control = nonconditioned medium, bio-fluid/tissue from control donors, as applicable

6-Reporting

- Submission of methodologic details to EV-TRACK (evtrack.org) with EV-TRACK number provided (strongly encouraged)
- Submission of data (proteomic, sequencing, other) to relevant public, curated databases or open-access repositories
- Data submission to EV-specific databases (e.g., EVpedia, Vesiclepedia, exRNA atlas)
- Temper EV-specific claims when MISEV requirements cannot be entirely satisfied (Section 6-b)

- Quantitative comparison of functional activity of total fluid, vs EV-depleted fluid, vs EVs (after high recovery/low specificity separation)
- Quantitative comparison of functional activity of EVs vs other EPs/fractions after low recovery/high specificity separation
- Quantitative comparison of activity of EV subtypes (if subtype-specific function claimed)
- Extent of functional activity in the absence of contact between EV donor and EV recipient