MISEV2018 Checklist

Numbers refer to sections listed in the Table of contents from:

C. Théry and K.W.Witwer, et al, "Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines", J Extracell Vesicles 2018;7:1535750.

+++ Mandatory ++ Mandatory if applicable + Encouraged

1-Nomenclature ✓

Mandatory

✓ +++ 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

✗ +++ Generic term, e.g., extracellular particle (EP): no intact cells but MISEV guidelines not satisfied

Encouraged (choose one)

✓ + Generic term extracellular vesicle (EV) + specification (size, density, other)

✗ + Specific term for subcellular origin: e.g., ectosome, microparticle, microvesicle (from plasma membrane), exosome (from endosomes), with demonstration of the subcellular origin

✗ + Other specific term: with definition of specific criteria

2-Collection and pre-processing ✓

Tissue Culture Conditioned medium (CCM, Section 2-a) ✗

General cell characterization (identity, passage, mycoplasma check…)

Medium used before and during collection (additives, serum, other)

++ exact protocol for depletion of EVs/EPs from additives in collection medium

+++ Nature and size of culture vessels, and volume of medium during conditioning

++ specific culture conditions (treatment, % O2, coating, polarization…) before and during collection

+++ 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)

+++ Frequency and interval of CM harvest

Biofluids or Tissues (Sections 2-b and -c) ✓

✓ ++ Donor status if available (age, sex, food/water intake, collection time, disease, medication, other)

✓ +++ Volume of biofluid or volume/mass of tissue sample collected per donor

✓ ++ Total volume/mass used for EV isolation (if pooled from several donors)

✓ +++ All known collection conditions, including additives, at time of collection

✓ +++ Pre-treatment to separate major fluid-specific contaminants before EV isolation

✓ +++ Temperature and time of biofluid/tissue handling before and during pre-treatment

✗ ++ For cultured tissue explants: volume, nature of medium and time of culture before collecting conditioned medium

✗ ++ For direct tissue EV extraction: treatment of tissue to release vesicles without disrupting cells

Storage and recovery (Section 2-d) ✓

✓ +++ Storage and recovery (e.g., thawing) of CCM, biofluid, or tissue before EV isolation (storage temperature, vessel, time; method of thawing or other sample preparation)

✓ +++ Storage and recovery of EVs after isolation (temperature, vessel, time, additive(s)…)

3-EV separation and concentration ✓

Experimental details of the method ✓

✓ ++ 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

✓ ++ Density gradient: nature of matrix, method of generating gradient, reference (and size) of tubes, bottomup (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

✓ ++ Chromatography: matrix (nature, pore size,…), loaded sample volume, fraction volume, number

++ Precipitation: reference of polymer, ratio vol/vol or weight/vol polymer/fluid, time/temperature of incubation, time/speed/temperature of centrifugation

✓ ++ Filtration: reference of filter type (=nature of membrane, pore size…), time and speed of centrifugation, volume before/after (in case of concentration)

++ Antibody-based : reference of antibodies, mass Ab/ amount of EVs, nature of Ab carrier (bead, surface) and amount of Ab/carrier surface

✓ ++ Other…: all necessary details to allow replication

✓ ++ Additional step(s) to concentrate, if any

✗ ++ Additional step(s) to wash matrix and/or sample, if any

Specify category of the chosen EV separation/concentration method (Table 1): ✗

+ High recovery, low specificity = mixed EVs and non EV components OR

+ Intermediate recovery, intermediate specificity = mixed EVs with limited non-EV components OR

+ Low recovery, high specificity = subtype(s) of EVs with as little non-EV as possible OR

+ High recovery, high specificity = subtype(s) of EVs with as little non-EV as possible

4-EV characterization ✓

Quantification (Table 2a, Section 4-a) ✓

✓ +++ Volume of fluid, and/or cell number, and/or tissue mass used to isolate EVs

✓ +++ 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

✗ +++ Ratio of the 2 quantification figures

Global characterization (Section 4-b, Table 3) ✓

✓ +++ Transmembrane or GPI-anchored protein localized in cells at plasma membrane or endosomes

✓ +++ 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, biofluid/tissue from control donors, as applicable

+++ 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

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)