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, 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

++ 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)