

## 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, % O<sub>2</sub>, 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, bio-fluid/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)