Framework Criteria	What to report	Please complete each criterion
1.1 Preanalytical variables conforming to MISEV guidelines.	Preanalytical variables relating to EV sample including source, collection, isolation, storage, and any others relevant and	
	available in the performed study.	
1.2 Experimental design according	EV-FC manuscripts should provide a brief description of the	
to MIFlowCyt guidelines.	experimental aim, keywords, and variables for the performed FC	
	experiment(s) using MIFlowCyt checklist criteria: 1.1, 1.2, and	
	1.3, respectively	
2.1 Sample staining details	State any steps relating to the staining of samples. Along with	
	the method used for staining, provide relevant reagent	
	descriptions as listed in MIFlowCyt guidelines (Section 2.4	
	Fluorescence Reagent(s) Descriptions).	
2.2 Sample washing details	State any steps relating to the washing of samples.	
2.3 Sample dilution details	All methods and steps relating to sample dilution.	
3.1 Buffer alone controls.	State whether a buffer-only control was analyzed at the same	
	settings and during the same experiment as the samples of	
	interest. If utilized it is recommended that all samples be	
	recorded for a consistent set period of time e.g. 5 minutes,	
	rather than stopping analysis at a set recorded event count e.g.	
	hotware controls and comparisons of total particle counts	
2.2 Duffer with recent each train	Ctate whether a huffer with respect control was and state	
3.2 Buffer with reagent controls.	State whether a burier with reagent control was analyzed at the	
	same settings, same concentrations, and during the same	
3.3 Unstained controls	State whether unstained control samples were analyzed at the	
	same settings and during the same experiment as stained	
	samples. If used, state what the results were, preferably in	
	standard units.	
3.4 Isotype controls.	The use of isotype controls is applicable to immunofluorescence	
	labelling only. State whether isotype controls were analyzed at	
	the same settings and during the same experiment as stained	
	samples. If utilized, state which antibody they are matched to,	
	the concentration used, and what the results were (Section 4.2,	
	4.3, 4.4). Due to conjugation differences between	
	manufacturers if should be stated if the isotype controls are from	
	the same manufacturer as the matched antibodies.	
3.5 Single-stained controls.	State whether single-stained controls were included. If used	
	state whether the single-stained controls were recorded using	
	the same settings, dilutions, and during the same experiment as	
	standard units (Section 4.2, 4.3, 4.4)	
3.6 Procedural controls	State whether procedural controls were included. If used, state	
	the procedure and if the procedural controls were acquired at	
	the same settings and during the same experiment as stained	
	samples.	
3.7 Serial dilutions.	State whether serial dilutions were performed on samples and	
	note the dilution range and manner of testing. The fluorescence	
	and/or scatter signal intensity would ideally be reported in	
	standard units (see Section 4.3, 4.4) but arbitrary units can also	
	be used. This data is best reported by plotting the recorded	
	number events/concentration over a set period of time at	
	different sample dilution. The median fluorescence intensity at	
	each of the dilutions should also ideally be plotted on the same	
	or a separate plot.	
3.8. Detergent treated EV-samples	State whether samples were detergent treated to assess lability.	
	If utilized, state what detergent was used, the end	
	concentration of the detergent, and what the results were of the	
	lysis.	

4.1 Trigger Channel(s) and Threshold(s) used for event detection. Preferably, the fluoresconce calibration (Section 4.3) and/or scatter calibration (Section 4.4) should be used in order to report the trigger channel(s) and threshold(s) in standardized units. 4.2 Flow Rate / Volumetric State if the flow rate was quantified/validated and if so, report duratification. 4.3 Fluorescence Calibration. State whether fluorescence calibration was implemented, and if so, report the materials and methods used, catalogue numbers, lot numbers, and supplied reference units for the standards. Fluorescence parameters may be reported in standardized units of MESF, ERF, or ABC beads. The type of regression used, and the resulting scatter pick of arbitray data ws standard data for the reference particles should be supplied. 4.4 Light Scatter Calibration. State whether and how EV diameter, surface area, and/or volume has been calculated using FC measurements. 5.1 EV diameter/surface State whether and how EV diameter, surface area, and/or volume has been calculated using FC measurements. 5.2 EV refractive index and how W was done. State whether EV epitope number has been approximated and how this was done. 6.1 Completention of MFlowCyt checklist criteria 1 to 4 using the MiFlowCyt checklist criteria 1 to 4 using the MiFlowCyt checklist criteria 1 to 4 using the MiFlowCyt checklist criteria. 6.2 Calibrated channel detection range State whether EV epitope number has been carried out, a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale action the sole and parking to a calibrated scaled, as discussed in Section 4.3 and 4.4, and provid			
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