Review of: "A simple immunoassay for extracellular vesicle liquid biopsy in microliters of non-processed plasma"

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The manuscript by Campos-Silva et al. describes a protocol by which exogenous charged polymers can influence the immunocapture with antibodies recognizing EV surface markers.
The first part of the study (colloids versus ab binding) tries to provide a rationale or associations to explain this phenomenon. The second part (test on plasma samples), that coul work without the first one, describes comparisons that require major revisions.
Major points:
The observation that cell-secreted EVs could be considered "a colloid suspension" is not sufficient to demonstrate that the binding affinity of one antibody depends on the stability of the particles in solution. In other words, how the stability of colloidal suspension relates to the non-interaction/interaction in immunoassays (since the concept is intended as general by the authors)? There is no mechanistic prove of this. Attached to this point please see also the following three points:
How to explain an improved antibody binding in the presence of an induced aggregation of particles? The binding also depends on "accessibility". One could allude that the immunocaptured material is constituted by a relevant bulk of non-antigen presenting particles.
Where do the authors show the antibody-independence? The data in Figure 3 show indeed that the effect is antibody-dependent.
In addition, the antibodies against CD81 or CD9 gave the best signal in WB (Figure 1D) rather than CD63. Why not also using those two ones in comparison? It seems all of those antibodies are suitable for immunophenotyping by flow cytometry.
When considering immunocapture, the incubation time is expected as the most important parameter: What is the difference between your method (exogenous polymers) and increased incubation times with antibodies?
Why EDTA and polybrene, which induce opposite effects in solution, result in a "better signal". This is in contrast with the conclusions drawn by the authors.
One could expect to detect the ab signal in the absence or the presence of a titration of exogenous polymers and show that the immunocapture increases consistently while preserving the binding specificity: Where do the authors show it?

The authors only use CD proteins and no controls, for example, including luminal markers.

Blood from healthy subjects? Age-matched?

The first paragraph of the results lets the readers hypothesize that the authors think that the particles detected through NTA are all vesicles. Is it correct?

Data in Figure 4 have polybrene?

The term "we used flocculation methods" in the abstract is misleading.

Have you considered aggregation of exogenous polymers?

The NTA profiles after the addition of exogenous polymers cannot be "not shown".

The discussion contains protocol details not mentioned in the appropriate sections.

"Such association studies, requiring the
analysis of samples from large patient cohorts, are hampered by the current available methods which require either relatively large sample volumes or long protocols of nanovesicle pre-enrichment together with the use of sophisticated equipment or specialised personnel" This sentence is outdated, since many different protocols that are easily adaptable to each laboratory have been presented, therefore the rationale of the study here presented should be carefully revised.