# Supplemental information to:

## Centrifugation affects the purity of liquid biopsy-based tumor biomarkers

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## Sample properties

Platelets have a log-normal volume distribution (1), and a discoid shape with an aspect ratio of 4-10 (2). For the model, we assumed platelets to be an oblate spheroid with aspect ratio 7, leading to an *S* for platelets of 2.0 assuming the platelet moves at sufficiently low speeds such that all orientations are equally feasible (3). If this assumption is false, and the platelet is oriented in the energetically most favorable orientation, *S* would need to be 1.5 (3). Platelets can have a volumetric mass density of 1.05-1.09 g/mL, where the lower density is associated with platelets that have secreted their α-granule content but with a unaffected platelet volume distribution (4, 5). Typical mass densities of EVs determined in Optiprep are found between 1.08 and 1.11. This seems very high compared to typical volumetric mass densities for cells of 1.05-1.08 (6). However, because of the highly skewed particle size distribution this likely pertains to EVs smaller than 200 nm, which have a relatively large membrane content. Modelling an EV as a small cell, i.e. a 8 nm thick membrane consisting of 50% protein (volumetric mass density 1.30), 50% phospholipids (volumetric mass density 1.01) and filled with cytoplasm (volumetric mass density 1.06) (6), would mean that 100 and 200 nm EV have volumetric mass densities of 1.099, and 1.081 respectively. These densities are in good agreement with literature values, while a 1000 nm EV would have a density of 1.064, which is within the range of densities of a cell. Lastly, the mass density of DNA is only available in cesium chloride media, and the length of ccfDNA is unknown. Assuming a length shorter than 1000 basepairs, the ccfDNA would be smaller than 50 nm.

**Table S1. Literature values for components in model.**

| | Property at 20°C, 1 bar | Values in article | Population/sample type | References |
|---|---|---|---|---|
| CTCs | | | | |
| Density (g/mL) | | | | |
| | 1.05 +/- 0.002 | | lung carcinoma cell line | (7) |
| | 1.056 +/- 0.004 | | cervical carcinoma cell line | (8) |
| Diameter (µm) | | | | |
| | median 10.7 | (measured in range 8-20) | 276 castration resistant PCa | (9) |
| | median 11.0 | (measured in range 8-20) | 464 colorectal cancer patients | (9) |
| | median 13.1 | (measured in range 8-20) | 177 breast cancer patients | (9) |
| Platelets | | | | |
| Density (g/mL) | | | | |
| | 1.05-1.09 @ 4°C | freq mean 1071 SD 6 | 17 normal donors | (4) |
| | 1.04-1.08 @ 23°C | | 30 normal donors | (10) |
| | 1.05-1.08 @ RT | freq mean 1067 SD 4.5 | 15 normal donors | (5) |
| Diameter (µm) | From spherical approximation of impedance based volume determinations | | | |
| | 2.5 +/- 0.6 (mean +/- SD) | range 1-21, mean 8.2, SD 6.1 fL | 55 normal donors | (11) |
| | 2.3 +/- 0.6 | lognormal mean 6.6 SD 4.3 fL | 50 normal donors | (1) |
| Shape factor | “Platelets are discoids 0.5 um thick and 2-5 um diameter” -> aspect ratio 4-10 | | | (2) |
| | 1.55, 2.03, 2.48 | aspect ratio 4, 7, 10 | | (3) |
| EVs | | | | |
| Density (g/mL) | Excluded last round culture with exosome containing media, included only iso-osmotic gradient media | | | |
| | 1.08-1.10 | | color. carcinoma cell line derived EVs | (12) |
| | 1.09-1.11 | sample in 0.25M sucrose before DGC | color. carcinoma cell line derived EVs | (13) |
| | 1.094-1.143 | | breast cancer cell line derived EVs | (14) |
| | 1.093 | some sucrose from previous steps | colorectal carcinoma ascites | (15) |
| | 1.087 | some sucrose from previous steps | melanoma in mouse model | (16) |

**Table S1 (continued)**

| | **Property @ 20°C, 1 bar** | **Values in paper** | **Population/sample type** | **References** |
|---|---|---|---|---|
| ccfDNA | | | | |
| Density (g/mL) | | | | |
| | 1.7 | | in CsCl gradient | (17) |
| Size (nm) | | | | |
| | 2 by 304 (1k base pairs) | | | (6) |
| PBS | | | | |
| Density (g/mL) | 1.004 | | | (18) |
| Viscosity (mPa . s) | 1.193 | | | (18) |
| Plasma | | | | |
| Density (g/mL) | | | | |
| | 1.026 (CI 1.024-1.027) | @ 4, 25,37 °C | 25 normal donors | (19) |
| | 1.0245 (CI 1.0215-1.0262) | | 32 normal donors | (20) |
| | 1.0253 (CI 1.0217 - 1.0273) | | 122 pathological donors | (20) |
| Viscosity (mPa . s) | Converted to 20°C through 2.2% increase per °C (Arrhenius) | | | (21) |
| | 1.6 - 1.9 | 1.1 - 1.3 @ 37°C | 'normal range' | (22) |
| | 1.70 | 1.52 @ 25°C | 57 normal, no influence of sex, age | (23) |
| | 1.80 (1.75/1.91) | 1.24 (25p-75p 1.20-1.31) @ 37°C | Unspecified | (24) |

*25p-75p: 25-75 percentile, ccfDNA: circulating cell-free DNA, CI: 95% confidence interval, Color.: Colorectal, CTCs: circulating tumor cells, DGC: density gradient centrifugation, EVs: extracellular vesicles, PBS: phosphate-buffered saline, PCa: prostate cancer, RT: room temperature, SD: standard deviation.*

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