These supplementary materials have been updatet on the 4th of September 2017 to correct the values of 2 cells in Table 1. Therefore, these supplementary materials differ from the original supplementary materials published by Wiley in J. Thromb. Haemost. **12**.7 (2014): 1182-1192.

1 Methods

1.1 Transmission electron microscopy (TEM)

To analyze the vesicle standard by TEM, vesicles were isolated by centrifugation ($12 \cdot 1000$ µL, 60 minutes, $18,900 \cdot g$, 4 °C). From each aliquot, 900 µL was collected for ultra-centrifugation, the next 75 µL was discarded, and the remaining 25 µL contained the relatively large vesicles. The 900 µL aliquots were ultra-centrifuged (60 minutes, $154,000 \cdot g$, 4 °C) to collect smaller vesicles from the pellet. All pellets present in the remaining 25 µL from both centrifugation speeds were resuspended in 975 µL PBS citrate and centrifuged as before. For both centrifugation speeds, the washed pellets were pooled to a final volume of 300 µL. Each pool was prepared as follows: 145 µL was diluted with 145 µL PBS containing 0.2% paraformaldehyde (w/v). After fixation for 24 hours, 10 µL was applied to a formfar-carbon coated 300 mesh grid (Electron Microscopy Sciences, Hatfield, USA) for 7 minutes, followed by staining with 1.75% uranyl acetate (w/v). Samples were allowed to dry at room temperature for 2 hours and imaged with TEM (CM-10, Philips, Eindhoven, The Netherlands) at 100 kV.

Next, 1000 vesicles were segmented manually using the Quick selection tool of PHOTOSHOP v11.0.2 (Adobe Systems, San Jose, CA). Overlapping vesicles were included in the analysis as their boundaries could be clearly observed. A custom-made Javascript saved the surface area of the segmented vesicles to a .csv file. The vesicle size d was calculated from the surface area A using $d = \sqrt{4A/\pi}$, thereby assuming that vesicles are spherical. The overall PSD was obtained by summation of the PSDs obtained by both centrifugation speeds.

1.2 Flow cytometry detector settings

For the conventional flow cytrometer (FACSCalibur, BD, Franklin Lakes, USA), the trigger was set on SSC, with a photomultiplier tube voltage of 400 V, a gain of 1, and a trigger threshold of 0 [**?**]. To account for the noise background, the intensity histogram of a background measurement with de-ionized water was subtracted from the intensity histogram of each dataset.

For the flow cytometer dedicated to detecting sub-micrometer particles (A50-Micro, Apogee, Hemel Hempstead, UK), a trigger was set on both the FSC and SSC detector. The gains were 1, the applied voltages were 290 V and 415 V, and the thresholds were 5 and 32 for the FSC and SSC detectors, respectively. Based on the counts from de-ionized water, a gate was set to reduce the noise background. This approach is preferable, but was not possible on the FACSCalibur due to high noise on especially the FSC detector.

2 Mathematical function to fit the particle size distribution of vesicles

To select the mathematical function that optimally describes the relationship between the concentration and diameter of vesicles, we have fitted the particle size distribution (PSD) of the vesicle standard as measured by TEM and RPS with six empirical functions that are frequently used to describe PSDs of particles in suspension [1]: the exponential function, the gamma function, the Lorentzian function, the power-law function, the Weibull distribution, and the log-normal distribution. We selected TEM and RPS as the most accurate methods to determine the PSD of vesicles. The exponential function is given by

$$C(d) = ce^{-bd} \tag{1}$$

where C is the concentration of vesicles as function of the size d, c is a scale factor for the particle concentration, and b is a fit parameter. The exponential function becomes a gamma function by adding an extra slope parameter a:

$$C(d) = cd^a e^{-bd} \tag{2}$$

The Lorentzian function is given by

$$C(d) = \frac{2C_{tot}}{\pi} \frac{w}{4(d-d_c)^2 + w^2} \tag{3}$$

where C_{tot} is the total concentration, and d_c and w specify the location of the peak and the full width at half maximum of the distribution, respectively. The power-law function is given by

$$C(d) = k \left(\frac{d}{d_0} \right)^{-m} \tag{4}$$

where k has the same dimension as C and m is a non-dimensional constant. Parameter $d_0 \equiv 1$ and has the same dimension as d to create a dimensionally homogeneous equation. The Weibull distribution is given by

$$C(d) = \frac{ca}{b} \left(\frac{d-d_s}{b} \right)^{a-1} \exp \left[- \left(\frac{d-d_s}{b} \right)^a \right] \tag{5}$$

where c is a scale factor for the particle concentration, a is a non-dimensional constant, and d_s is the smallest particle diameter of the distribution. Parameter b has the same dimension as d to create a dimensionally homogeneous equation. The log-normal distribution is given by

$$C(d) = \frac{C_{tot}}{\sqrt{2\pi}\sigma d} \exp - \frac{\ln(d/\mu)^2}{2\sigma^2} \tag{6}$$

where C_{tot} is the total concentration, and μ and σ are the mean and standard deviation of the natural logarithm of the distribution, respectively.

The PSD of vesicles is an asymmetrical distribution, but the Lorentzian function is symmet-

Function	Parameter	TEM	RPS
Exponential function	b	$2.389{\cdot}10^{-2}$	$2.079\ {\cdot}10^{-2}$
	c	$3.416{\cdot}10^{8}$	$2.050\ {\cdot}10^{9}$
Gamma function	a	-2.229	-4.371
	b	$9.040{\cdot}10^{-3}$	$-1.870{\cdot}10^{-3}$
	c	$1.929{\cdot}10^{12}$	$1.666{\cdot}10^{17}$
Lorentzian function	C_{tot}	$9.201{\cdot}10^{9}$	$5.824{\cdot}10^{10}$
	d_c	30.04	77.34
	w	56.43	28.95
Power-law function	k	$2.494{\cdot}10^{14}$	$3.992{\cdot}10^{16}$
	m	3.508	4.024
Weibull distribution	a	0.3400	0.5294
	b	0.1977	10.05
	c	$6.199{\cdot}10^{12}$	$7.279{\cdot}10^{10}$
	d_s	-13.18	66.28
Log-normal distribution	C_{tot}	$9.676{\cdot}10^{9}$	
	μ	0.6284	
	σ	48.99	

Table 1: Parameters used to fit the particle size distribution of the vesicle standard measured by transmission electron microscopy (TEM) and resistive pulse sensing (RPS) with an exponential function, a Gamma function, a Lorentzian function, a power-law function, a Weibull distribution, and a log-normal distribution.

rical, the exponential function, gamma function, and power-law function diverge as the particle size tends to 0, and the Weibull distribution diverges as the particles size tends to d_s. Thus, the shape of these functions differs from the shape of the PSD of vesicles. However, these functions can still be used to describe a part of the PSD. In fact, for all methods except TEM the full PSD is not measured due to the presence of a lower detection limit. Therefore, functions are fitted to data on the right-hand side of the peaks only. Fitting was done by taking the natural logarithm of both the function and the data and performing a least square fit. Fig. 1 shows the PSD of the vesicle standard measured by TEM and RPS fitted by an exponential function, a Gamma function, a Lorentzian function, a power-law function, and a Weibull distribution. Due to the "long tail" at the right-hand side of the log-normal distribution, the fit did not converge for data on he right-hand side of the peak. Therefore, the log-normal distribution is fitted to the full PSD obtained by TEM only, since the PSD by TEM is not affected by the lower detection limit. Fig. 2a shows the PSD of the vesicle standard measured by TEM fitted by a log-normal distribution. The obtained fit parameters are listed in table 1. To determine the goodness-of-fit, the reduced χ^2 and the adjusted R^2 were calculated for each fit and listed in Table 2 .

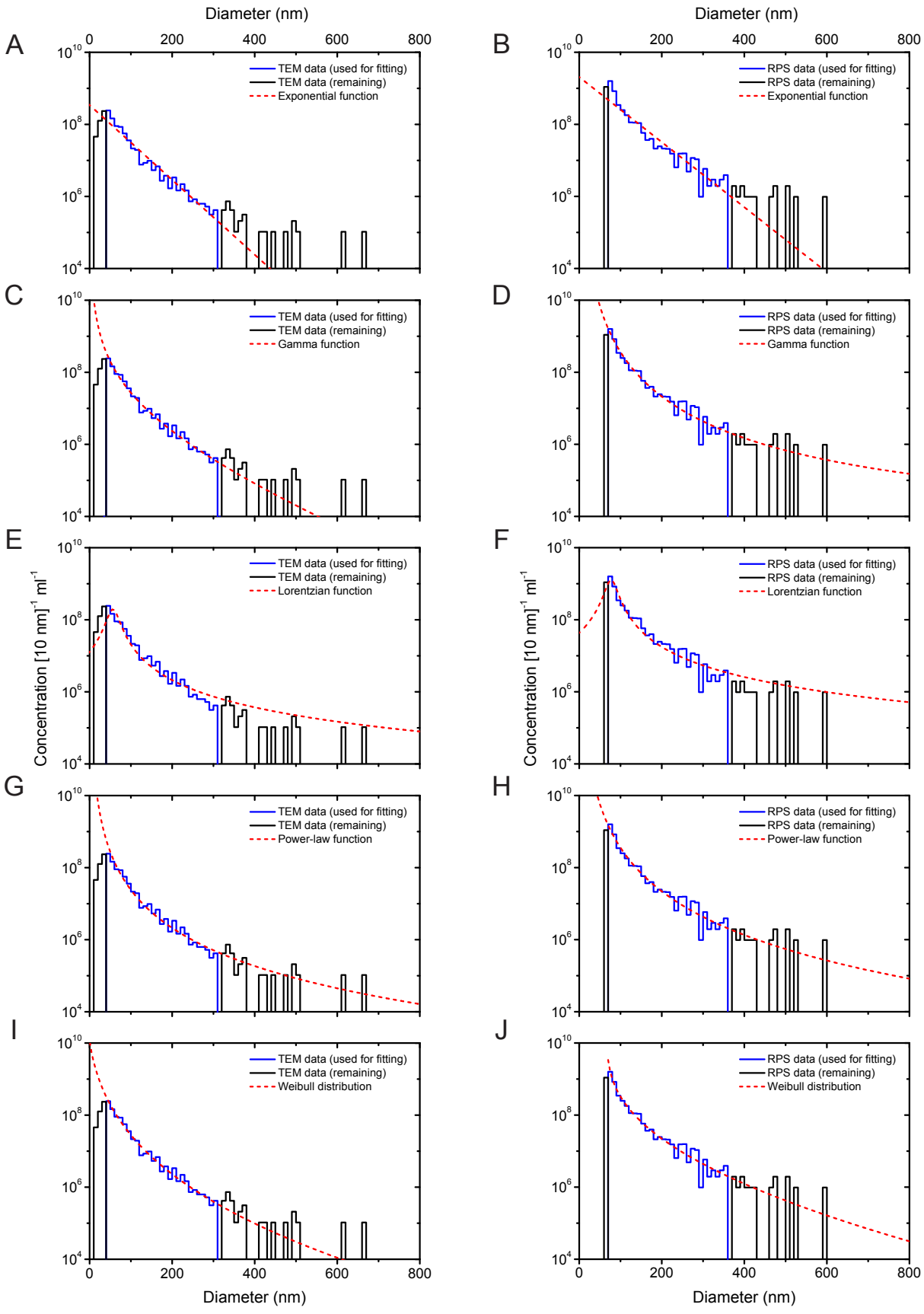

Figure 1: Particle size distribution of the vesicle standard measured by transmission electron microscopy (TEM) and resistive pulse sensing (RPS) fitted by (a,b) an exponential function, (c,d) a Gamma function, (e,f) a Lorentzian function, (g,h) a power-law function, and (i,j) a Weibull distribution.

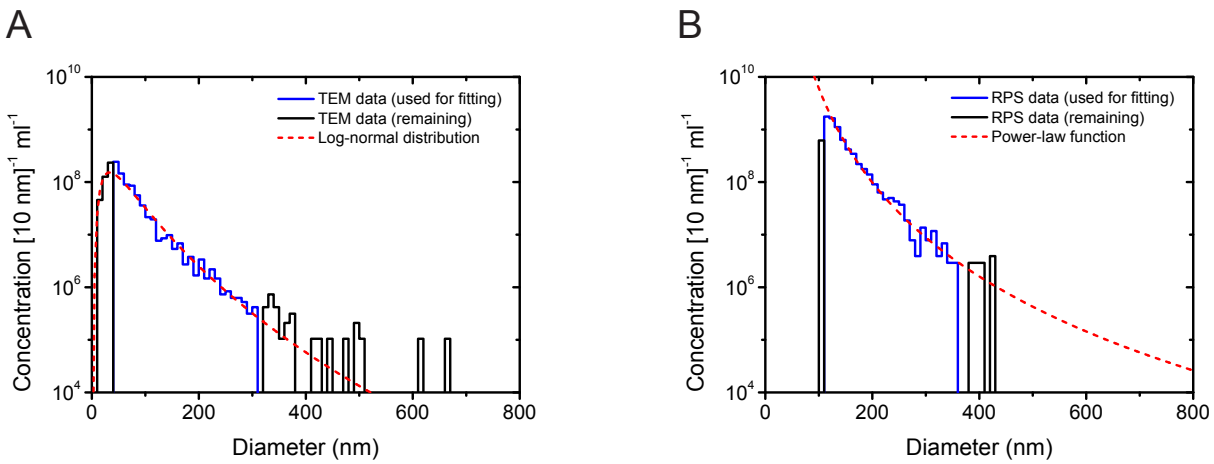

Figure 2: (a) Particle size distribution of the vesicle standard measured by transmission electron microscopy (TEM) and fitted by a log-normal distribution. (b) Particle size distribution of vesicles from platelet poor plasma measured by resistive pulse sensing (RPS) and fitted by a power-law function using fit parameters $k = 4.520 \cdot 10^{21}$ and $m = 5.938$, giving a χ^2_{red} of 0.14 and a R^2_{adj} of 0.97.

	TEM		RPS	
Function	χ^2_{red}	R^2_{adj}	χ^2_{red}	R^2_{adj}
Exponential function	0.16	0.96	0.39	0.89
Gamma function	0.07	0.98	0.23	0.93
Lorentzian function	0.20	0.95	0.30	0.92
Power-law function	0.10	0.97	0.23	0.94
Weibull distribution	0.07	0.98	0.24	0.93
Log-normal distribution	0.20	0.97		

Table 2: Results of goodness-of-fit tests applied to fitting a function to the PSD of the vesicle standard as measured by transmission electron microscopy (TEM) and resistive pulse sensing (RPS). χ^2_{red}, reduced χ^2; R^2_{adj}, adjusted R^2.

Method	Smallest detectable vesicle size (nm)	Detected concentration (vesicles mL^{-1})	Expected concentration (vesicles mL^{-1})	Ratio (-)
TEM	40	$1.2{\cdot}10^{9}$	$1.8{\cdot}10^{10}$	0.1
Conventional flow cytometry	340	$1.6{\cdot}10^{7}$	$2.7{\cdot}10^{7}$	0.6
Novel flow cytometry	160	$3.3{\cdot}10^{8}$	$2.8{\cdot}10^{8}$	1.2
NTA	70	$4.6{\cdot}10^{9}$	$3.4{\cdot}10^{9}$	1.3
RPS	60	$5.0{\cdot}10^{9}$	$5.5{\cdot}10^{9}$	0.9

Table 3: Total concentration of vesicles from urine as detected by transmission electron microscopy (TEM), conventional flow cytometry, novel flow cytometry, nanoparticle tracking analysis (NTA), and resistive pulse sensing (RPS) and the expected concentration calculated by bounding the power-law fit of RPS on urine vesicles between the minimum detectable vesicle size and 800 nm. For all methods except TEM, the calculated concentration was within 40% of the detected concentration, indicating that the detected concentrations were primarily constrained by the minimum detectable vesicle size.

Best fits were obtained with the gamma function, power-law function, and the Weibull distribution. For further analysis, we selected the power-law function, since it is least susceptible to the minimal detectable vesicle size and it has the least fit parameters. To show that the power-law function is not only applicable to fit the PSD of the vesicle standard, we have applied it to vesicles from plasma. Fig. 2b shows that also the PSD of vesicles from platelet poor plasma as measured by RPS can be well-described by a power-law function.

To show that the detected concentrations of vesicles in Fig. 4 of the manuscript where primarily determined by the minimum detectable vesicle size, we have calculated the vesicle concentration of the power-law fit of RPS between the minimum detectable vesicle size of each method and 800 nm. Table 3 shows the actual detected vesicle concentration and the concentration expected from the minimum detectable vesicle size. For all methods except TEM, the expected concentration was within 40% of the detected concentration, confirming that the detected concentrations were primarily determined by the minimum detectable vesicle size.

References

[1] M. Jonasz and G.R. Fournier. *Light scattering by particles in water.* Academic Press, Great Britain, London, 1 edition, 2007.