Step up your EV characterization with Leprechaun

Introduction

Analyzing all the EVs in your sample without missing critical particles is a challenge due to the small size of EVs and the abundance of similar-sized, non-EV nanoparticles present in complex samples. This problem is made even trickier by contaminants that co-purify with EVs – including lipoproteins, protein aggregates and bovine EVs [1]. Most common particle characterization tools, such as NTA and TRPS, can't tell EVs apart from other particles meaning they count the garbage as well as the gold.

Leprechaun utilizes an immuno-capture step to separate EVs from other particles to ensure you only count the vesicles you care about. It is the only platform that provides vesicle concentration, size and phenotypic analysis (including cargo) on a single vesicle basis, without the need for sample purification (Figure 1). Building on the technology underpinning its predecessor, the ExoView R200, Leprechaun boasts an improved lower sizing limit of 35 nm, opening the door to comprehensive, purification-free analysis of EVs from exosomes to exomeres.

This app note describes how Leprechaun sizes up even the smallest EVs to shed light on previously undetected particles, and compares Leprechaun performance relative to the ExoView R200.

EV analysis on Leprechaun

Leprechaun's Human Tetraspanin Luni consumable captures EVs on anti-CD81, -CD9 and -CD63 antibody spots. This immuno-capture step on the Luni surface means it is not necessary to purify samples prior to analysis, allowing Leprechaun to characterize EVs from crude or pure samples. Once EVs have been captured on the Luni surface the Luni Washer performs automated washing to remove nonspecifically bound particles, EVs are then counterstained with fluorescent antibodies, before being dried by the washer ready for analysis on Leprechaun. Particles are sized using single particle interferometric reflectance

Figure 1: Leprechaun: The next step in EV analysis.

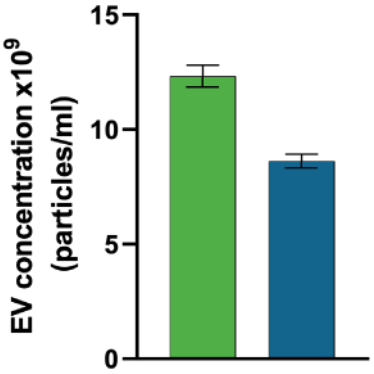
imaging while fluorescence microscopy provides information on EV phenotype and cargo content.

For this experiment an EV standard with endogenous GFP was analyzed (Sigma-Aldrich) and fluorescent anti-CD81-CF555 added to confirm tetraspanin expression. The EV standard was diluted 1 in 25 in kit provided Incubation Solution (Unchained Labs) and incubated on the Luni for 1 hour prior to washing and staining. Data was analyzed in Leprechaun Exosome Analysis (Unchained Labs) where colocalization analysis was performed to determine the number of tetraspanin particles which are GFP tagged.

Leprechaun vs ExoView R200

The biggest step forward with Leprechaun is the ability to size EVs all the way down to 35 nm – so particles invisble to the ExoView R200 can now be seen, sized, and added up. Both instruments utilize single particle interferometry to measure the diameter of individual vesicles captured on the Luni surface. The signal enhancing silicon dioxide surface of the Luni maximizes the interference of light reflected from the Luni surface and scattered by the captured EV [2]. Updated optics and refined background subtraction allow Leprechaun to reduce

A



B

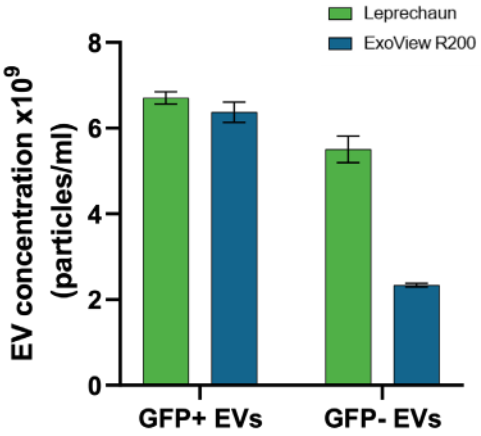


Figure 2: (**A**) Total EV concentration as determined by Leprechaun and the ExoView R200. (**B**) Comparison of the concentration of GFP positive and GFP negative EVs calculated by each platform. For both plots n=3 and error bars are SD, green bars = Leprechaun, blue bars = ExoView R200.

the lower limit of particle sizing from 50 nm, as found on the R200, to the improved 35 nm.

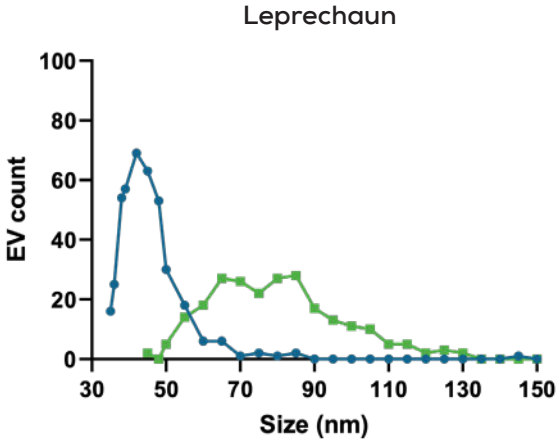
The total concentration of EVs in the sample, as determined by each platform, is shown in Figure 2A. Here, an EV is defined as a tetraspanin positive particle, which has a diameter within the sizing range of the respective platform and may or may not express GFP. There is no difference between the configuration, power, or sensitivity in the fluorescence channels of the Leprechaun and ExoView, meaning fluorescence detection is not a driver of any differences in measured EV concentration.

Subpopulation analysis shows that while the concentration of GFP positive EVs is comparable between both platforms (6.5 x 10^9 particles/ml), there are significantly fewer GFP negative EVs detected by the ExoView (27% of total CD81+ EVs) compared to Leprechaun (45%) (Figure 2B).

Particle size analysis performed on both instruments reveals that the GFP positive and negative populations have distinct size profiles (Figure 3A & 3B). On both platforms the mean diameter of GFP positive EVs is measured to be 82 nm, compared to under 60 nm for GFP negative EVs. While the sizing peak for the GFP positive EVs sits comfortably in the 50 – 200 nm range of the ExoView, a significant proportion of the smaller, GFP negative EVs appear to be below the 50 nm cut off.

Leprechaun's ability to detect and size sub 50 nm particles means the true mean and mode of the

A



B

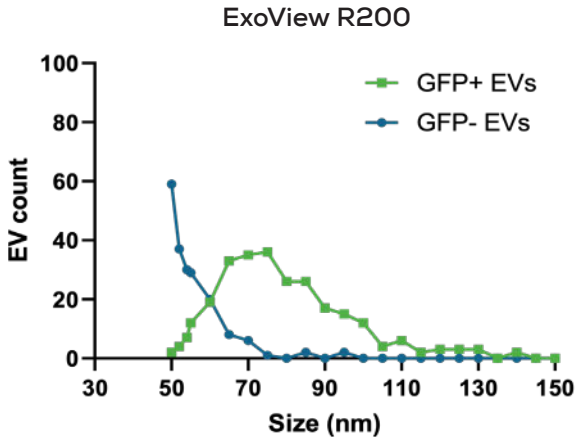


Figure 3: Size distributions of GFP positive and GFP negative EVs, acquired on Leprechaun (**A**) and ExoView R200 (**B**). For both plots green line = GFP positive EVs, blue line = GFP negative EVs.

	Leprechaun		ExoView R200	
	Mean (nm)	Mode (nm)	Mean (nm)	Mode (nm)
GFP+ EVs	82	72	82	75
GFP- EVs	48	42	58	50

Table 1: Mean and mode comparison for both GFP subpopulations on Leprechaun and ExoView R200.

GFP negative population can be obtained (Table 1). The inclusion of these small EVs accounts for the difference in total EV concentration between Leprechaun and ExoView (Figure 2A). Based on particle counts from Leprechaun, 61% of GFP negative EVs are smaller than 50 nm, equivalent to 3.35 x 10^9 particles/ml. This value matches the difference in the concentration of GFP negative EVs between ExoView (2.33 x 10^9) and Leprechaun (5.50 x 10^9).

Titration of the EV standard highlights the linear nature of the Leprechaun readout (Figure 4). EV concentration can be determined from crude or pure samples down to a lower limit of detection of 5 x 10^6 particles/ml.

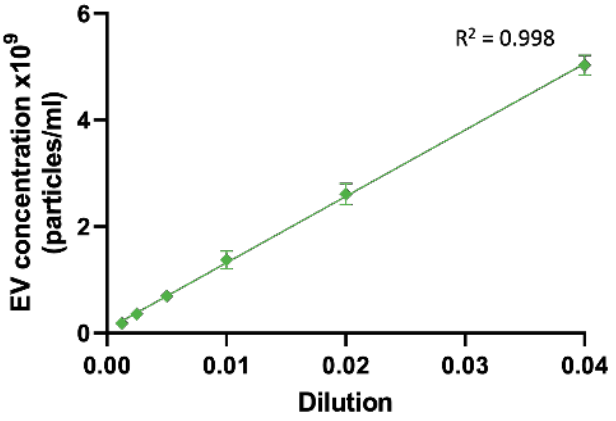


Figure 4: Linearity of EV concentration on Leprechaun. N = 3, mean values displayed, error bars = SD.

Conclusion

There is an increasing appreciation of the heterogeneity of EVs, not only in terms of surface markers expressed, but also vesicle size. The discovery of exomeres in 2018 highlighted the abundance of vesicles at the lower end of the size range [3]. Most analytical tools however, struggle to detect and size biological particles below 50 nm in diameter, and so provide no information on this particle population, leading to inaccurate characterization of EV samples.

Leprechaun provides the full picture for your EV sample, by sizing and phenotyping even the smallest vesicles on a single particle basis. Building on the technology of its predecessor, the ExoView R200, Leprechaun serves up EV concentration, size, surface marker phenotyping and cargo detection without the need for sample purification. No matter how rare or small your EV population, Leprechaun can give you the low down.

References

1 Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. Sodar, B et al. Scientific Reports, 2016. 6:24316.

2 Digital detection of exosomes by interferometric imaging. Daaboul, G et al. Scientific Reports, 2016. 6:37426.

3 Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. Zhang, H et al. Nature Cell Biology, 2018. 332-343.

UNCHAINED LABS

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

Rev A