Phenotyping of EVs by Multi-Wavelength Fluorescence Nanoparticle Tracking Analysis

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C. Helmbrecht^{1*}, S. Raschke¹, B. Giebel², M. Bremer², P. Guazzi³

ticle Metrix GmbH, Wildmoos 4, D-82266 Inning, Germany versity Hospital Essen, Institut for Transfusion Medicine, Virchowatr. 179, D-46147 Essen, Germany isaBioMed Life Science LTD, Akadeemia tee 15A, 12618 Tallinn, Estonia sponding author: helmbrecht@pahticle.metrix.de



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Introduction



Nanoparticle Tracking Analysis of fluorescent labelled bionanoparticles, such as vesicles or liposomes, is an efficient technique for quantification of size and total concentration. Analysis with single wavelength requires measurement of samples in sequence.

For the first time, we present an NTA instrument equipped with two lasers (TWIN-Laser NTA), allowing analysis of at least two different biomarkers on the same sample.

An NTA with the combination of 405 and 488 nm was evaluated with fluorescent standards as well as antibody-labelled EVs.

Material and Methods

NTA: ZetaView® TWIN Laser NTA with 405 and 488 nm Laser and long pass filters

HEK293-EVs (Hansabiomed LTD) were reconstituted in water by 30 s of vortexing.

MSC-EVs (AG Giebel) were purified by PEG precipitation followed by UC.



Standards – Fluorescent PS



Immortalized MSC derived EVs



Evaluation of directly labeled antibodies: influence of clone and fluorophore. Preliminary data from flow and image flow cytometry are in agreement with NTA data.

Comparison of tetraspanin detection procedures: sequence (488 nm, left) and by two wavelengths on same sample (405 and 488 nm, right).



6.0E+09 CD81 (blue channe 4.0E+09 2.0E+09 0.0E+00 100 200 300 400 Particle diameter (N) NTA / nm

vophilized EVs from HEK293



Conclusion

- Antibody conjugation has significant impact on the result. Parameters for quantifying antibody quality need to be determined and defined.
- Labeling protocol needs further optimization and standardization for reproducible quantitative data. SEC is not necessary when antibody concentration is opitmized.
- Multiwavelength NTA improves reproducibility, minimizes sample amount and increases information per sample.

Acknowledgement EFRE.NRW

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