

Standardization of extracellular vesicle measurements by quantifying the quality of scatter flow cytometry

Vascular Biology

- Person responsible (Chair / Principal Investigator): Dr. Rienk Nieuwland
- Design
Quantifying the quality of scatter flow cytometry to standardize extracellular vesicle measurements.
- Aim/Objective/Rationale (Needs assessment / Reason)
Improve the reproducibility of the measured concentration of extracellular vesicles (EVs) by flow cytometry by quantifying the quality of scatter flow cytometry.
- Methodology (Data expected to collect, sample size and statistical analysis):
 - Physics model
 - Implementation of the physics model in the research field
 - Application of the model to an earlier collected SSC dataset (Coumans 2014, 46 flow cytometers)
- Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):
 - Please see request of Coumans 2014
 - 33 laboratories, 46 flow cytometer, no labs excluded based on quality of flow cytometer
- Expected timeline:
 - The project will start at August 2017
 - A method allowing direct comparison of the quality of scatter-based flow cytometers is expected to be ready in January 2018
 - Validation of the method is expected to be ready in May 2018
 - Application of the method to the dataset of Coumans 2014 is expected to be ready in September 2018
 - The project will take till December 2018
- Expected outcomes (ie. publications):
 - The activity aims for two scientific manuscripts and three scientific presentations to guarantee knowledge dissemination
 - One technical paper submitted to Cytometry A
 - One formal SSC guideline to be published in JTH

- Oral presentations at an ISTH SSC meeting, the annual meeting of the International Society for Advancement of Cytometry (ISAC), and the annual meeting of the International Society for Extracellular Vesicles (ISEV).
 - This project also fits within the ongoing collaboration regarding standardization of detection of EVs by ISTH, ISEV and ISAC (www.evflowcytometry.org), and will further strengthen the role of the ISTH in the unique collaboration.
- Description of project set/up and management, needed infrastructure and resources (summary):

The quality of fluorescence flow cytometry is quantified by the efficiency (Q), background (B), and resolution limit. This QB -analysis is currently being implemented in the field and substantially improves reproducibility of EV measurements. However, a QB -analysis for scatter flow cytometry is lacking. Based on the underlying physics, this activity aims to develop and implement a QB -analysis for scatter. Validation of the QB -analysis requires availability of the same reference materials in both labs involved (shipping costs), a specialized light source with calibrated intensity (quantiFlash) and a neutral density filter set. Once the QB -analysis is developed and validated, this activity will use the dataset of an earlier funded SSC activity (Coumans 2014, manuscript in preparation) to demonstrate improved reproducibility.

The research will be conducted by Dr. Josh Welsch (NIH, Bethesda, USA) and Dr. Edwin van der Pol (AMC, Amsterdam, The Netherlands) under supervision of Dr. Rienk Nieuwland (AMC, Amsterdam, The Netherlands).

- Possible references: Coumans F et al. Methodological Guidelines to Study Extracellular Vesicles. *Circulation Research* **2017**; 120: 1632-1648. DOI: 10.1161/CIRCRESAHA.117.309417