Extracellular vesicle concentrations as a biomarker for cardiovascular diseases

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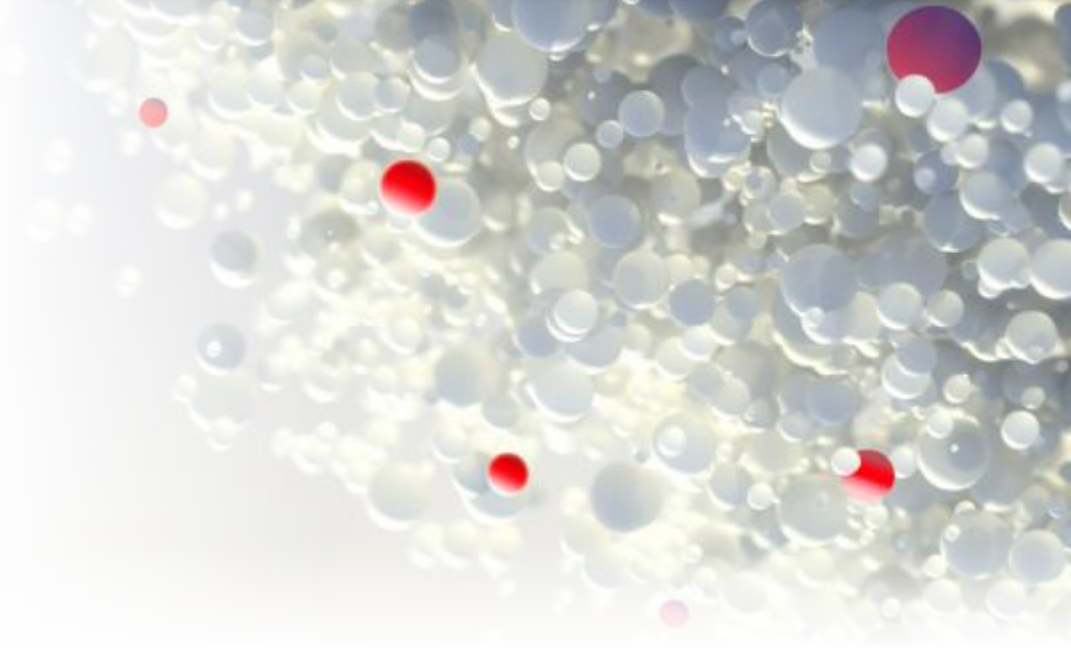
March 31st, 2023

Amsterdam UMC
University Medical Centers

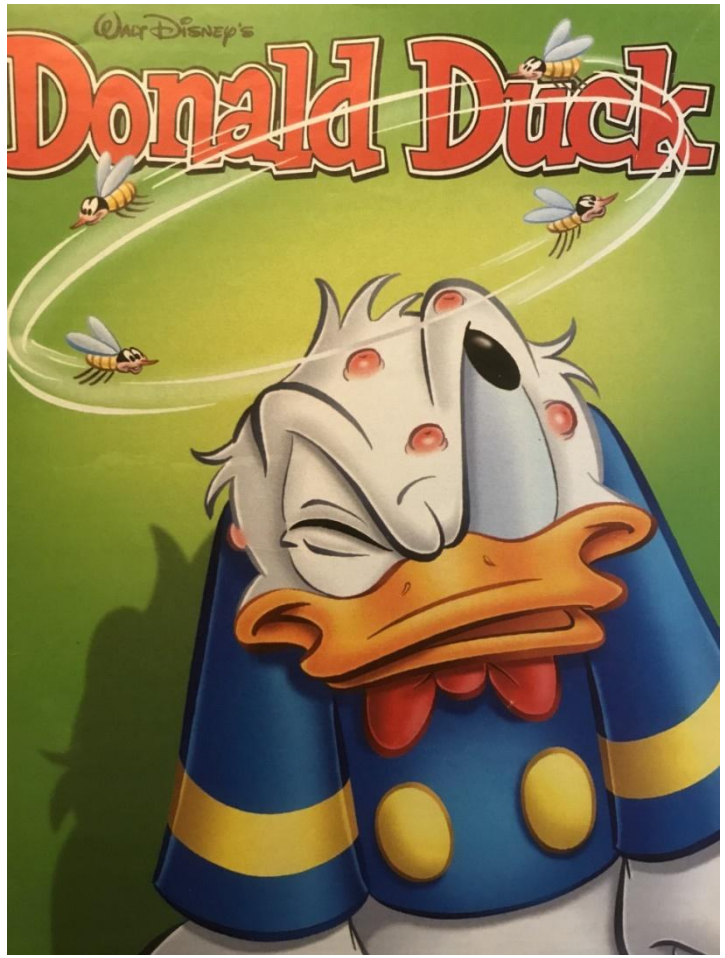
Amsterdam Vesicle Center

Outline



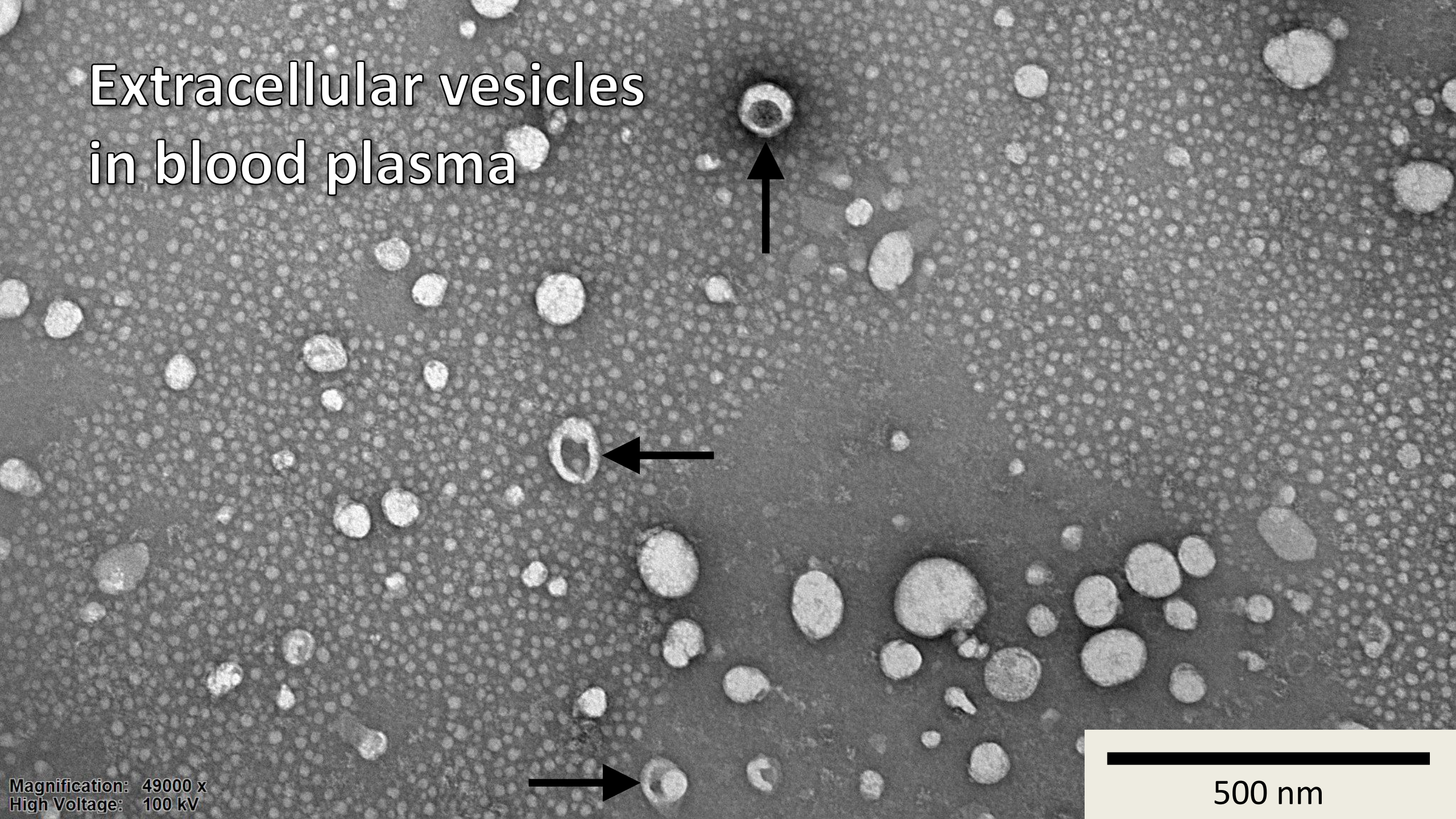
- What is a biomarker?
- Extracellular vesicles (EVs)
- Analytical challenges
- Pre-analytical challenges
- EV concentrations in cardiovascular diseases: acute coronary syndrome

What is a biomarker?

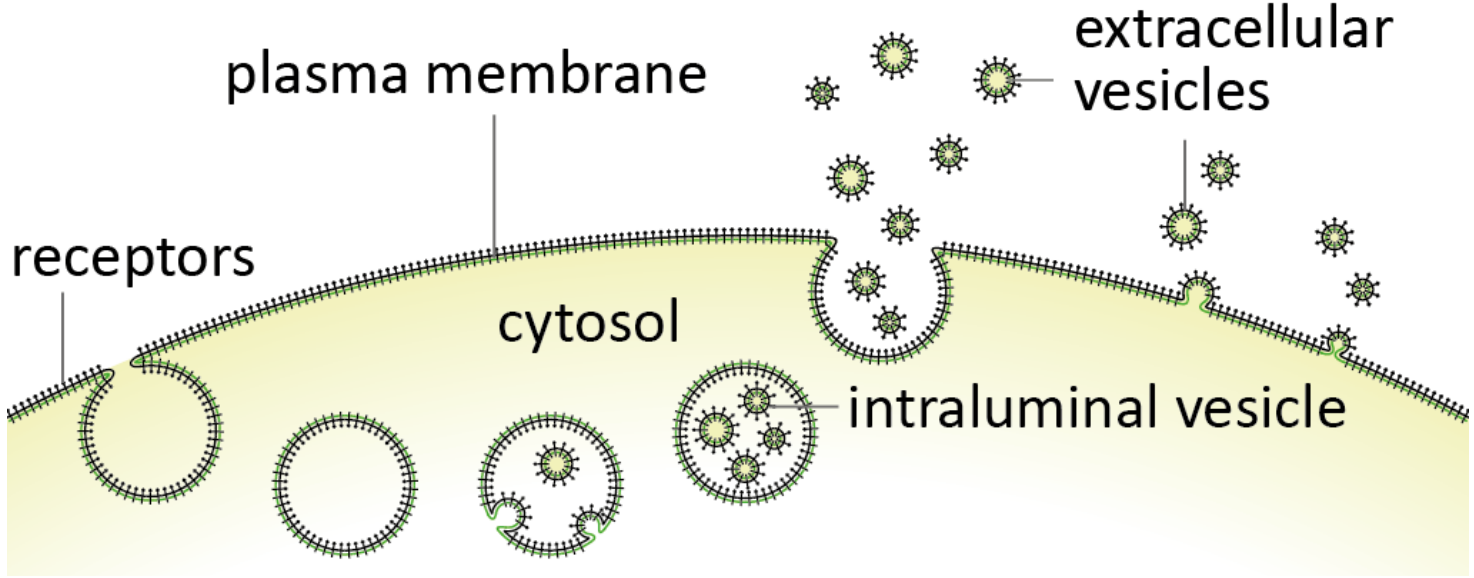


- Quantify mosquito bites
  - Number of bites
  - Height
  - Circumference
  - Color
  - Composition
- Association with clinical diagnosis or outcome

Donald Duck *2014* 37:1
Slide from Rienk Nieuwland


Extracellular vesicles
in blood plasma
Magnification: 49000 x
High Voltage: 100 kV
500 nm

Extracellular vesicles (EVs)



- cells release vesicles:
  biological nanoparticles with receptors, DNA, RNA
- specialized functions
- clinically relevant

van der Pol et al. *Pharmacol Rev* 2012

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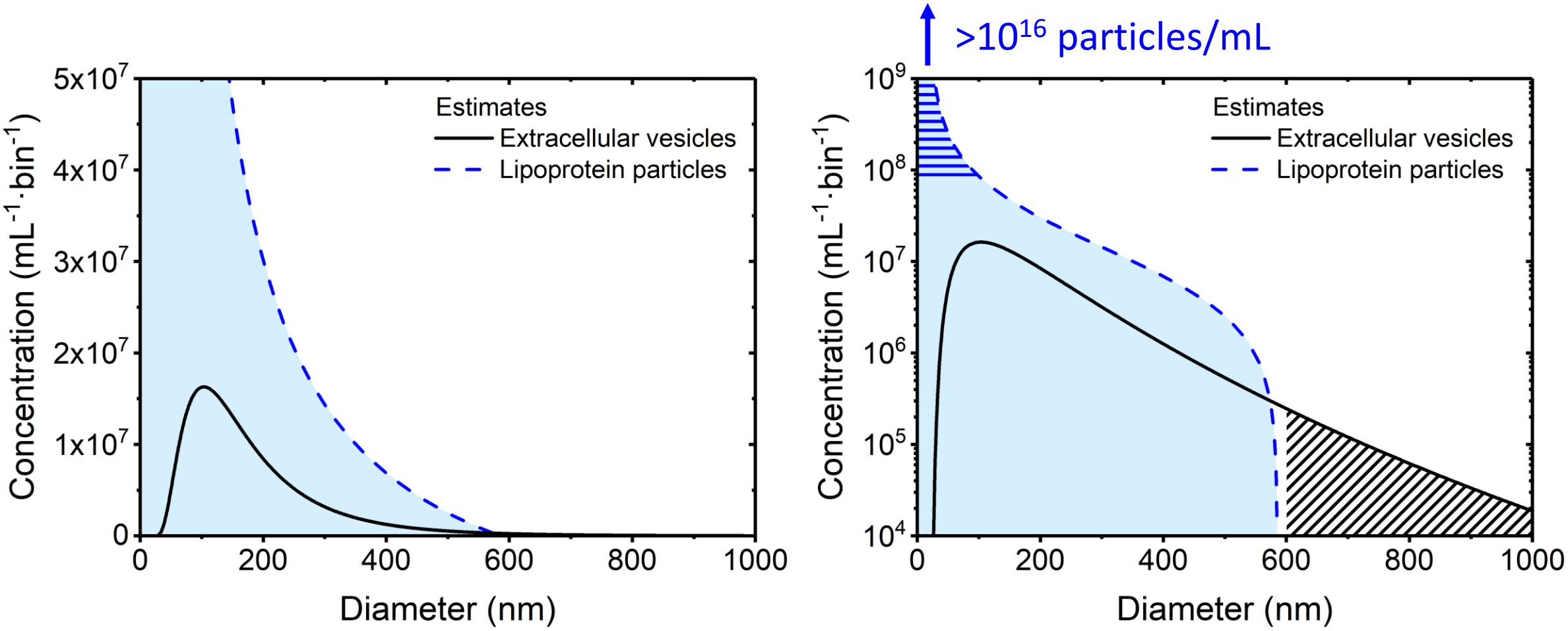
Extracellular vesicle samples and size distribution

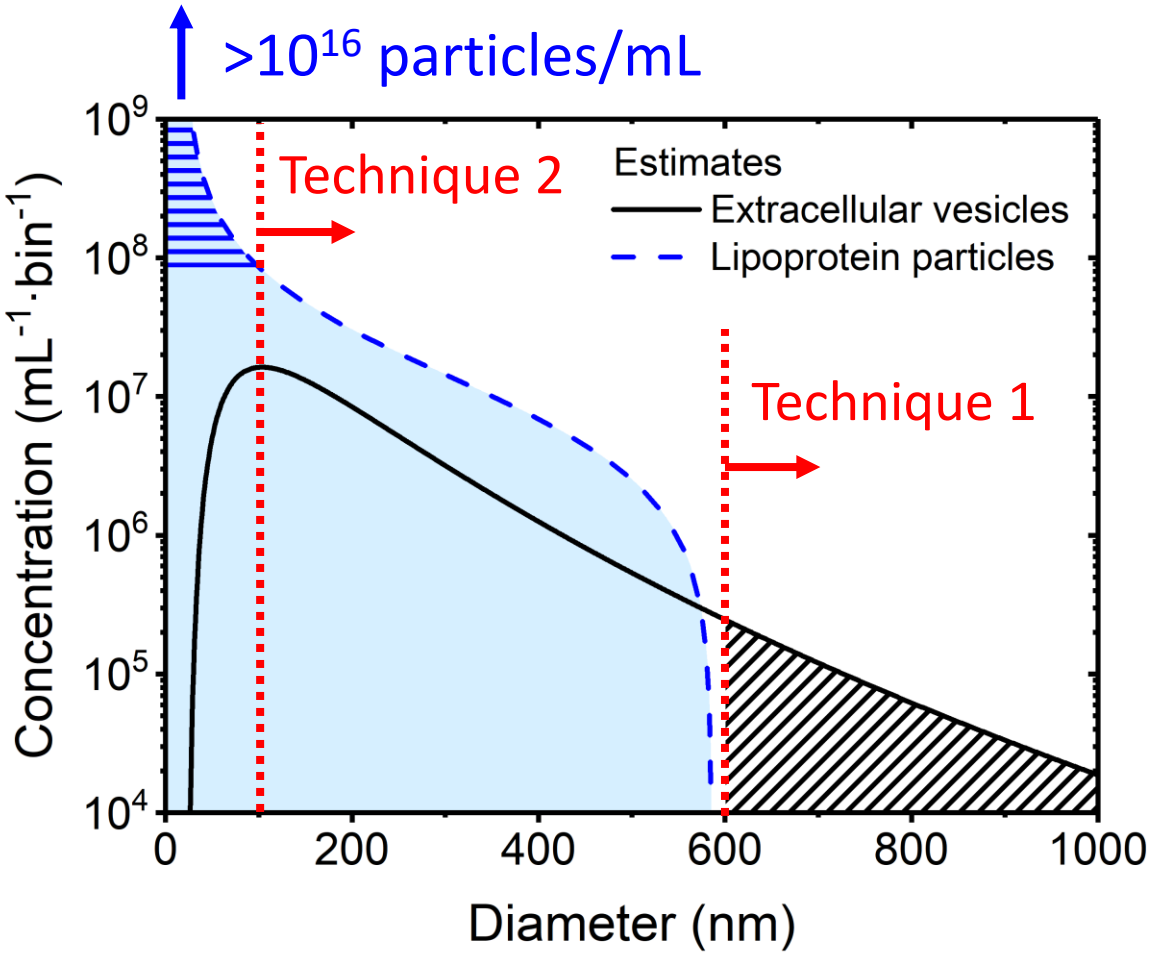


Welsh et al. *J Extracell Vesicles* **12**(2) 2023

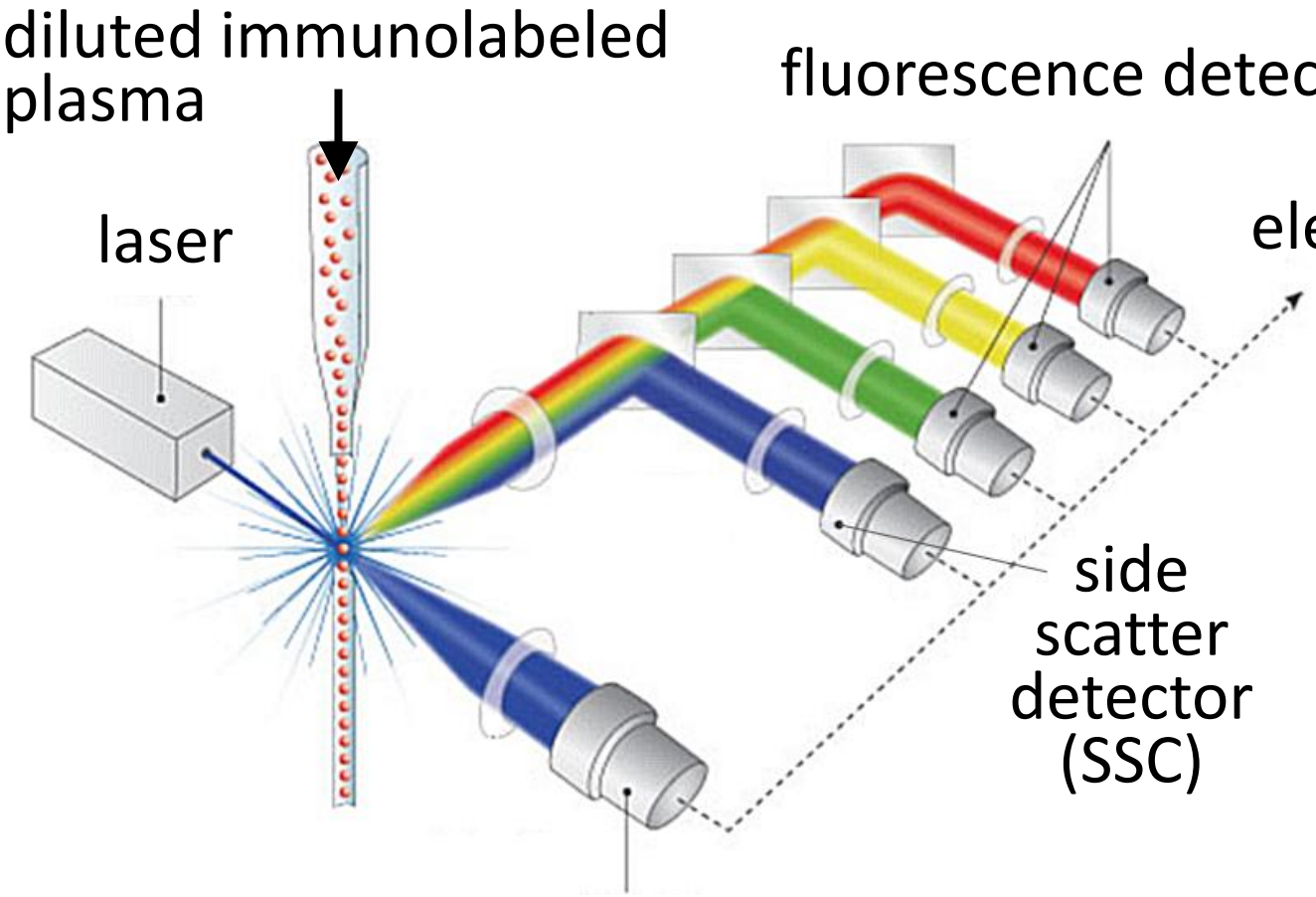
Bin width of graphs: 1 nm

Extracellular vesicle samples and size distribution

- Detection approaches
  - Isolate EVs and apply bulk technique (e.g. -omics)
  - Characterize particles <u>one by one</u>



Flow cytometry



Specialized instrument:
>~50 nm EVs | 200 particles/s

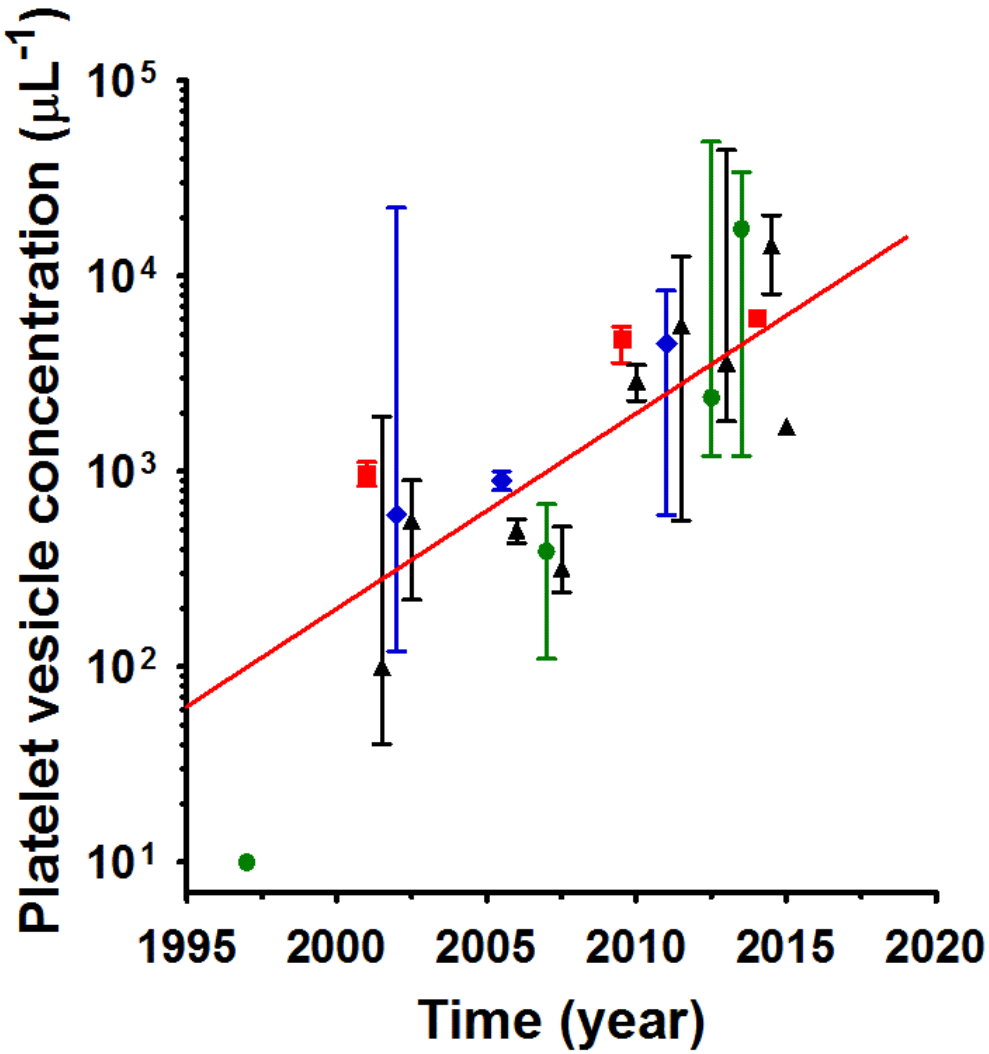
Modern flow cytometer:
>~100 nm EVs | 10.000 particles/s
~100 samples per day

Image: semrock.com

Clinical reality

- Reported concentrations of blood plasma EV differ >10^4-fold
- Clinical data cannot be compared
- Standardization required

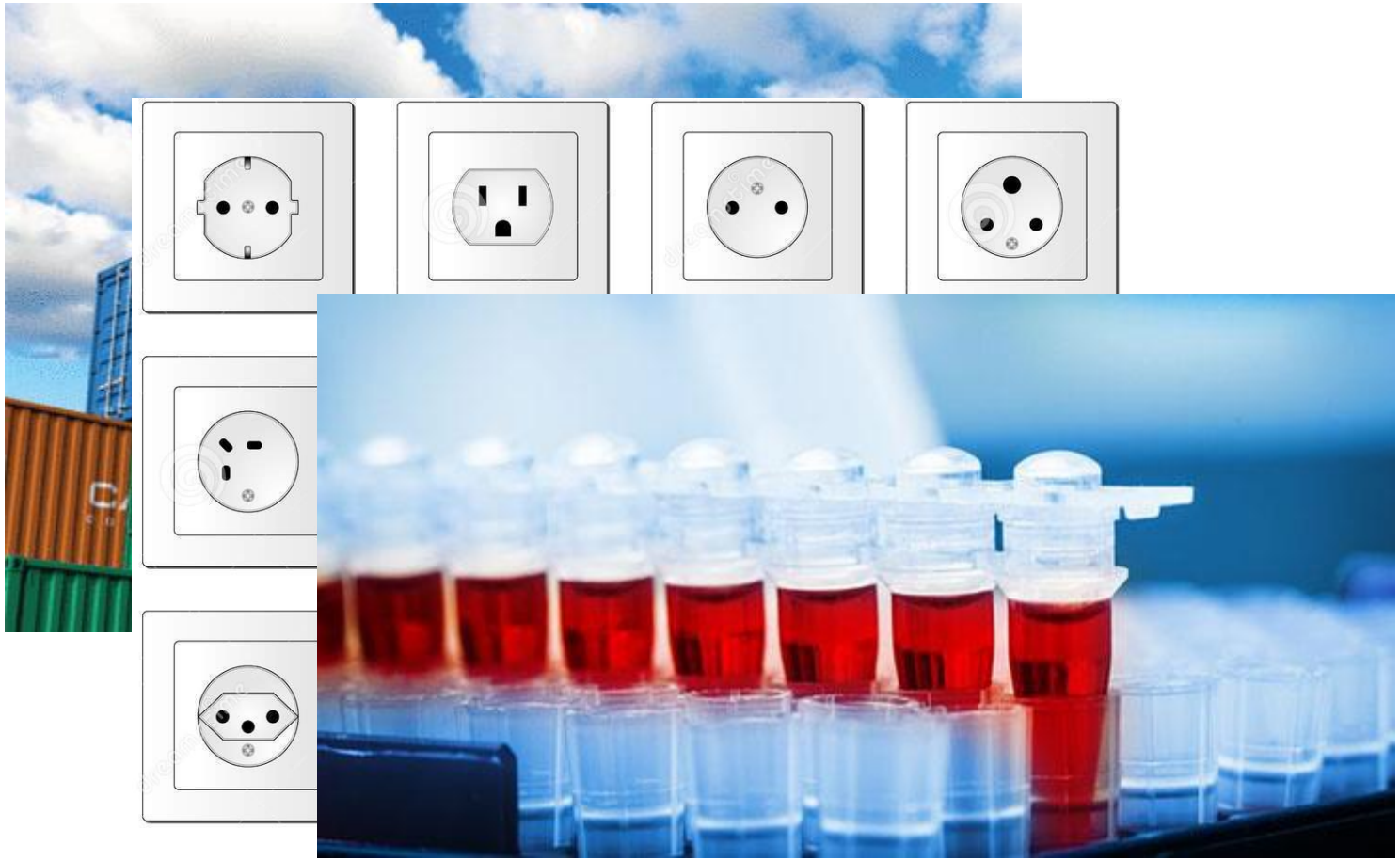
"Gąsecka's law"



Standardisation is boring (most biomedical scientists)

Slide from Rienk Nieuwland

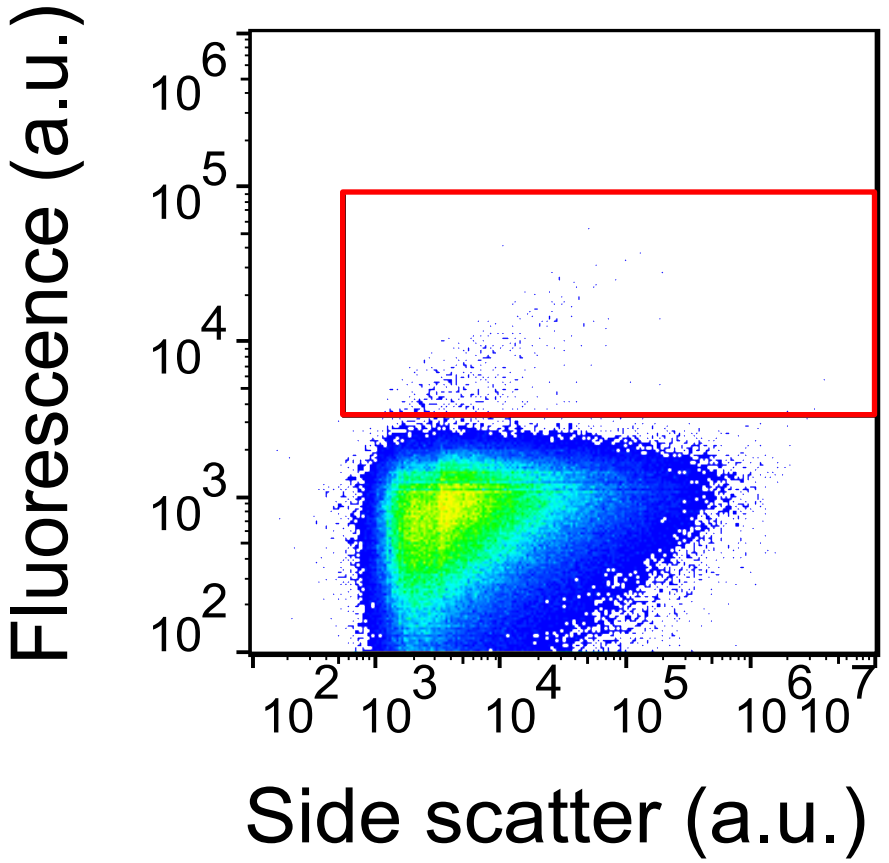
Standardization is important (everybody)



Example: unstandardized flow cytometry

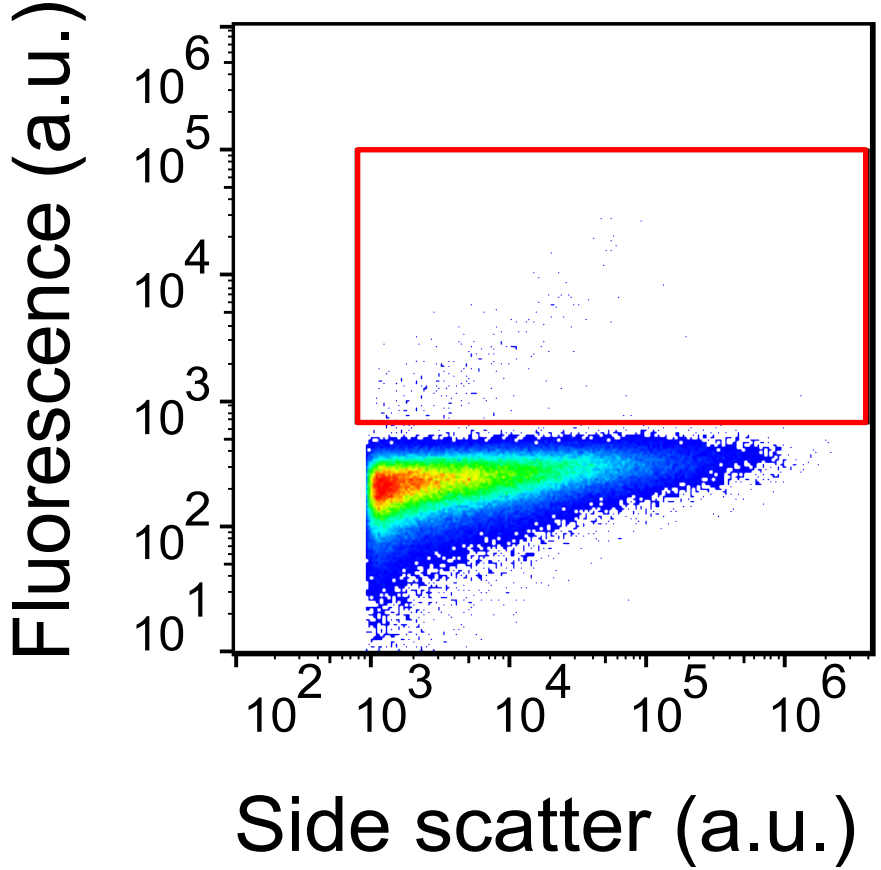
Apogee A60-Micro

Concentration = $3.8 \cdot 10^6$ /mL



Cytek Aurora

Concentration = $1.4 \cdot 10^7$ /mL

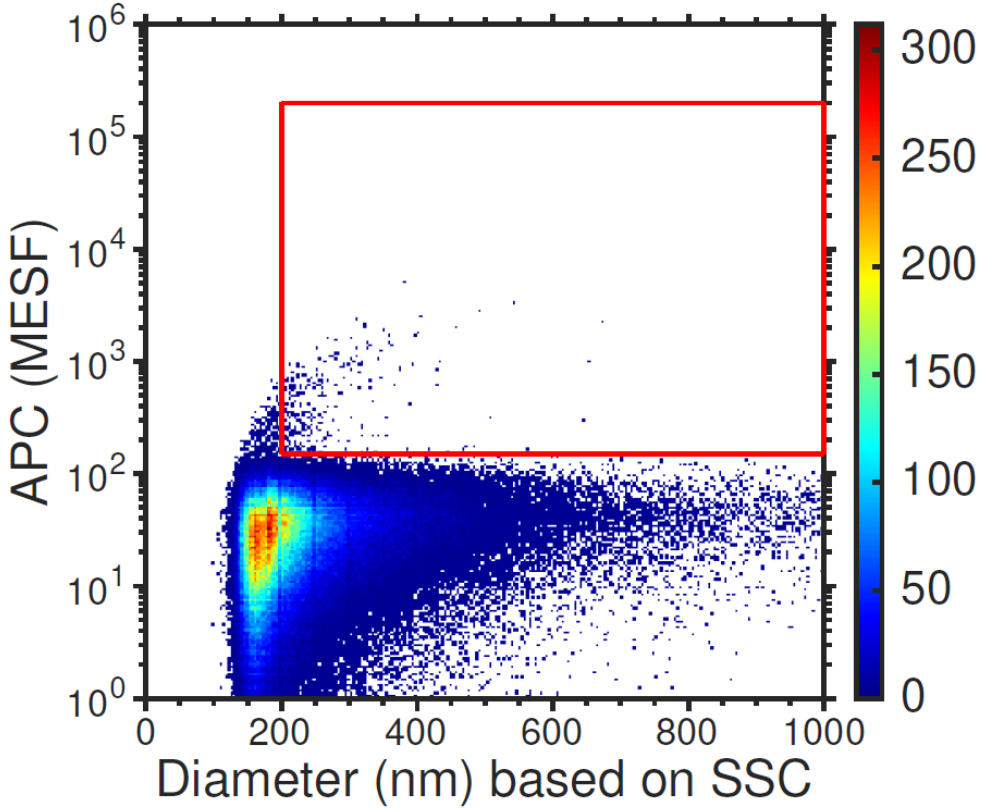


Example: unstandardized flow cytometry

Example: standardized flow cytometry

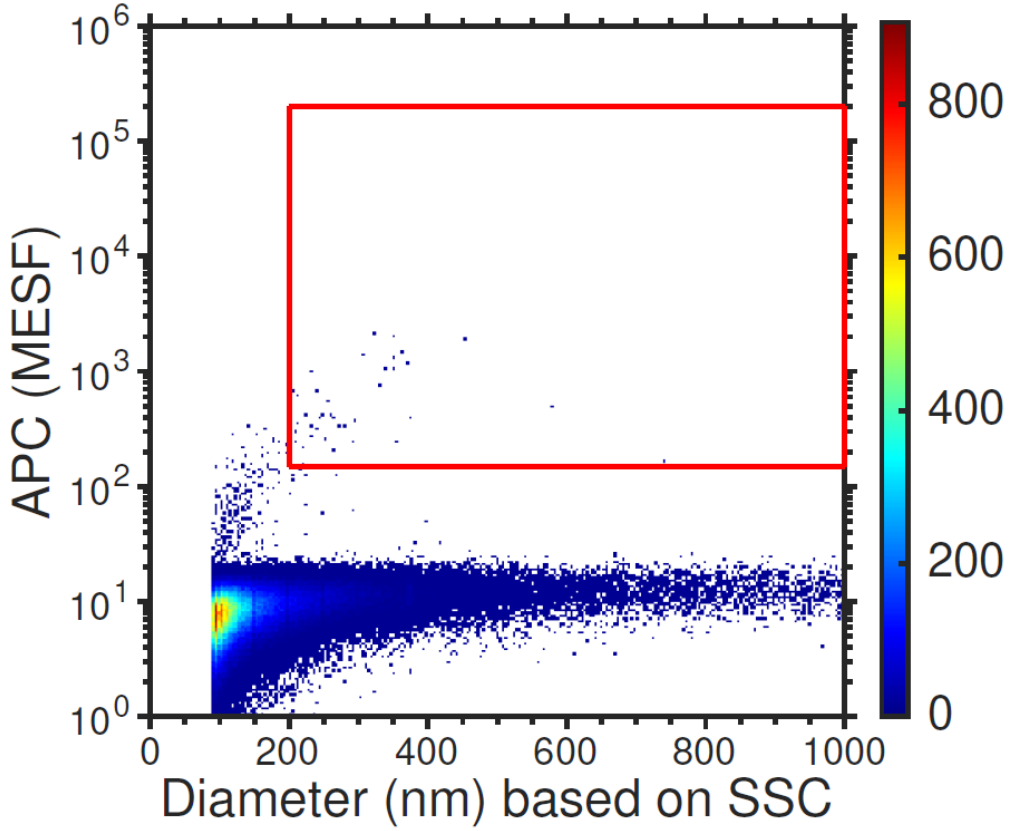
Apogee A60-Micro (50x)

Concentration = $2.7 \cdot 10^6$ /mL



Cytek Aurora (1,000x)

Concentration = $2.2 \cdot 10^6$ /mL



MESF: molecules of soluble fluorophore; SSC: side scatter

Learn to calibrate: Welsh et al. *J Extracell Vesicles* **12**(2) 2023

Amsterdam Vesicle Center EV flow cytometry summer school

- August 28th to September 1st
- Learn (almost) everything we know about EV flow cytometry
- More information: c.m.hau@amsterdamumc.nl
- Still few places available

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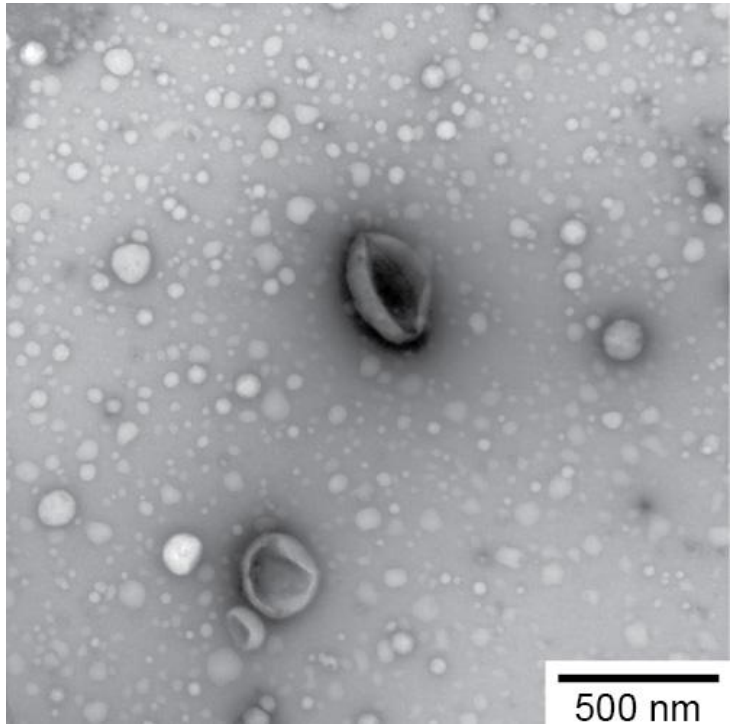
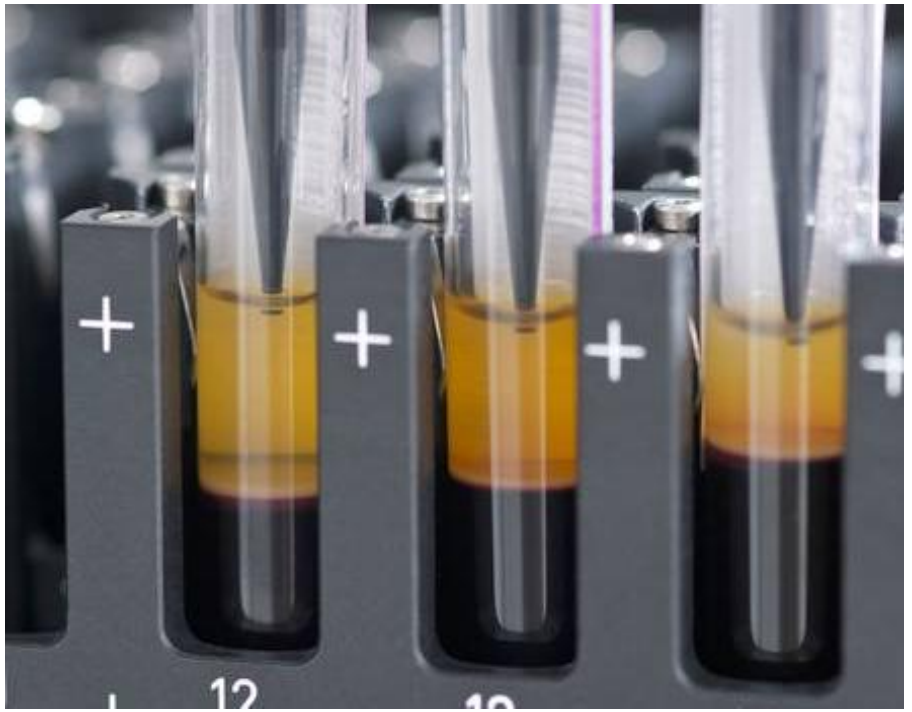
- ✔ Analytical challenges
- Pre-analytical challenges
- EV concentrations in cardiovascular diseases: acute coronary syndrome



Blood plasma preparation for biobanks

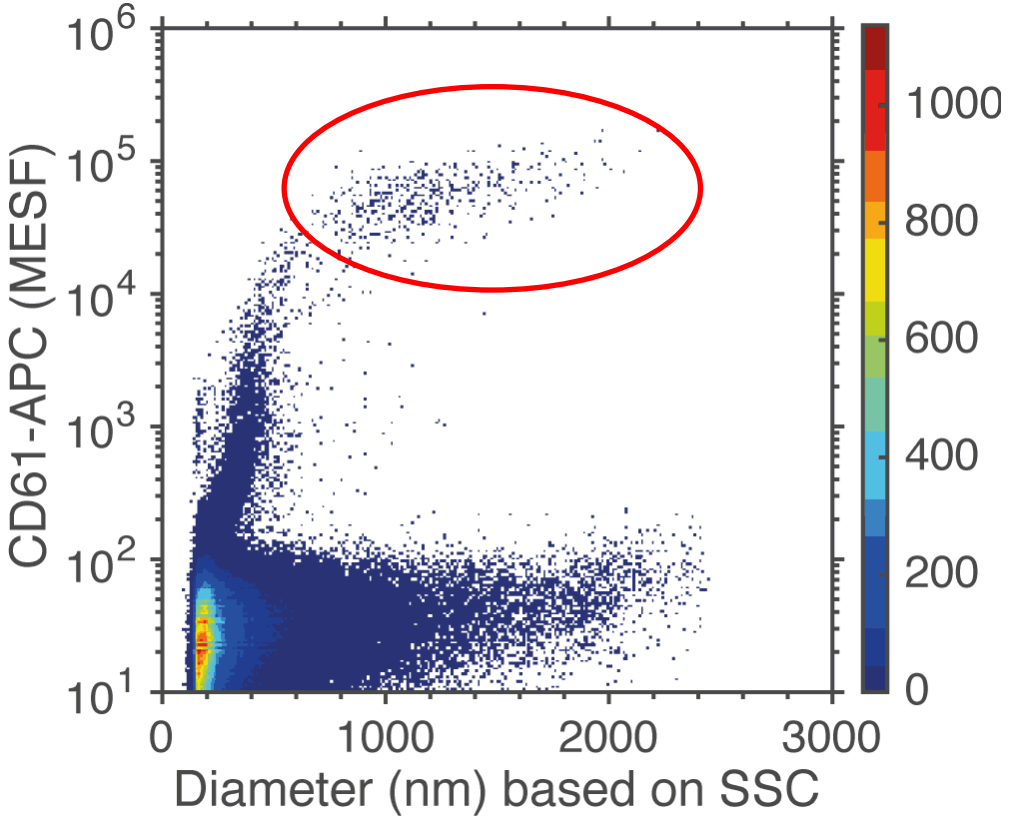
- Plasma prepared according to ISTH[1]/AHA[2] protocol
- Double centrifugation
  - 15 minutes
  - 2,500 *g*
  - 20 °C



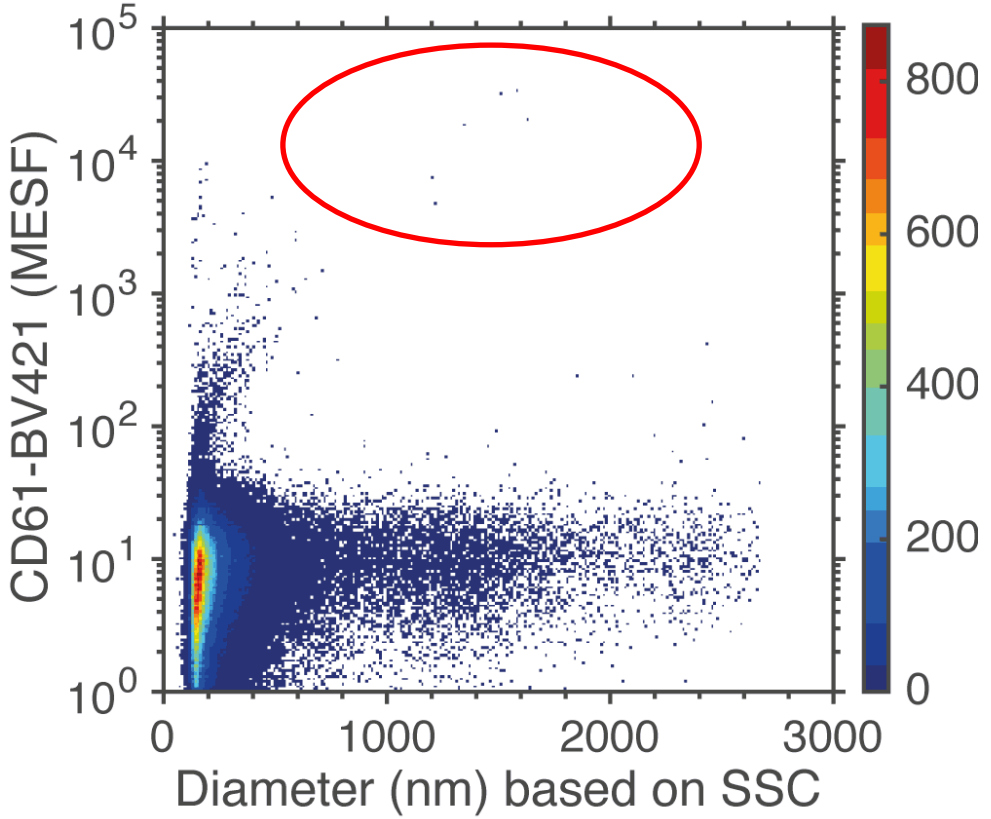
[1]Lacroix et al. *J Thromb Haemost* 2013
[2]Coumans et al. *Circ Res* 2017

“Platelet free plasma” contains platelets

Unmaintained centrifuge



Maintained centrifuge



Right data: Gasecka et al. *J Thromb Haemost* 2020

“Platelet free plasma” contains platelets

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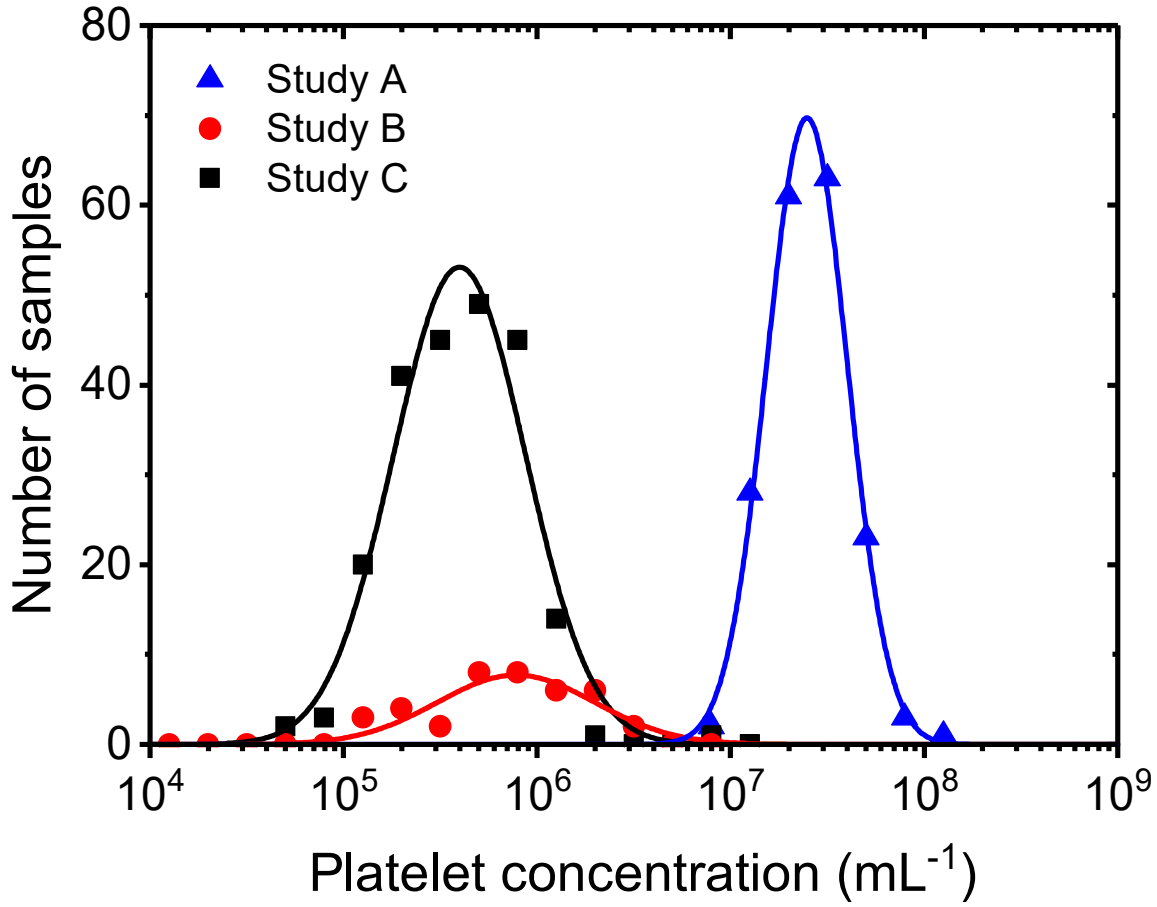
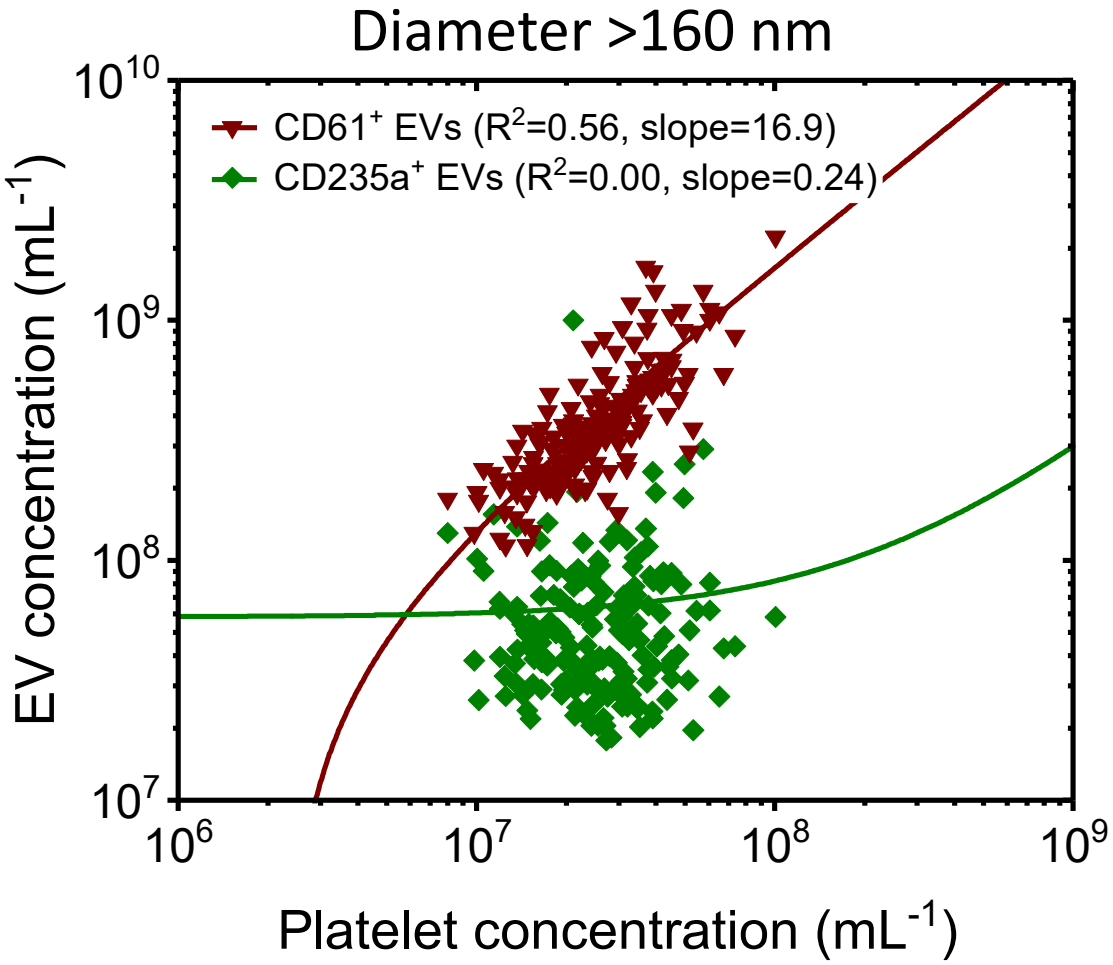


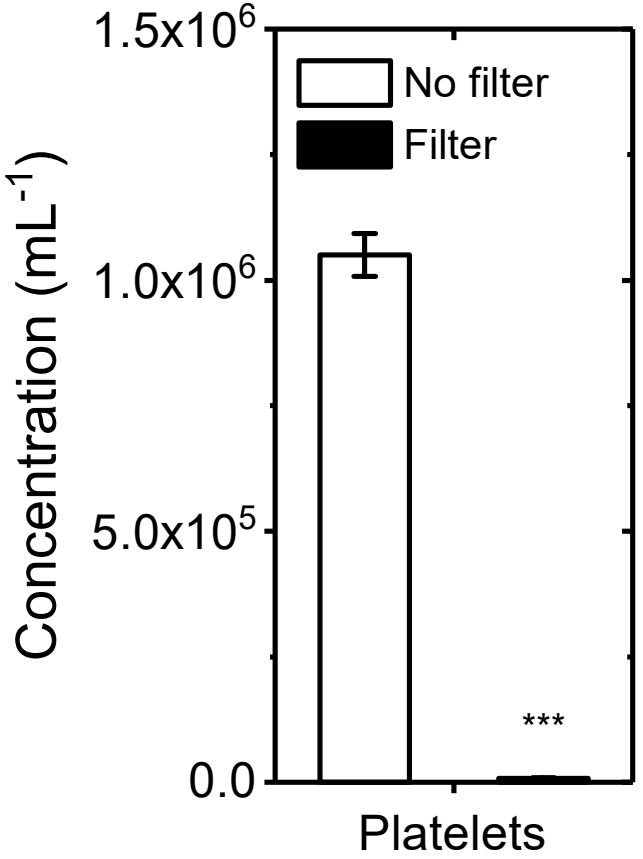
[1]Lacroix et al. *J Thromb Haemost* 2013
[2]Coumans et al. *Circ Res* 2017

Platelets in plasma affect downstream analyses



Bettin et al. *J Thromb Haemost* 2022
Bracht et al. *J Extracell Vesicles* 2023

Platelets can be removed by filtration



Polycarbonate track-etched membrane filter with 800-nm pores

Bettin et al. *J Thromb Haemost* 2022
Bracht et al. *J Extracell Vesicles* 2023

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Platelet EVs to diagnose acute coronary syndrome

- LEMONADE trial[1,2]. Problem:

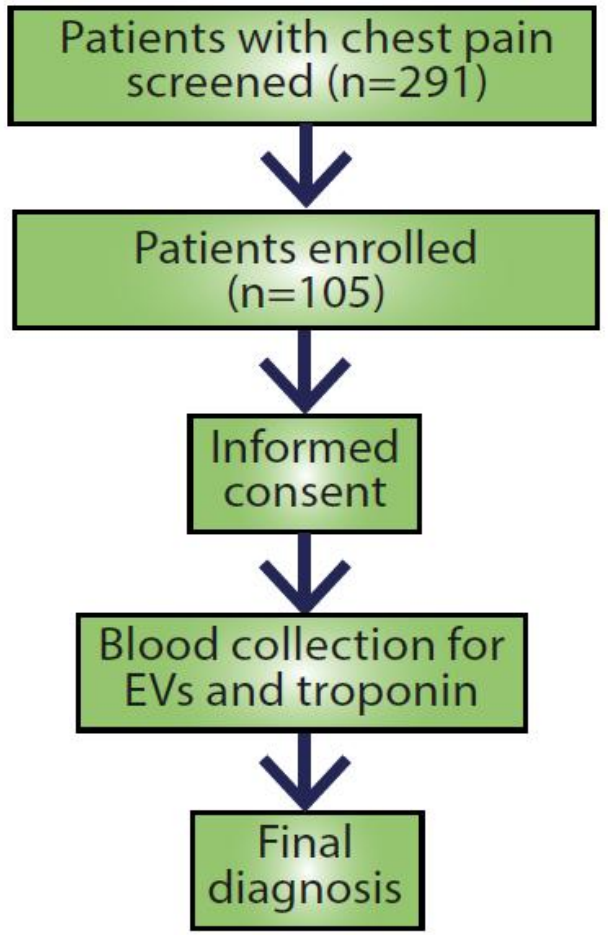
	Troponin concentration
Acute coronary syndrome (ACS)	
• Acute myocardial infarction	+
• Unstable angina	-
Other conditions	+

- Goal: to determine the diagnostic value of circulating platelet EVs as a biomarker for ACS

[1]Aleksandra Gąsecka, Medical University of Warsaw
[2]Funding: Polish Cardiac Society

LEMONADE[1,2] overview

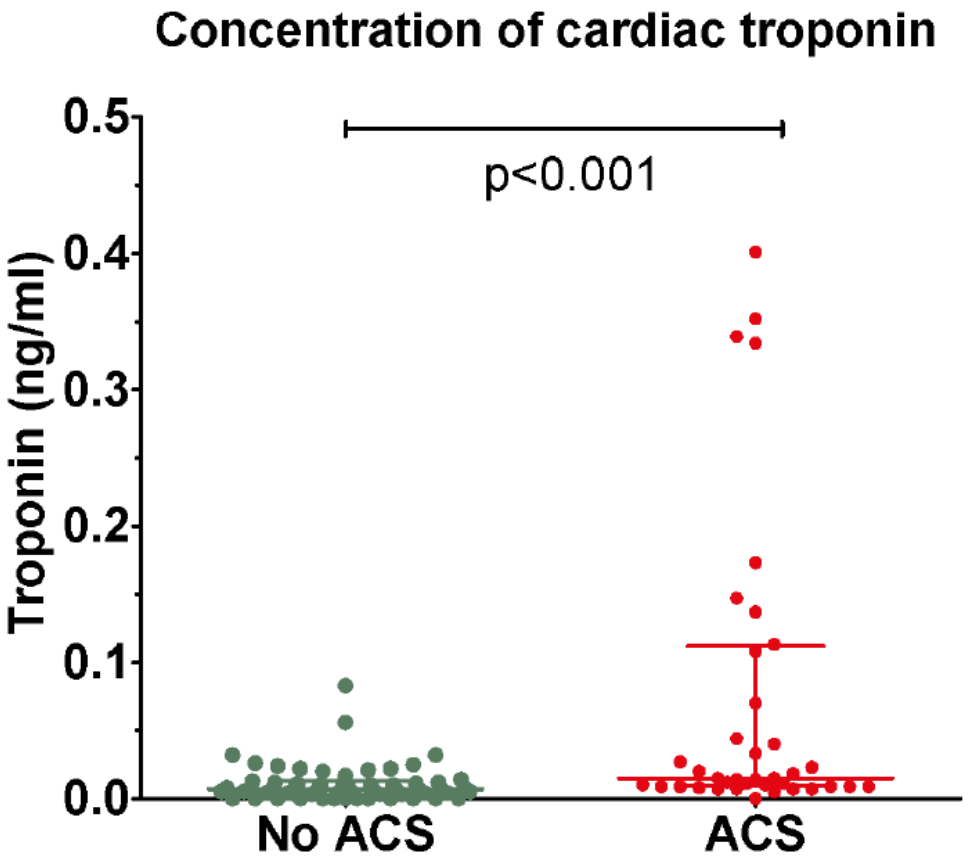
- Trial design
  - prospective
  - multicenter
- Analytical method
  - flow cytometry

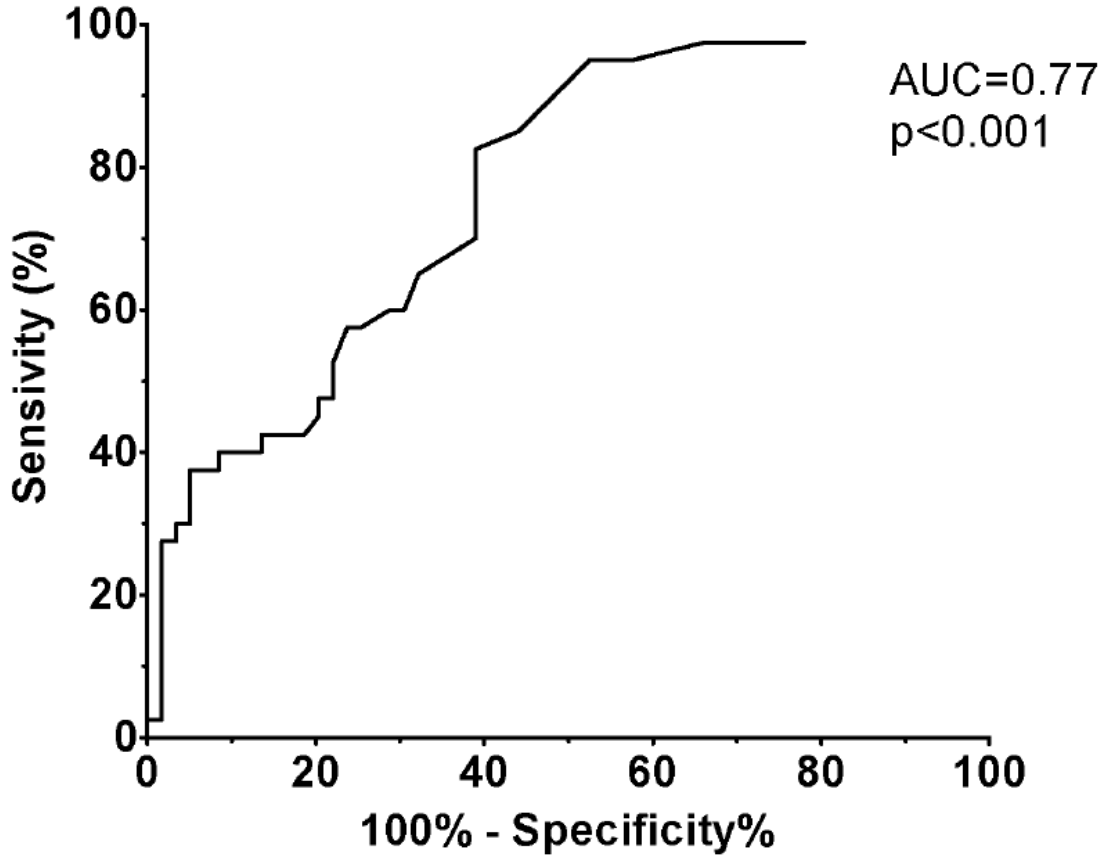


ACS: n=41 (39%)
Other cardiological: n=31 (30%)
Non-cardiological: n=33 (31%)

[1]Aleksandra Gąsecka, Medical University of Warsaw
[2]Funding: Polish Cardiac Society

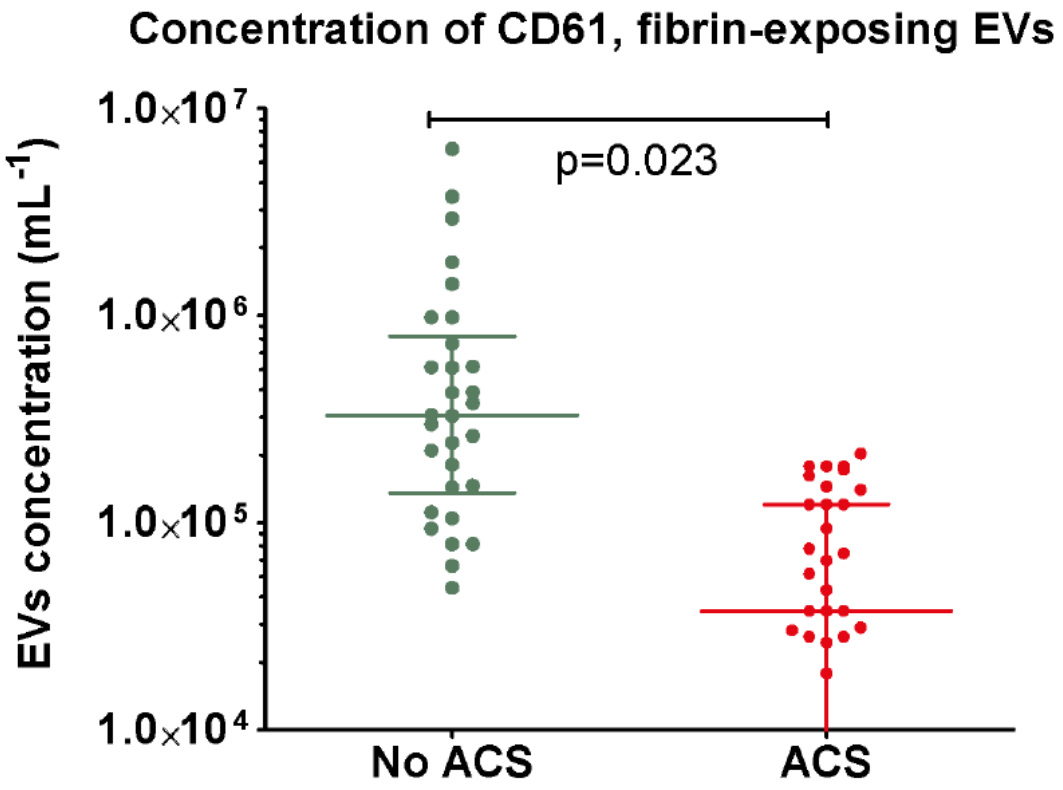
Results patients presented <2 hours after onset of chest pain

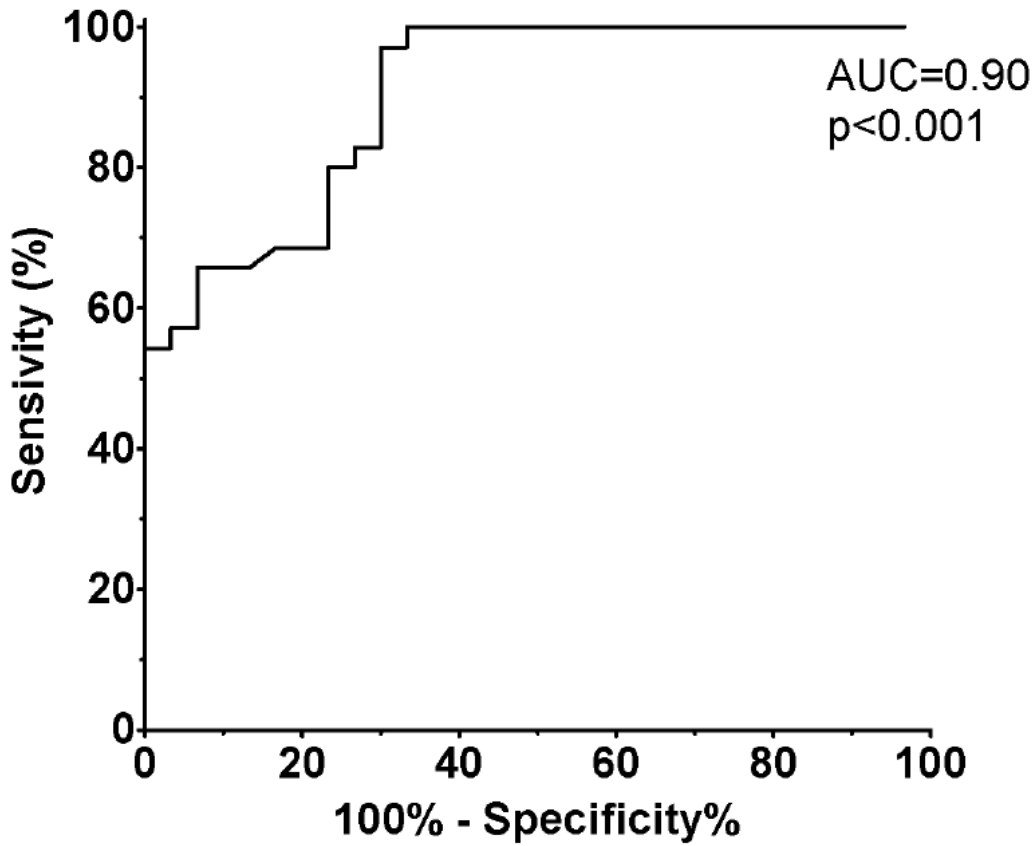




ACS: acute coronary syndrome

Results patients presented <2 hours after onset of chest pain





ACS: acute coronary syndrome

Preliminary conclusions of LEMONADE

- Fibrin^{+} EVs outperforms cardiac troponin as early biomarkers of developing ACS in patients presented <2 hours after onset of chest pain
- EV measurements might improve thrombotic risk stratification in the emergency setting

ACS: acute coronary syndrome

Summary

- Blood plasma contains extracellular vesicles (EVs): potential biomarkers in cardiovascular diseases
- (Pre-)analytical challenges (!)
  - Avoid arbitrary units
  - Look, think, do
- Fibrin^{+} EVs outperforms cardiac troponin as early biomarkers of developing ACS

Acknowledgements

- Amsterdam University Medical Centers
  - Amsterdam Vesicle Center
    - Britta Bettin
    - Chi Hau
    - Jillian Bracht
    - Rienk Nieuwland
- Medical University of Warsaw
  - Department of Cardiology
    - Aleksandra Gąsecka

Amsterdam UMC
University Medical Centers

- More information
  - edwinvanderpol.com
  - evflowcytometry.org
  - isev.org
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  - Dutch Research Council
    VIDI project 19724

Amsterdam
Vesicle Center