ISEV
INTERNATIONAL SOCIETY FOR
EXTRACELLULAR VESICLES

UNIVERSITEIT
GENT

# ISEV Workshop: Open, Reproducible and Standardized EV Research

2-3 December 2019

Ghent University, Belgium

Chair: An Hendrix | IOC: Clotilde Théry, Marca Wauben, Olivier de Wever, Kenneth Witwer

# Abstract book

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# WELCOME

Dear colleagues,

It is with great pleasure that we welcome you in Ghent to participate in the ISEV workshop: Open, transparent and reproducible EV research.

Research into extracellular vesicles has yielded important biological insights and raised the prospect of developing novel diagnostics and therapeutics for a wide range of diseases. As with other emerging and transformative fields in research, it requires a broad, supportive base for EV research and biomedical application to evolve.

To further steer open, reproducible and standardized EV research this workshop will cover four focus themes: 1) minimal experimental guidelines and transparency tools, 2) interlaboratory validation and benchmarking studies, 3) reference standards, and 4) regulatory affairs for clinical applications.

The workshop will feature a dialogue between an outstanding and diverse group of academic research scientists, clinicians and industrial partners involved in open, reproducible and standardized research in extracellular vesicles and any other field. Morning sessions will consist of focus theme-based keynote presentations interspersed with selected abstract presentations. Afternoon sessions will be dedicated to round-table discussions covering the four focus themes outlined above.

The workshop is expected to lead to one or more publications, such as position papers in the Journal of Extracellular Vesicles.

Through your participation, interest and support, you will highly contribute to the success of the workshop and the advancement of the extracellular vesicle field.

Sincerely yours,

An Hendrix (Chair)

Clotilde Théry

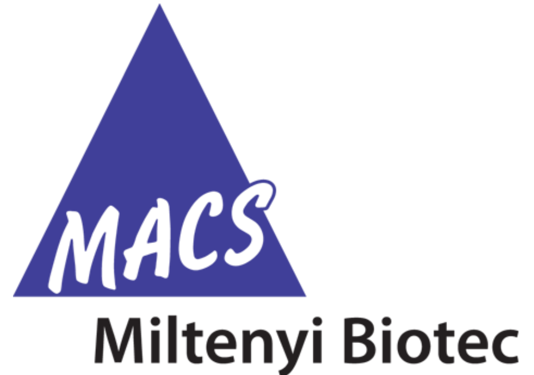
Marca Wauben

Olivier De Wever

Kenneth Witwer

SPONSORS

COMPANIES



INSTITUTIONS

# PROGRAM

## 2nd December 2019

8u00-8u45: Registration

8u45-9u00: Welcome *August Vermeylen*

9u00-11u00: Keynote presentations (35’ presentation + 5’ discussion) *August Vermeylen*

- **9u00 Anne Plant**
  Striving for confidence in biomedical research
- **9u40 An Hendrix, Clotilde Théry and Kenneth Witwer**
  EV-TRACK (transparency tools) and MISEV (guidelines)
- **10u20 Edwin Van der Pol and Marca Wauben**
  Interlaboratory and benchmarking studies: experience from ISAC-ISEV-ISTH

11u00-11u30: Networking coffee break *Kloostergang*

11u30-12u30: Selected abstracts (12’ presentation + 3’ discussion) *August Vermeylen*

- **11u30 Joshua Welsh**
  FCMPASS v3 utilizes MIFlowCyt-EV criteria and extracts flow cytometry setting information to allow transparent reporting irrespective of file sharing repository
- **11u45 Dave Carter**
  EVs from cultured cells: where do the Western Blot signals come from?
- **12u00 Rikke Baek**
  Influence of pre-analytical sample handling on small EVs
- **12u15 Luca Musante**
  Circadian variation of extracellular vesicles secreted in urine: Analysis of time point collection and normalization strategy

12u30-13u30: Networking lunch break *Kloostergang*

13u30-16u15: Round table 1 and 2 (1h15min per round table)

**Round table 1**
Topic: Minimal information guidelines and transparency tools
Moderators: An Hendrix, Clotilde Théry, Jo Vandesompele and Kenneth Witwer

**Round table 2**
Topic: Interlaboratory validation and benchmarking studies
Moderators: Olivier De Wever, Anne Plant , Edwin Van der Pol and Marca Wauben

- **13u30 Round table 1: group 1** *Oude Infirmerie*
- **13u30 Round table 2: group 2** *Priorzaal*
- **15u00 *Round table 1: group 2*** *Oude Infirmerie*
- **15u00 *Round table 2: group 1*** *Priorzaal*

16u15-16u45: Networking coffee break *Kloostergang*

16u45-17u15: Sponsor talks (12’ presentation + 3’ discussion) *Oude Infirmerie*

- **16u45 *BD: Tina Van Den Broeck***
- **17u00 *Izon: Emma Blundell***

17u15-18u30: Wrap up round table 1 and 2 + workshop product 1 *Oude Infirmerie*

18u30-20u00: Networking reception with poster *Kloostergang*

## 3rd December 2019

8u45-9u00: Welcome *August Vermeylen*

9u00-11u00: Keynote presentations (15' or 35' presentation + 5' discussion) *August Vermeylen*

- **9u00 Jo Vandesompele**
  Lessons learned from quantitative and digital PCR MIQE reporting guidelines

- **9u20 Anna Nowocin**
  Considerations for establishing EV reagents as International Standards or Reference Reagents

- **9u40 Olivier De Wever and Edwin Van der Pol**
  Reference materials

- **10u20 Bernd Giebel and Eva Rohde**
  Regulatory affairs for clinical applications

11u00-11u30: Networking coffee break *Kloostergang*

11u30-12u30: Selected abstracts (12' presentation + 3' discussion) *August Vermeylen*

- **11u30 Andre Görgens**
  New Biological Reference Materials for Standardisation of Imaging Flow Cytometry-based Single EV Analyses

- **11u45 Michael Pfaffl**
  miREV: a web-based tool for EV meta-analyses to find stable reference miRNAs

- **12u00 Diana Pham**
  From Bench to Clinic, and Everything in Between. A Regulatory Perspective

- **12u15 David Haylock**
  Bridging science and regulations

12u30-13u30: Networking lunch break *Kloostergang*

13u30-16u15: Round table 3 and 4 (1h15min per round table)

**Round table 3**
Topic: Reference materials
Moderators: Olivier De Wever, An Hendrix, Anna Nowocin and Edwin Van der Pol

**Round table 4**
Topic: Regulatory affairs for clinical application/listing questions to raise to regulatory agencies
Moderators: Bernd Giebel and Eva Rohde

- **13u30 Round table 3: group 1** *Oude Infirmerie*
- **13u30 Round table 4: group 2** *Priorzaal*
- **15u00 *Round table 3: group 2*** *Oude Infirmerie*
- **15u00 *Round table 4: group 1*** *Priorzaal*

16u15-16u45: Networking coffee break *Kloostergang*

16u45-17u15: Sponsor talks (12' presentation + 3' discussion) *Oude Infirmerie*

- **16u45 *NanoView: George Daaboul***
- **17u00 *Sysmex/Malvern: Tom Dennison***

17u15-18u30: Wrap up round table 3 and 4 + workshop product 2 *Oude Infirmerie*

18u30-19u30: Closing drink *Kloostergang*

# PARTICIPANTS

## Keynote speakers and moderators

| | |
|---|---|
| De Wever Olivier | Théry Clotilde |
| Giebel Bernd | Van der Pol Edwin |
| Hendrix An | Vandesompele Jo |
| Nowocin Anna | Wauben Marca |
| Plant Anne | Witwer Kenneth |
| Rohde Eva | |

## Attendees

| | |
|---|---|
| Aubert Dimitri | Lässer Cecilia |
| Baek Rikke | Lee Wai-Leng |
| Bedina Zavec Apolonija | Lenassi Metka |
| Bettin Britta | Li Bo |
| Blundell Emma | Llorente Alicia |
| Brichard Vincent | Lozano-Andrés Estefanía |
| Bruce Terri | Maaninka Katariina |
| Caigny Katrien | Musante Luca |
| Carter Dave | Mussack Veronika |
| Crescitelli Rossella | Nazarenko Irina |
| Cunha Raquel | O'Driscoll Lorraine |
| Daaboul George | Pfaffl Michael W. |
| Davies Owen | Pham Diana |
| Dennison Tom | Pinheiro Cláudio |
| DeRita Rachel | Puhka Maija |
| Douterloigne Karen | Radeghieri Annalisa |
| Falcon-Perez Juan | Roux Quentin |
| Frelet-Barrand Annie | Sódar Barbara |
| Geeurickx Edward | Tertel Tobias |
| Görgens André | Van den Broeck Tina |
| Haylock David | Van Gele Mireille |
| Hotham William | van Royen Martin |
| James Patrick | Van Schie Margo |
| Jones Jennifer | Varga Zoltan |
| Karnas Elżbieta | Welsh Joshua |
| Kelwick Richard | Wild Stefan |

# ABSTRACTS

## Oral presentations (alphabetical order)

1/ Baek Rikke
2/ Carter Dave
3/ Görgens André
4/ Haylock David
5/ Musante Luca
6/ Pfaffl Michael W.
7/ Pham Diana
8/ Welsh Joshua

## Poster presentations (alphabetical order)

Poster Session time: Monday 2nd December 8:00 – Tuesday 3rd December 20:00
Room: Kloostergang, Het Pand
Your poster board area is approximately 2 meters in height by 1 meter in width.

9/ Bedina Zavec Apolonija
10/ Bettin Britta
11/ Bruce Terri
12/ Crescitelli Rossella
13/ Cunha Raquel
14/ Davies Owen
15/ DeRita Rachel
16/ Frelet-Barrand Annie
17/ Geeurickx Edward
18/ Hotham William
19/ Jones Jennifer
20/ Karnas Elżbieta
21/ Kelwick Richard
22/ Lässer Cecilia
23/ Lee Wai-Leng
24/ Lenassi Metka
25/ Li Bo
26/ Lozano-Andrés Estefanía
27/ Maaninka Katariina
28/ Mussack Veronika
29/ Pinheiro Cláudio
30/ Puhka Maija
31/ Radeghieri Annalisa
32/ Roux Quentin
33/ Sódar Barbara
34/ van Royen Martin
35/ Varga Zoltan

## Sponsor abstracts (alphabetical order)

36/ Blundell Emma
37/ Wild Stefan

# 1
**Baek Rikke**

## Influence of pre-analytical sample handling on small EVs

Rikke Baek (1), Malene Møller Jørgensen (1), Kim Varming (1)

(1) Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark

With every new publication and research project in the EV field the potential for the future use of vesicles in the clinical setting increases as does the demand for EV analyses with short performance times. However, as most researchers in the field has realized by now, these vesicles are not straightforward to work with in terms of isolation, characterization etc. A major challenge when working with EVs is the pronounced impact that the pre-analytical treatment has on the analysis outcome. There are many factors to consider when preparing a protocol and there are pros and cons of any choice regarding sample collection and preparation. It is also important to keep in mind that what is most optimal for one analysis may not be appropriate for other types of analyses. I will share results from our work with small EVs analyzed on our in-house developed antibody-based platform as an example of the impact of pre-analytical sample preparation. The work related to testing the influence of different steps has primarily been performed on blood samples and includes type of sample collection tube, incubation time before initial centrifugation, centrifugation protocol, storage temperature and length, freeze-thaw cycles and transport-related stress. All parameters tested displayed varying degree of influence depending on the type of blood collection tube used. Hence, to compare results it is important to ensure that all samples are of the same type and have been handled similarly. I will also share some of our considerations for building a protocol to be used in the clinical setting of our department. One thing is strict protocols for research use with limited number of well-trained personnel. Another thing is protocols to be adapted among other diagnostic analyses being performed by a broad range of personnel and with less controlled and strict performance of the sample preparation and to some extent the analysis.

# 2

**Carter Dave**

## EVs from cultured cells: where do the Western Blot signals come from?

Elise Padbury (1), Elizabeth Dellar (1,2), Emanuela Carollo (1), Paschalia Pantazi (1,3), GenevieveMelling (1), Alberto Baena (2), Esther Becker (2), Ryan Pink (1), Dave Carter (1)

(1) Oxford Brookes University, Oxford, UK
(2) University of Oxford, Oxford, UK
(3)Imperial College London, London, UK

The current MISEV guidelines state that Western Blotting is an appropriate method for confirming the presence of extracellular vesicles (EVs). The guidelines also suggest that when using conditioned media from cultured cells, a non-condition control (where cells are omitted from the culture media) should be included. However, most studies do not include this crucial control. Here I present the results of our investigation into the importance of controls for verifying the presence and number of EVs when using cultured cells.
Our key findings are that:
a) ‘cleared bovine serum’ still contains EVs and numerous particles that are detectable using nanoparticle tracking analysis.
b) to quantify EV levels that are above the background requires at least 48 hours of conditioning
c) Western Blotting can in some cases detect proteins, including tetraspanins, in some non-conditioned media, and this may be influenced by batch-to-batch variations in the bovine serum being used or by the efficiency of EV-preclearing from serum.
I discuss the implications of these findings and make recommendations for improving the validity, reproducibility and transparency of EV research.

## 3
**Görgens André**

## New Biological Reference Materials for Standardisation of Imaging Flow Cytometry-based Single EV Analyses

André Görgens (1), Michel Bremer (2), Manuela Gustafsson (3), Dhanu Gupta (4), Oscar Wiklander (5), Bernd Giebel (6), Samir El Andaloussi (7)

(1) Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, and University Hospital Essen, Essen, Germany
(2) University Hospital Essen, Essen, Germany
(3) Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
(4) Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
(5) Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
(6) University Hospital Essen, Essen, Germany
(7) Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

The extracellular vesicle (EV) field has seen a dramatic increase of interest and research output within recent years. Nowadays, EVs are known to be released by all cell types and have relevant functions in both physiological and pathophysiological contexts related to intercellular communication. Due to their small size and heterogeneity, it is still a major challenge in the field to define sensitive and robust methods being suitable for accurate single EV analysis, especially for small EVs (<200 nm) such as exosomes. Most conventional flow cytometers are not suitable for detection of particles <300 nm, and the limited availability of suitable reference materials hampers the definition of sEV analysis techniques. In this webinar, we will share our experience in investigating the usability of a method called Imaging Flow Cytometry (IFCM) for single EV analysis. By using GFP-tagged EVs as biological reference material we optimized and defined various methodological parameters related to sample preparation, data acquisition, and analysis. We showed that fluorescently labelled, single EVs and EV subpopulations can be accurately quantified by IFCM in both isolated EVs and unprocessed EV-containing samples. Thus, IFCM-based single EV analysis will improve our understanding of EV heterogeneity and facilitate sEV biomarker identification for various diseases. Limitations and potential pitfalls for this and related methods will be summarized, and our approaches of developing and using new biological reference materials for optimization and standardization of IFCM will be shared as a proof-of-concept example how EV analysis methods can be optimized and standardized.

## 4
**Haylock David**

## Bridging science and regulations

Xenia Sango, David Haylock

VivaZome Therapeutics

The global guidelines and regulations pertaining to the development of human cells, tissues and cellular and tissue-based products, do not explicitly define how they are to be applied to products derived from extracellular vesicles (EVs). EV-based therapeutics can be defined as a biological medicine and potentially belong to the pharmaceutical class of biologicals. Regulatory frameworks for manufacturing and clinical trials for biologicals exist in Europe, Australia and the United States, but special guidelines targeting EV-based therapeutics may be needed. While the active substance in EV-based therapeutics determines their pharmaceutical classification, other important features will inform the regulatory categorisation of EV-based therapeutics. Such considerations include the type of cell they are derived from, the cargo they carry such as the type of proteins, nucleic acid, lipids as well as the nature of therapy whether it be autologous or allogenic. To ensure production of safe and effective EV-based therapeutics, manufacturing process development, target product profiles and potency tests should be based on extensive scientific analysis of EV properties and characteristics. There is an urgent need to bridge science and data with regulation. The differences that exist within the global regulatory arena adds to the level of complexity in the global development of products derived from EVs. Information and data should be jointly assessed by regulators and companies developing the EV therapeutics in order to develop a regulatory framework that facilitates the production and clinical translation of this new therapeutic modality. The relevant guidelines and regulatory pathways that may impact aspects of the safety and regulatory requirements - from the starting material to the finished product - for the pharmaceutical manufacturing and clinical application will be addressed.

## 5
**Musante Luca**

# Circadian variation of extracellular vesicles secreted in urine: Analysis of time point collection and normalization strategy

Luca Musante (1), Sabrina La Salvia (1), Uta Erdbrügger (1)

(1) Division of Nephrology and Center for Immunity, Inflammation and Regenerative Medicine, Department of Medicine, University of Virginia, Charlottesville, VA, USA

Urinary extracellular vesicles (uEVs) are an ideal source of biomarkers for kidney and urogenital diseases. Despite the great deal of interest generated by uEVs, little is known about its collection time and normalization approach. The majority of the studies on uEVs focus on spot urine collection based on the assumption that it accurately reflects the renal function, although time point of collection is not standardized. Therefore the practice to collect spot urine does not allow for calculating and standardizing accurately the uEV excretion rate which may vary during the day. In addition, no research has been carried out yet to show the quantitative and qualitative difference of uEVs between spot urine and 24h collections. The aim of this study is to compare uEVs excreted in all single voids during a 24 hour collection period and compare it with 24 hour collection performed. uEVs were enriched by differential centrifugation and electron microscopy, western blot, nanoparticle tracking analysis, tuneable resistive pulse sensing, imaging flow cytometry and miRNA quantitation by qPCR were used to quantify uEVs and associated markers variation during the 24 hour. Creatinine and particle concentration (NTA, TRPS and using 8-ANEPPS lipid dye) were used to normalize the assessed analytes. Electron microscopy showed a heterogeneous population of EVs and western blot confirmed the presence of EV markers (TSG101, ALIX and CD9). RNA was extracted by a column-based method (miRNA extraction kit Qiagen) and cel-39 miRNA was spiked in each sample. A multiparametric detection of nephron markers podocalyxin, aquaporin-2 and uEVs pan tetraspanins (CD9 + DC63 + CD81) was performed utilizing imaging flow cytometry. Whereas the uEV composition did not change across the 24 hours analysis, the quantity of uEVs and related markers fluctuated during the day depending on the hydration and excretion rate. The results of a 24 hour urine collection reflected the average results of all single voids over a 24 hr period. Creatinine and particle count normalization failed to normalize “outliers”. In conclusion, this study represents the very first report which compares single void urine versus 24 hour uEV analysis. We concluded that the 24 hour collection is the preferred choice for a robust and rigorous assessment of uEVs and its associated markers.

## 6

**Pfaffl Michael W.**

## miREV: a web-based tool for EV meta-analyses to find stable reference miRNAs

Alex, Hildebrandt (1), Benedikt, Kirchner (1), Chenna, R., Galiveti (2), Esther N., Nolte-'t Hoen (2), Michael W., Pfaffl (1)

(1) Division on Animal Physiology and Immunology, Technical University of Munich, Freising, Germany.
(2) Department of Biochemistry and Cell Biology, Utrecht University, Utrecht, The Netherlands.

To validate the results of RNA-seq studies, candidate transcripts are commonly confirmed using RT-qPCR. In order for the results to be as accurate as possible, normalization by stable reference transcripts, which are still elusive for extracellular vesicles (EV), is required. Different studies and experimental setups will likely yield distinct candidate reference miRNAs, which hampers comparability across studies. We collected in-house and publicly available metadata on pre-analytical variables, EV isolation, RNA isolation, and small RNA-seq. In this study data sets from a variety of human blood EV studies were analyzed from healthy donors and various patients cohorts suffering from diverse diseases. The obtained suggestions for stable reference miRNAs are made available in an easy-to-use web tool, named miREV. Small RNA-seq datasets comprising a total of 654 samples were processed by a standardized data analysis pipeline to account for a variety of experimental setups. Robust filter steps to remove data of poor quality, as well as six different normalization methods (total count, median, quantile, upper quartile, trimmed mean of M-values and median of expression ratios) and three stability algorithms (BestKeeper, geNorm, NormFinder) for finding robust reference transcripts were implemented. Results can be further refined by e.g. EV isolation method or disease/treatment context to match experimental setups as close as possible to individual users. Suggested reference candidate lists are visualized to easily facilitate comparisons and to detect influences of different experimental procedures or the disease context. miREV represents a useful tool for scientists working with EVs, to find suitable reference transcripts for their own experiments without the need to priorly run RNA-seq. The inclusion of additional data sets in the future that span a variety of patient populations, sample or tissue types, or EV isolation methods will boost flexibility and increase the statistical validity and robustness of suggested reference transcripts for all kinds of experimental setups.

## 7
**Pham Diana**

## From Bench to Clinic, and Everything in Between. A Regulatory Perspective.

Diana Pham (1), Desmond Pink (1), Catalina Vasquez (1), Robert Paproski (1), Colin Coros (1), Michael Wong (1), Renjith Pillai (1), Rebecca Hiebert (1), Aditya Muley (1), Stepan Hlushak (1), Seah Rah (1), Leanne Stifanyk (2), Sylvia Koch (2), Perrin Beatty (3), and John Lewis (1,3)

(1) Nanostics, Edmonton, Canada;
(2) DynaLIFE Medical Labs, Edmonton, Canada
(3) University of Alberta, Edmonton, Canada

Since 2014, extracellular vesicle (EV) research has brought together experts to establish guidelines to ensure reproducibility and transparency of EV research. MISEV (Minimal Information for Studies of EVs) requirements have been central in promoting standardization in this area. Research on EVs has shown promising clinical applications for disease diagnosis and prognosis. As interest in the clinical utility of EVs continues to grow, quality assurance criteria need to be established to successfully implement EV-based assays into clinic. Translation of an EV assay from the bench to the clinic requires the implementation of rigorous validation and regulation parameters to ensure the safety and efficacy of the assay when used for its intended purpose. Nanostics has developed a diagnostic platform that incorporates clinical and blood-based EV microflow data with advanced machine learning algorithms to generate a fingerprint score that determines the patients' risk for disease. Our first diagnostic blood test, ClarityDX Prostate, identifies men with clinically significant prostate cancer. Nanostics has partnered with a major Canadian medical laboratory called DynaLIFE to conduct an ongoing, 2800 patient, clinical study to validate ClarityDX Prostate. With the help of regulatory consultants and the vast information available from regulatory bodies such as FDA and Health Canada, Nanostics has introduced regulation to the ClarityDX Prostate test. There were many facets to consider during each stage of development to ensure a safe, effective, and high-quality product. From software and instrument qualification, assay validation, establishment of a Quality System; our ongoing experience implementing regulation into our workflow has drastically changed our perspective on translational research. It is imperative to follow the standards set forth by regulatory bodies to ensure the successful development of our assay into a medical laboratory-grade platform test for prostate cancer.

**8**
**Welsh Joshua**

## FCMPASS v3 utilizes MIFlowCyt-EV criteria and extracts flow cytometry setting information to allow transparent reporting irrespective of file sharing repository.

Welsh J.A. (1), Jones J.C. (1)

(1) National Institutes of Health, Bethesda, USA

EVs are small, heterogeneous, and therefore difficult to measure. Flow cytometry (FC) is a powerful method that provides accurate and precise enumeration of single cells, and measurement of their molecular components. For these reasons there is an interest in applying FC to the measurement of individual EVs. However, most commercial flow cytometers, and the assays that employ them, (i) were designed for the analysis of cells that are orders of magnitude larger than EVs, (ii) are not readily adapted to the measurement of EVs, and (iii) generate data which can only be interpreted if all experimental details are reported. Limitations in instrument sensitivity, assay specificity, and a general failure to adequately report experimental details together have produced a scientific literature that is rife with artefacts. Reference materials for calibration and quality control are desperately needed in the field along with software to enable calibration and metadata extraction. The FCMPASS software package was developed to enable a free, ergonomic, light scatter calibration of flow cytometry data, along with extracting diameter or refractive index from light scatter signal. With the development of the MIFlowCyt-EV framework the FCMPASS package has been expanded to allow calibration of light scatter and fluorescence parameters, writing the information to the flow cytometry files for downstream analysis and importantly sharing the data. The utilization of the FCMPASS software provide a method of transparent report of EV data in publications, but also for the standard characterization of reference materials. The extraction of flow cytometry settings from the files also makes it possible to easily share all meta data related to an experiment and makes it possible to share experimental data in any online repository.

# 9

**Bedina Zavec Apolonija**

## Analysis of EVs by Microscale Thermophoresis

Apolonija Bedina Zavec (1), Marjetka Podobnik (1), Gregor Anderluh (1)

(1) Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Slovenia

Microscale Thermophoresis (MST) is recently developed biophysical technology for the analysis of molecular interactions. The motion of molecules in microscopic temperature gradients allows the quantification of biomolecule interactions by changes in conformation, charge and size of a molecule induced by a binding event. MST is a quick method, easy to handle, has a low sample consumption, has no limitation on molecule size, and it enable the measurements in a free solution, in a buffer or complex biological liquid. These properties make MST an interesting tool for research of EVs; therefore, our aim is to apply this method to EVs. The problem comes from tiny capillaries for sample mounting and adhesion of EVs to a large surface of capillaries. We have tested all capillaries available on the market and capillaries homemade coated by different solutions. However, the results are not so convincing yet to be presented.

## 10
**Bettin Britta**

## Off-the-shelf: stable, ready-to-use, pre-labeled human plasma and urine-derived EVs

B. Bettin (1,2)*, K. Maaninka (3)*, C. Hau (1,2), R. Nieuwland (1,2), P. Siljander (3), E. van der Pol (1,2,4)

(1) Department of Clinical Chemistry, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands;
(2) Vesicle Observation Center, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands;
(3) Department of Biosciences, Division of Biochemistry and Biotechnology and Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland;
(4) Department of Biomedical Engineering and Physics, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
(*: authors contributed equally)

Introduction: Extracellular vesicles (EVs) in body fluids are explored as biomarkers of diseases. Many laboratories use flow cytometers (FCMs) to characterize single EVs, but the measured concentrations of EVs are incomparable between FCMs. To improve comparability, development of reference materials and methods are needed. In addition, the validation of new reference materials and methods requires stable biological test samples. Our aim is to develop stable, ready-to-use, and well-characterized biological test samples containing EVs.
Methods: In a preliminary experiment, urine and plasma were collected from healthy donors, and EVs were labeled with lactadherin-FITC. Labeled EVs were isolated by size-exclusion chromatography, after which the isolated EVs were mixed with the cryopreservation agents dimethyl sulfoxide (DMSO), ExoCap, or trehalose, frozen in liquid nitrogen, and stored at -80°C for 1 day and 5 months. After thawing, EV concentrations were measured by a calibrated FCM (Apogee A60-Micro).
Results: The measured EV concentrations stored for 1 day decreased 27% (p=0.04; mean of the 3 cryopreservation agents) in plasma and 35% (p=0.05) in urine, relative to the fresh starting materials. After 5 months of cryopreservation, the concentration of plasma-derived EVs decreased 2.0% (DMSO and Exocap) and 8.5% (trehalose) compared to 1 day of storage. The concentration of urine-derived EVs decreased of 6-18% after 5 months cryopreservation compared to 1 day of storage.
Conclusions: These preliminary results show that pre-labeled plasma-derived EVs can be stably stored for up to 5 months when using appropriate cryopreservation agents. Although urine-derived EVs seem less stable, other cryopreservation agents will be tested to assess their cryopreservation stability. Our optimized biological test samples will be key to validate newly developed reference materials and methods.
Acknowledgements: This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme.

## 11
**Bruce Terri**

# High Throughput Isolation and Purification of Extracellular Vesicles Using Hydrophobic Interaction Chromatography on Capillary-Channeled Polymer (C-CP) Fiber Phases

Terri Bruce (1), Lei Wang (2), Sisi Huang (2), Ken Marcus (2)

(1) Department of Bioengineering, Clemson University, Clemson, SC, USA
(2) Department of Chemistry, Clemson University, Clemson, SC, USA

Ovarian Cancer (OC) is extremely difficult to diagnose in its earliest stages because it is largely asymptomatic until late stages of development. When diagnosed at a late stage, the 5-year survival rate for OC is ~15% as compared to nearly 90% if diagnosed early. Unfortunately, 67% of OC patients are diagnosed at an advanced stage. A straightforward, affordable screening test for early detection could lead to vast improvements in survival rates. One promising tool for development of an early-stage screening test is the capture of extracellular vesicles (EVs). Found in bodily fluids, including blood, urine, and cervical mucus, preliminary studies indicate they may be used to diagnose and monitor diseases, such as OC, via a non-invasive, liquid biopsy. On a cellular level, EVs are also attributed with playing a key role in intercellular communication, and may eventually be exploited for targeted drug delivery. In order for exosomes to become useful in disease diagnostics, and as burgeoning drug delivery platforms, they must be isolated efficiently and effectively without compromising their structure. Unfortunately, current EV isolation is normally costly and time-consuming, making a straightforward, inexpensive test presently unfeasible. To address issues associated with the isolation of EVs from bodily fluids, we have developed a more efficient, cost effective method of isolation using patented poly(ethylene terephthalate) (PET) capillary-channeled polymer (C-CP) fibers in a hydrophobic interaction chromatography (HIC) protocol to remove EVs from bodily fluids. The fibers are inexpensive, can be modified to provide specific affinities to biomarker proteins, and may be packed into columns to create an efficient isolation platform. Our initial results demonstrate the ability to isolate EVs with comparable yields and size distributions and on a much faster time scale when compared to traditional isolation methods, while also alleviating concomitant proteins and other impurities. As a demonstration of the potential quantitative utility of the approach, a linear response (particles injected on-column vs peak area) using a commercial exosome standard was established using a standard UV absorbance detector. Based on the calibration function, the concentration of a human plasma sample was determined and subsequently confirmed by NTA measurement. We have also confirmed the potential for scalable separations covering sub-milliliter spin-down solid phase extraction tips to the preparative scale.

# 12

**Crescitelli Rossella**

## Reassessment of extracellular vesicle subpopulations present in human tumor tissues

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Background: Extracellular vesicles (EVs) are released from all cells and mediate intercellular communication. To identify relevant EV-based biomarkers, tissue-derived EVs are preferred over cell lines because EV functions may be affected by long time cell cultivation. The aims of this study were to establish a method to isolate and characterize EV subpopulations from human tumor tissues. Methods: We established a novel protocol for EV isolation from metastatic melanoma using enzymatic treatment (Collagenase and DNase), differential ultracentrifugation and density separation. Small and large EVs were separated and further divided into high and low density fractions. All EV subpopulations were characterized by electron microcopy (EM), Bioanalyzer and quantitative mass spectrometry. Results: EM showed that the interstitial space of metastatic melanoma tissue contained vesicles. Large vesicles (200-800 nm) and low density EVs showed a RNA profile with 18S and 28S ribosomal subunits. By contrast, small vesicles (40-100 nm) and high density EVs carried small RNA but no or small peaks for ribosomal RNA. Quantitative proteomic results showed the presence of EV-proteins such as TSG101, RAB proteins and Flotillin in both large and small low density EVs, while ADAM10, Prostaglandin F2 receptor negative regulator and transferrin receptor protein 1, were exclusively enriched in small low density EVs. Interestingly, several proteins commonly detected in EVs such as Enolase 1, GAPDH and AGO2 were identified in the high density fractions. Conclusions: Collagenase and DNase treatments enhance EV isolation from fibrotic tissues, without any effect on molecular and morphology EV characteristics. Using size and density separations, we found different proteins enriched in specific EV subpopulations and several proteins expressed in more than one subpopulation. This depth categorization of EV subpopulations from tumor tissues may help to understand the EV functions and their possible use as biomarkers.

# 13

**Cunha Raquel**

## Towards the development of standardized and robust platforms for the production and characterization of mesenchymal stromal cell-derived extracellular vesicles

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Cellular therapies using mesenchymal stromal cells (MSC) held great promise. However, the efficacy of MSC therapies failed the expectations partially due to loss of cell viability and potency upon administration. MSC-derived extracellular vesicles (MSC-EV) were reported to mimic the regenerative and immunomodulatory potential of the cells of origin, paving the way for their use as cell-free therapy. The use of MSC-EV in the clinics requires the development of robust, scalable, standardized and good manufacturing practice-compliant processes for their production and isolation, coupled with product characterization platforms to evaluate their quality and potency. To advance the field towards an optimized and scalable process for the production of MSC-EV with higher potency, we studied the effect of MSC source (adipose tissue, bone marrow, umbilical cord matrix), culture system (2D static culture flask, 3D dynamic systems), culture medium (serum containing medium, xenogeneic-free medium), culture conditions (oxygen tension, shear stress). MSC-EV were phenotypically characterized according to MISEV2018 guidelines, using techniques as NTA, protein and lipid quantification, purity assessment, western blot and imaging techniques. Another technique being implemented for MSC-EV characterization is the Fourier-transform infrared spectroscopy, which is able to detect differences in EV biomolecules (nucleic acids, lipids, proteins, carbohydrates) when produced under different conditions. Moreover, different potency assays are also been developed for the functional characterization of the MSC-EV produced under different culture conditions. In summary, this work contributes to the development of methods for the large scale production of MSC-EV, their isolation and characterization.

## 14
**Davies Owen**

## Osteoblast-derived vesicles as specialised sites of mineral nucelation and maturation

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Extracellular vesicles (EVs) were first identified in mineralising cartilage in 1967. This was one of the very first examples of the physiological and developmental roles of EVs. However, they were frequently dismissed as mere tissue processing artefacts for decades after their initial discovery and it was not until 1991 that their function as early sites of mineral nucleation was more widely appreciated. In mineralising tissues such as bone and dentine, research has historically been focused on vesicles bound within the extracellular matrix. Consequently, research into the roles that unbound osteoblast EVs play in mineralisation have largely been ignored. This has also meant that the evolution of this field has not typically aligned with ISEV guidelines. However, recently roles for unbound EVs EVs have emerged in bone communication, remodelling and regeneration. We sought to determine the capacity of unbound osteoblast-derived EVs to drive mineralisation in MSC cultures. EVs were isolated from the culture medium of mineralising osteoblasts (16/SS/0172) over 3 weeks using differential ultracentrifugation. Temporal variations in the EV proteome were analysed using liquid chromatography tandem-mass spectrometry (LC-MS/MS). The capacity of 5 µg/mL EVs to induce mineralisation in stem cell cultures was assessed against a clinical gold-standard, BMP-2. Mineral phase was analysed using X-ray fluorescence (XRF) and infrared spectroscopy (IR). EVs significantly enhanced alkaline phosphatase levels, mineralisation rate and mineral volume beyond BMP-2. XRF elemental mapping showed enriched areas of calcium and phosphorus co-localisation in EV supplemented cultures. Infrared spectroscopy analysis of the mineral phase confirmed the presence of octacalcium phosphate, an intermediate phase in the formation of hydroxyapatite. Principal component analysis and accompanying TEM-coupled energy dispersive X-ray spectroscopy (EDX) localised mineralisation to the EV phospholipid membrane, implicating EVs as sites of nucleation. Proteomic analysis of EVs revealed the presence of bridging collagens, calcium chelating proteins and extracellular binding proteins. The relative intensity of these proteins was significantly ($P<0.05$) upregulated as mineralisation advanced. Our data suggests that unbound EVs also function as sites of mineral nucleation.

## 15
**DeRita Rachel**

## The role of core facilities and emerging technologies in maximizing rigor and reproducibility of EV quantification and characterization

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It remains very clear in the field of extracellular vesicle (EV) research that the rapid rate of increase in publications and expansion of interdisciplinary clinical EV interest has created the need for increased standardization and access to the appropriate technologies to uphold these standards. As the first core facility in the United States (to the best of our knowledge) with the sole intention of creating a space where users can both isolate and characterize EVs, we provide a central location for the facilitation of EV research via access to multiple technologies (both established and emerging) such as resistive pulse sensing, nanoparticle tracking analysis, ultracentrifugation, high-performance liquid chromatography, immunophenotyping. We surveyed a group of leading scientific investigators and researchers in varying stages of their scientific careers in the mid-Atlantic region of the US and present data demonstrating areas of largest interest and also what technology is most desired and needed in a shared resources facility and difficult to access otherwise. Our data indicate a large attraction to EV-based flow cytometry, followed by a need for better access to pure isolation techniques and improved EV size and concentration quantification using techniques beyond nanoparticle tracking alone, such as resistive pulse sensing. We additionally propose strategies for shared resource facilities to facilitate multifactorial and rigorous EV characterization in accordance with MISEV guidelines and also encourage collaboration among EV researchers. In order to answer the larger remaining questions in the EV field such as the isolation of specific EV subsets, EV tracking between cells and the use of EVs for biomarker discovery and drug delivery, it is essential that shared resource facilities interact not only with investigators, but with each other to integrate the necessary resources to progress.

## 16

**Frelet-Barrand Annie**

## The Nanobioanalytical Platform, a tuneable tool for a deep characterization of extracellular vesicles

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The NanoBioAnalytical (NBA) platform combines different complementary highly sensitive biophysical technologies for in-depth investigation of biological samples at the nanoscale level. It is mainly devoted to the qualification of biological samples including extracellular vesicles (EVs), allows biodetection, phenotyping and sizing of EVs subsets by multiplexed immunocapture monitored by surface plasmon resonance (SPR) on biochip, followed by a subsequent investigation by Atomic Force Microscopy (AFM). Moreover, a proteomic analysis of biological samples specifically captured on biochip is further achieved through nano-liquid chromatography-tandem mass spectrometry. In parallel, a characterization in solution, giving size and concentration of the biological species of interest, helps to normalize the conditions of sample injection process on the NBA platform. This label-free system allows the qualification of biological samples including the difficult EVs samples, without limitation in size, from diverse origins [1, 2] and at a dynamic range from 106 to 109 particles /mL. The utility of the NBA platform was also recently highlighted by the EVs community in the latest MISEV guidelines [3]. Overall, the NBA platform is a modular, versatile and upgradable tool, enabling to envision sorting and/or manipulation of nanobioobjects in addition to their deep size and phenotype qualification. 1 Obeid et al, B&B 2017 2 Obeid et al, NBM 2019 3 Thery et al, JEV 2018

## 17
**Geeurickx Edward**

## Recombinant extracellular vesicles as biological reference material

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Introduction: EV data interpretation and extrapolation is challenging owing to the complexity of biofluids and the technical variation introduced during EV sample preparation and analysis. To evaluate and mitigate this variation, we developed recombinant EV (rEV) as a biological reference material with unique trackability, and physical and biochemical similarity to sample EV.
Methods: rEV are purified by velocity gradient centrifugation from cell culture supernatant of HEK293T cells expressing an eGFP-tagged self-assembling protein that directs its own release. We studied the similarity of rEV and EV using electron microscopy, zeta potential analysis, nanoparticle tracking analysis (NTA), lipidomics and proteomics. We assessed the trackability, stability and commutability of rEV using fluorescent NTA (fNTA), flow cytometry (FC), fluorescent microplate reader, quantitative real time PCR (qRT-PCR) and ELISA. rEV was used to asses EV stability and spiked in plasma to calculate the recovery efficiency of EV isolation methods and to normalize EV numbers in plasma using fNTA and ELISA.
Results: rEV show biophysical and biochemical similarity to EV and are 100% fluorescent. rEV can be accurately quantified by fNTA and FC in EV-comprising samples. In addition, rEV behaves linearly with fluorescent intensity levels (R2=0.969) and ELISA concentrations (R2=0.978), and semi-logarithmic with qRT-PCR for eGFP mRNA (R2=0.938). rEV is stable during multiple freeze-thaw cycles at -80°C and can be lyophilized without changes in morphology, concentration and aggregation. EV recoveries from plasma for size-exclusion chromatography, differential ultracentrifugation, DG and ExoQuick were respectively 100%, 10%, 30% and 100%. For the first time, we could calculate the normalized EV concentration for breast cancer patients, which was significantly higher than healthy individuals (1.46x1011 vs 5.83x1010 particles/mL plasma).

Conclusion: We developed rEV, a biological reference material for EV research which can be used as positive control, spike-in material or calibrator to asses pre-analytical variables and ensure standardized EV measurements in various applications.

## 18

**Hotham William**

## Comparing extracellular vesicles produced by bone marrow mesenchymal stem cells of human, ovine, bovine, equine and murine origin and demonstrating interspecies uptake.

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Objectives: In this study, we aimed to isolate and characterise extracellular vesicles (EVs) from bone marrow Mesenchymal Stem Cells (MSC) from various species and to demonstrate interspecies EV uptake by chondrocytes.
Methods: Bone Marrow MSC isolation; Bone marrow of human, ovine, bovine, murine and equine origin was isolated from the femur. EV Harvest; Upon reaching passage 3 and 80% confluency, MSCs were cultured in serum free media for 48 hours. The media underwent differential sequential ultracentrifugation at speeds up to 100,000g. Characterising EVs; Transmission Electron Microscopy and flow cytometry enabled the investigation of EV surface phenotypes. Flow cytometry, EVs were coupled to latex beads before staining. Protein, Protein levels were calculated using BCA assays. Investigating particle size: concentration; all data was gathered using a nanosight NS3000. EV internalisation; To assess EV uptake, EVs were stained with PKH67 before co-culture with primary chondrocytes. Cell membranes were stained with WGA-55. EV uptake was assed via confocal microscopy.
Results: We obtained bone marrow derived MSCs from all species and demonstrated their tri-lineage potential and surface phenotype was in accordance with international standards. The EVs derived from these MSCs were characterised via single and multiple EV analysis. We have shown subtle differences in the sizes of EVs from different species (40-300nm), they all have similar morphologies however, our data suggests that total EV protein levels differ between species and the ratio of EV surface protein to internal protein varies between species. Via confocal microscopy, we have ascertained that EVs are readily internalised by chondrocytes regardless of EV species origin.
Conclusion: We have shown subtle differences in the proteomics of EV obtained from of different species and that EVs are internalised by chondrocytes regardless of species origin indicating that cross-species applications of EV are realistic.

## 19
**Jones Jennifer**

# EV Cargo Standards- Lessons from the cfDNA, exRNA, and CTC liquid biopsy experience

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EVs are heterogeneous “packets” of lipids, proteins, and nucleic acids. Whether the cargo of different types of EVs arise due to systematic cargo sorting and EV packaging or due to various stochastic “shedding” mechanisms, it is widely recognized that there are important differences in the cargo that is bourne by different EV populations. Precise characterization of EV cargo profiles in health and disease is a major goal for researchers who hope to utilize EV-associated signatures for liquid biopsies, disease monitoring, or the study of basic biological mechanisms. The liquid biopsy field is far more advanced in the analysis of cell-free DNA (cfDNA), extracellular RNA (exRNA), and circulating tumor cells (CTCs), than our EV field is in the analysis of EV cargo. Most studies of EV-protein, -DNA, and -RNA in biofluids (serum, plasma, etc) to date use methods for bulk EV isolation which often fail to separate EVs from lipoproteins, and which do not provide quantitative estimations of the abundance or the distribution of different cargo elements among EV populations. We are investigating the role of established liquid biopsy validation tools for cfDNA, exRNA, and CTC assays, such as digital PCR, spike in controls, and synthetic or biological reference materials, for use in EV cargo studies. Rigorous and straightforward EV cargo validation tools, along with well-designed orthogonal assays, including single molecule detection and enumeration methods, are essential for understanding EV cargo profiles. We will present the use of these tools and assays to demonstrate the relevance of lipoprotein and other co-isolates in common EV isolation methods for the cargo analysis of biofluids.

## 20
**Karnas Elżbieta**

## Multiparameter characterization of stem cell-derived extracellular vesicles using classical and imaging flow cytometry platforms

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Extracellular vesicles (EVs) are heterogeneous group of small vesicular structures released by different types of cells, including stem cells (SCs), that may enclose and transfer bioactive content to the target cells. Thus, growing interest is placed on the utilization of EVs in the field of biomedical research. However, there is still lack of standardized methods of EVs characterization. As an example, typical flow cytometry-based protocols, commonly used for cells phenotyping, may be inadequate for the characterization of EVs as particles with size close to the detection limit of conventional cytometers. Thus, the aim of this study was to optimize and compare the use of two flow cytometry platforms for the multiparameter analysis of EVs isolated from different types of SCs populations. EV samples were obtained from human iPS- and mesenchymal SCs (MSCs)- conditioned media by ultracentrifugation method. Next, high resolution flow cytometry systems: Apogee (A50 and A60 Micro-Plus) and Image Stream Mk II were utilized to examine EVs phenotype, including expression of tetraspanins and surface markers. Furthermore, RNA Select dye was used to evaluate the content of RNA and the integrity of analyzed vesicles. Our results have revealed that two tested flow cytometry systems may be utilized for the phenotypic characterization of EVs secreted by SCs populations, demonstrating the difference in the RNA content and the expression of exosomal markers. However, the conventional staining and gating strategy protocols have to be thoroughly optimized. Additionally, the analysis of calibrating beads allowed to compare detection limits of tested cytometers. Finally, imaging flow cytometry platform was also employed to visualize EVs on the single particle level. In conclusion, we have demonstrated that tested high-resolution flow cytometry platforms are convenient methods for the multiparameter characterization of EVs produced by different types of SCs populations.This study was funded by NCBR grant STRATEGMED III (STRATEGMED3/303570/7/NCBR/2017) to EZS.

## 21
**Kelwick Richard**

# Heterogeneity and batch variation of extracellular vesicles manufactured from hollow fibre cell culture

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Heterogeneity and batch variation of extracellular vesicles manufactured from hollow fibre cell culture. We have cultured HEK293 cells at scale within a Holowfibre bioreactor. Conditioned media has been continuously harvested (almost daily) over a period of 4-6 weeks and we are isolating extracellular vesicles using several different methods (UC, IC and TFF). Upon isolation we are also using several different methods to examine exosome/EV batch heterogeneity (NTA, qNano, DLS etc.). If selected we will present our most recent findings and hope they will serve as a discussion around batch variability and EV heterogeneity.

## 22

**Lässer Cecilia**

## How similar are serum and plasma when it comes to EVs?

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Introduction: The ability to isolate extracellular vesicles (EVs) from blood is paramount in the development of EVs as disease biomarkers. Both serum and plasma can be used but few studies have compared them in terms of amount and type of EVs. We have previously developed a method to isolate EVs from plasma with minimal contamination of lipoprotein particles (Karimi et al 2018). The aim of this study was to compare the amount of EVs and their protein cargo isolated from plasma and serum.
Methods: Blood was collected from healthy subjects, from which plasma and serum were isolated. EVs were isolated using a combination of density cushion and size exclusion chromatography (SEC) or a combination of density cushion and density gradient. Purity and yield of EVs were determined by nanoparticle tracking analysis (NTA), Western blot, electron microscopy (EM), and mass spectrometry (LC-MS/MS). Additionally, Cy7-labelled cell line-derived EVs were spiked in to blood prior to isolation of plasma and serum to compare the recovery.
Results: As determined by NTA and protein measurement more EVs could be isolated from plasma. This result was supported by experiments were labelled EVs were spiked in to blood, which demonstrated that less labelled EVs could be retrieved from serum compared to plasma. Additionally, more proteins were identified in plasma EVs with mass spectrometry, as 1789 proteins could be identified in plasma-derived EVs, while only 628 proteins could be identified in serum-derived EVs. Both proteomes were strongly associated with the GO term “Extracellular exosome”, while the serum derived EVs were more associated with “Complement activation”.
Conclusion: This study shows that a larger amount of EVs could be isolated from plasma compared to serum. We currently don’t have the explanation why this is so, however it might be due to the fact that EVs get trapped in the clot during serum formation. Future studies are needed to answer how this affects the use of blood-derived EVs as biomarkers from serum and plasma.

## 23

**Lee Wai-Leng**

## A Pilot Study of Prostate Cancer-Derived Extracellular Vesicles in Urine Using IR Spectroscopy

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Prostate cancer (PCa) is the third most frequent cancer in men in Malaysia. Currently the only biomarker for PCa screening is prostate-specific antigen with a low specificity. Urinary extracellular vesicles (UEVs) have lately emerged as potential biomarkers for PCa detection because it is non-invasive and therefore easily obtained from patients. In this study, we aimed to study UEVs using IR spectroscopy to obtain a signature spectrum for early detection of PCa. Urine samples collected from PCa patients and healthy volunteers were subjected to ultracentrifugation for isolation of UEVs. Isolated UEVs were characterized using transmission electron microscopy (TEM), Litesizer, Nanosight and western blotting. To examine the difference between healthy and patient UEVs, their FTIR spectra were collected using Attenuated Total Reflection Fourier-Transform Infrared (ATR-FTIR) Spectroscopy and analyzed using Principal Component Analysis (PCA). The spectra obtained were used to derive a diagnostic classifier for PCa using Linear Discriminant Analysis (LDA). TEM analysis revealed the membranous vesicles in round shape, the diameter of the vesicles ranged within 30-500 nm and the EV protein markers including CD10 and CD9 were detected from the isolate. The FTIR spectra of UEVs from PCa patients were found to be different from those of healthy individuals. PCA loadings plots indicated the discrimination was based on the difference at the following spectral peaks: amide I peak (1640 cm-1), RNA ribose peak (1120 cm-1), C-C, C-N stretch peak (967 cm-1) and C4–C5/C=N, imidazole ring peak (1610 cm-1). This suggests a difference in the protein content of the PCa samples as the cancer progresses. Further, the diagnostic classifier generated using LDA in this study achieved a sensitivity of 83.33% and a specificity of 60%, suggesting the ATR-FTIR technique has potential utility as a point-of-care test for PCa in urine. The technology offers a novel strategy for development of a non-invasive urine test for early screening of prostate cancer.

## 24

**Lenassi Metka**

## Development of a method for extracellular vesicle separation from human blood plasma and its validation in a clinical biomarker study

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Introduction: Extracellular vesicles (EVs) are ideal candidates for minimally invasive biomarkers. However, they are largely unexplored due to lack of simple, reproducible and standardized separation methods from complex fluids. Here, we established a simple method for reproducible recovery of pure EVs from blood plasma and validated it in a pancreatic ductal adenocarcinoma (PDAC) EV biomarker study. PDAC is one of the cancers with worst prognosis, thus better preoperative characterization of patients would aid in treatment optimization.
Methods: For method development, EVs were isolated from plasma of 10 healthy subjects, using density-based (ultracentrifugation on 20% sucrose cushion; sUC) and size-based (size exclusion chromatography; SEC) isolation methods. Size, concentration, and purity of EV isolates were determined by NTA, AF4-MALS/UV, TEM and miRNA levels with qPCR. For PDAC biomarker study, levels and size of EVs, isolated by sUC from plasma of 34 PDAC patients collected before, 1 and 6 months after surgery, were analysed by NTA. Mann-Whitney test, Spearman's rho and Cox regression were used in statistical analysis. All subjects provided informed consent and the study was approved by NMEC.
Results: SEC method led to a higher number but lower quality of isolated particles compared to sUC ($p<0.001$), due to contamination with lipoproteins and aggregates. sUC method was highly reproducible and resulted in purer EV isolates with more miRNA cargo. NTA detected 3.46*109 particles/mL (mean size 109 nm) and AF4 0.73*109 particles/mL (2xRgeom 195 nm). In PDAC study, coefficients of variation for EV concentration and size measurements were 5% and 2-6%, respectively. Higher EV concentration correlated with smaller EVs (mean diameter: $\rho=-0.363$, $p=0.035$). Preoperatively, patients with poorly differentiated tumors had significantly larger plasma EVs when compared to patients with well/moderately differentiated tumors (mean diameter 176.9 vs. 149.2 nm, $p=0.021$), the EV size even enabling discrimination of the two groups (AUC=0.742, 95% CI=0.560-0.923). Plasma EVs characteristics were also a significant predictor of overall survival in multivariable analysis.
Conclusion: sUC method led to reproducible recovery of pure EVs from blood plasma and was successfully validated in the PDAC biomarker study.

## 25
**Li Bo**

## A facile procedure to avoid swarm detection for extracellular vesicle concentrations in human plasma by flow cytometry

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Background: At present, the most favored instrument used to detect single extracellular vesicle (EV) in a clinical setting is a flow cytometer. Human plasma, containing huge numbers of EVs, is an important resource of EVs biomarkers for clinical disease diagnosis. However, there are still some problems that hamper the application of the flow cytometry for EV measurements in human plasma. The main issue of EV concentration detection is the “swarm” effect on the flow cytometry. Specifically due to the wide size range, from about 30 to 2000 nm, a vast amount of EVs are smaller than the detection limit of flow cytometers may appear simultaneously in the focused laser beam, thereby triggering the light scatter and fluorescence signal. This effect leads to both an underestimation of the EVs number and an overestimation of EVs size. Therefore, serial dilutions are recommended to find the optimal dilution, avoid swarm detection and achieve statistically significant counts within a clinically applicable measurement time. Because the particle concentration in samples differs between individuals, it is unfeasible to operate serial dilutions in each sample for a study with hundreds of samples. Therefore, it is necessary to establish a new procedure for EVs concentration measurements to avoid swarm detection in human plasma.
Objectives: Recently, we developed software to calibrate flow cytometers, which for the first time makes the fluorescence signal and flow rate calibrated and compared. Based on this calibration software, we aim to find the maximum count rate at which swarm detection does not occur in the concentration measurement of the EVs from human plasma.
Methods: First, we investigated what the maximum concentration of particles exceeding the side scatter (SSC) trigger threshold is without having coincidence detection. Therefore, we measured serial dilutions of 150 nm polystyrene beads (3000 Series Nanosphere; Thermo Fisher, Waltham, MA, USA). Based on the scatter signals, we could differentiate between singlets (1 particle is illuminated and detected) and doublets (2 particles are simultaneously illuminated and detected). Therefore, the maximum concentration, for which the ratio of measured doublets to singlets is acceptable, could be calculated to a corresponding count rate of particles. However, PFP also contains particles that are too small to exceed the SSC trigger threshold, which is different from the homogenous solution of polystyrene beads. Therefore, secondly, to confirm the absence of swarm detection in PFP in maximum count rates of beads, we measured serial dilutions of PFP pooled from 5 male and 5 female donors. The relationship between reciprocal dilution of PFP samples and particle concentration or median scatter signals were explored, respectively. The concentration scales linearly with the reciprocal dilution and the median scatter signals were confirmed based on the above results. Third, according to the linear range achieved above, we find the maximum count rate and diluted each PFP sample accordingly with phosphate-buffered saline (PBS), and then measured the total concentration of particles for 30 seconds without staining.
Results: Firstly, singlets follow the expectation value between a concentration range of √( 〖10〗 ^5 ) to 108 beads mL-1, indicating the dynamic concentration range of the flow cytometer. The maximum

concentration, for which the ratio of measured doublets to singlets is acceptable (≤1%), is 1.0×108 particles/mL, corresponding to a count rate of 5.0×103 events per second. Secondly, the concentration scales linearly with the reciprocal dilution and the median scatter signals are similar with concentrations ≤ 108 mL-1 and count rates ≤ 5.0×103 events per second according to results of PFP samples dilutions, confirming the absence of swarm detection for a typical PFP sample. Thirdly, PFP samples were diluted 2,000-fold in PBS to make the count rate ≤5.0×103 events per second and measured the total concentration of EVs on the flow cytometry. Conclusions: Herein, we established a facile procedure to avoid swarm detection for EVs concentration measurements in plasma samples with flow cytometry. We demonstrate this procedure reduced the affection from swarm" effects on the measurement of EVs concentration with the flow cytometry. Therefore, this standardized procedure is essential for the reproducible of EV concentration measurements in plasma samples on flow cytometers, which will encourage the comparison and acceptance between differences instruments and institutions.

## 26

**Lozano-Andrés Estefanía**

# Benchmarking Analysis of sub-micron sized particles: a cross-platform study for single extracellular vesicle flow cytometric characterization

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# TRAIN-EV Marie Skłodowska-Curie Action-Innovative Training Network, train-ev.eu

The analysis of single extracellular vesicles (EV) is crucial to exploit their potential as biomarkers. However, their small size, low refractive index and phenotypic heterogeneity challenge single EV detection by currently available techniques. Flow cytometry (FC) allows for a high-throughput quantitative and qualitative multiparametric characterization. Despite FC being widely used, most commercial instruments lack sensitivity when measuring sub-micron sized particles. Furthermore, the lack of calibration and standardization tools, together with the scarce appropriate reference materials (RM), make it very difficult to compare results. To work towards successful applications for EV biomarker profiling by FC, benchmarking studies including state-of-the-art flow cytometers are required. Besides the comparison of suitable flow cytometers for single EV-based analysis, a great challenge is the appropriate sample preparation which might have different requirements for different flow cytometers. Therefore, we performed a side-by-side cross-platform study in the lab, to avoid inter-laboratory variation, in which we evaluated the performance of 3 different instruments in terms of light scattering and fluorescence by analyzing polystyrene and silica submicron beads next to an EV-RM and fluorescently labeled EVs. Although the fundamental findings were consistent between the different platforms, e.g. the detection of low- and high-abundance surface epitopes on EVs, we identified several pro's and con's for each platform with respect to single EV analysis. Furthermore, proper calibration and RM are of utmost importance to ensure a reliable interpretation of EV flow cytometric data and comparison between different platforms.

## 27

**Maaninka Katariina**

## Off-the-shelf:stable, ready-to-use, pre-labeled human plasma- and urine-derived EVs (joint Abstract with Britta Bettin)

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Introduction: Extracellular vesicles (EVs) in body fluids are explored as biomarkers of diseases. Many laboratories use flow cytometers (FCMs) to characterize single EVs, but the measured concentrations of EVs are incomparable between FCMs. To improve comparability, development of reference materials and methods are needed. In addition, the validation of new reference materials and methods requires stable biological test samples. Our aim is to develop stable, ready-to-use, and well-characterized biological test samples containing EVs.
Methods: In a preliminary experiment, urine and plasma were collected from healthy donors, and EVs were labeled with lactadherin-FITC. Labeled EVs were isolated by size-exclusion chromatography, after which the isolated EVs were mixed with the cryopreservation agents dimethyl sulfoxide (DMSO), ExoCap, or trehalose, frozen in liquid nitrogen, and stored at -80°C for 1 day and 5 months. After thawing, EV concentrations were measured by a calibrated FCM (Apogee A60-Micro).
Results: The measured EV concentrations stored for 1 day decreased 27% ($p=0.04$; mean of the 3 cryopreservation agents) in plasma and 35% ($p=0.05$) in urine, relative to the fresh starting materials. After 5 months of cryopreservation, the concentration of plasma-derived EVs decreased 2.0% (DMSO and Exocap) and 8.5% (trehalose) compared to 1 day of storage. The concentration of urine-derived EVs decreased of 6-18% after 5 months cryopreservation compared to 1 day of storage.
Conclusions: These preliminary results show that pre-labeled plasma-derived EVs can be stably stored for up to 5 months when using appropriate cryopreservation agents. Although urine-derived EVs seem less stable, other cryopreservation agents will be tested to assess their cryopreservation stability. Our optimized biological test samples will be key to validate newly developed reference materials and methods.
Acknowledgements: This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

## 28
**Mussack Veronika**

## Evaluation of differentially isolated small urinary EVs and its effect on downstream transcriptomics

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Urine holds great potential for profound biomarker identification allowing for disease detection and progress monitoring, as it is non-invasively accessible and resembles a person's (patho-)physiological condition, just as urinary extracellular vesicles (EVs) do. Thereby, the EVs' RNA cargo is of special interest, since microRNAs (miRNAs) have been shown to be protected by EVs, contain highly conserved regions, and show great regulatory impact on most protein-coding genes (> 60 %) on the post-transcriptional level. In overall EV research, however, more than staggering 190 different EV isolation strategies have been applied in more than 2,000 experiments. Picking up on this, there does not exist any scientific consensus on what strategy for urinary EV isolation reaches highest purity and data reproducibility. To bridge this knowledge gap, five different isolation strategies were applied to purify small EVs from urine samples provided by six healthy men: spin column chromatography, immunoaffinity, membrane affinity, precipitation, and ultracentrifugation combined with density gradient. Thereby obtained vesicles were characterized by nanoparticle tracking analysis, western blot analysis and transmission electron microscopy to achieve best sample knowledge before extracting RNA and subsequent small RNA sequencing (EV-METRICs: 75 % to 100 %). Comprehensive EV characterization established significant method-dependent differences in isolation efficiencies, size and concentration as well as variances in protein composition of isolated vesicles. Even though all purification methods captured enough total RNA to allow for small RNA sequencing, method-dependent differences were also observed with respect to library sizes, mapping distributions, number of miRNA reads and diversity of transcripts. Hence, the present study indicated the importance of benchmarking studies and a combined evaluation of biophysical and proteomic EV characteristics alongside transcriptomic results. Moreover, this study emphasized the pressing need for and adherence to minimal experimental guidelines and transparency tools, thus, allowing for improved results' comparability, reproducibility, and interpretation.

## 29

**Pinheiro Cláudio**

# A comprehensive analysis of disparate methods to separate extracellular vesicles from multiple biofluids - Extracellular Vesicle Quality Control (EVQC) study

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Research into extracellular vesicles (EV) has yielded important biological insights and raised the prospect of developing novel diagnostics and therapeutics for a wide range of pathologies. However, the plethora of methods to separate EV and the complexity of biofluids present considerable challenges for rigorous and reproducible research as a basis for clinical application. Several technologies have been developed to separate EV, but there are no appropriate and quantifiable performance metrics available for these technologies which hampers informed selection of the most appropriate method for the particular study objectives. We have designed the extracellular vesicle quality control (EVQC) study with the aim to systematically compare the performance of 14 frequently implemented separation methods. These methods are implemented to separate EV from blood, urine and cell culture supernatant spiked or not with recombinant EV (rEV). EV preparations are compared in terms of repeatability, sensitivity, accuracy, specificity and efficiency at protein, nucleic acid and structural level. The final goal of the EVQC consortium is the development of a guiding tool for the informed selection of a separation method for particular study goals.

# 30

**Puhka Maija**

## EV-RNA: variables and challenges in preanalytics, EV/RNA isolation and evaluation

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During the last decade, RNA has been one of the main interests of extracellular vesicle (EV) research. Despite the enthusiasm, the field is young and challenged by the constant flood of novel technologies and lack of basic understanding of all the variables. Our results suggest that sample storage, EV/RNA isolation and down-stream evaluation methods can all affect the obtained EV-RNA results. As any successful study in this field requires sufficient EV RNA quality and quantity, we find that these challenges call for more discussion about the best practices and inter-laboratory repeatability/validation testing.

## 31

**Radeghieri Annalisa**

# EV-Hub: a multi-laboratory hub for extensive characterization of extracellular vesicles

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A shared approach for grading EV formulations is a priority for EV full exploitation in every field, from clinical translation to food industry. The road to achieve such target is rough for basic and clinical laboratories as well. It is hard to fingerprint EV samples in a reproducible and robust fashion, given the huge amount of intra- and inter-laboratory pre-analytical and analytical variables related to EV separation and characterization. Therefore, when it comes to grade EV samples, we still lack (i) optimal reference standards, (ii) assay validation methods and (iii) cross validation studies to compare results among different bioanalytical approaches and/or laboratories. A consortium of laboratories located nearby - the EV-Hub consortium - has recently been established in Lombardy, Italy, to contribute to tackle the third issue (iii). EV-Hub platform set as its first goal to perform round-robin intercomparisons of a single EV sample using state-of-the art practices, from the gold standards in EV analysis to advanced methods. This experience is made possible by the physical proximity of the participating labs and aims at triggering the exchange of ideas and experience required for harmonization of measurement methods, towards best practices and (eventually) quality standards. In this workshop I will share a fresh update on the hub activities and the challenges it's facing, hoping that it will be a constructive experience-based contribution to the topic. The EV-Hub consortium (open to new members) is currently composed by 10 laboratories, featuring multiple core competences, from biophysics to clinics: Centro Cardiologico Monzino IRCCS, Milano; Ospedale San Raffaele, Urological Research Institute, Milano; ICRM-CNR : Chemistry and Technology for Biosciences (CTB) Group, Milano; Istituto CNR di Neuroscienze, lab Cell-to-Cell-signalling in the brain, Milano; Fondazione Don Carlo Gnocchi, IRCCS Santa Maria Nascente, Milano; Laboratory of Nanomedicine and Clinical Biophotonics (LABION), Milano; IRCCS Maugeri and Fondazione Mondino, IRCCS, Pavia; Biogenic Colloids, Surfaces and Interfaces Laboratory (BioCSI), Dipartimento di Medicina Molecolare e Traslazionale, Università degli Studi di Brescia, Brescia.

## 32

**Roux Quentin**

# EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research.

Quentin Roux (1,2), EV-TRACK consortium members, Olivier De Wever (1,2) and An Hendrix (1,2)

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Extracellular vesicles (EV), small membrane vesicles with a size between 30 and 1000 nm, are generated by cells using a variety of biogenesis pathways to transfer biologically active metabolites, proteins, lipids and nucleic acids to recipient cells in their vicinity and at a distance. Over the past decade, EV have been allocated diverse (patho)physiological functions. However, their separation from biofluids remains a challenge. Other macromolecular structures in the extracellular milieu, such as protein aggregates, ribonucleoproteins and lipoprotein particles overlapping in size and/or density with EV are frequent contaminants in EV preparations. A multitude of methods have become available to separate EV from biofluids, but each method achieves this with different specificity and efficiency, resulting in method-dependent identification of EV cargo. A complete and transparent reporting of separation and characterization methods employed in EV studies is essential for unbiased data interpretation and reproducibility. We recently released the community-based knowledgebase EV-TRACK. Currently, EV-TRACK includes experimental parameters of 2022 EV experiments from 1318 publications. For each experiment, the completeness of reporting of generic and method-specific information that facilitates interpretation and reproduction of the experiment is assessed by a checklist, summarized into the EV-METRIC. Supported by the community, EV-TRACK has been included in the 2018 update of the MISEV guidelines (Minimal Information for Studies of Extracellular Vesicles). The rapid growth of the EV research field requires the development of new EV-TRACK features that allow for 1) the integration of the EV-METRIC into EV-related databases and 2) text data mining to derive high-quality information from EV studies to assist EV-TRACK submission. To address the first point, we present here the importance, development and integration of the EV-TRACK summary add-on to summarize experimental information relevant to the interpretation of knowledge in databases and thus to facilitate true EV cargo and function discovery using publicly available data. To enable EV-TRACK submission we have created a user-friendly submission platform and are currently developing a text data mining tool to, at least in part, automate submission of experimental parameters from EV studies to the EV-TRACK knowledgebase.

## 33

**Sódar Barbara**

## Standardisation of extracellular vesicle assessment: from protein towards lipid quantification with special focus on associated lipoproteins

Barbara W. Sódar,(a) Tamás Visnovitz,(a) Xabier Osteikoetxea,(a) Judith Mihály,(b) Péter Lőrincz,(c) Krisztina V. Vukman,(a) Eszter Ágnes Tóth,(a) Anna Koncz,(a) Inna Székács,(d) Robert Horváth,(d) Zoltán Varga,(b) and Edit I. Buzása,(e)

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EV samples are most commonly quantified based on their protein content, particle number or the combination of both. Our group proposes a new, improved method for quantification of the total lipid content of EV samples. This improved sulpho-phospho-vanillin (SPV) assay provides an easy-to-use, sensitive and reproducible method, which can help to reduce inter-laboratory variations. This assay is capable of determining the total lipid content of a sample. According to our results, EV lipid measurements with the SPV assay are almost as sensitive and as easy as measuring proteins with the BCA test. However, when EVs are purified from biofluids, lipoprotein contamination of the samples is a serious issue. According to our results, EVs do not only co-purify with lipoproteins, but there is also an association between them, likely mediated by the apoB content of LDL. Given that the SPV assay also detects the lipid content of lipoproteins (e.g. LDL), and lipoproteins may co-purify and associate with EVs, quantification of EVs form biofluids remains particularly challenging.

# 34

**van Royen Martin**

## EVQuant; high throughput quantification of extracellular vesicle (sub)populations

Thomas A. Hartjes (1), Natasja Dits (2), Roy Van Der Meel (3), Ray M. Schiffelers (4), Adriaan B. Houtsmuller (1), Guido W. Jenster (2), Martin E. Van Royen (1)

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Extracellular vesicles (EVs) are an important biomarker source for a range of diseases. Proteins and RNAs in or on the surface of secreted organ- or disease-specific EVs in body fluids can be used for detection or monitoring disease. Although various methods exist to quantify EVs, their quantification in clinical samples remains challenging and importantly, current approaches are often unable to identify EV subpopulations. With the EVQuant assay, we provide a rapid and robust microscopy based assay to both quantify and characterize individual EVs (down to 50nm) in research and clinical samples without the need for extensive EV isolation and purification procedures. In short, EVs are fluorescently labeled using non-specific dyes or immune-specific antibodies, immobilized in a transparent gel and detected by confocal microscopy. The EVQuant assay allows multicolor fluorescence-based detection of individual EVs in high throughput to quantify total and biomarker-specific EV populations present in several biological fluids. Characterization of EV subpopulations in urines and prostatic fluid identified prostate derived urinary EVs, showing that the EVQuant assay allows the identification of organ and/or disease derived EVs in complex body fluids. Because of its low costs and general accessibility without the need for highly specialized equipment and skills, we believe that this assay can provide an important contribution to the EV community.

# 35
**Varga Zoltan**

## Synthetic reference materials for extracellular vesicles

Zoltan Varga (1), Edwin van der Pol (2), Rienk Nieuwland (2)

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There is an unmet need for standardization of concentration measurements of extracellular vesicles (EVs). Flow cytometry remains the clinically most applicable method for measuring it. To compare concentration measurements of EVs between flow cytometers, solid polystyrene reference beads are used. However, these polystyrene beads lead to false size determination of EVs due to the mismatch in refractive index between the beads and EVs. The objective of this study is to prepare, characterize and test hollow organosilica beads (HOBs) with nominal diameters with 200 nm (HOB200) and 400 nm (HOB400) as reference beads to set EV size gates in flow cytometry investigations. HOBs were prepared by a hard template sol-gel method and extensively characterized for morphology, size distribution and colloidal stability. The applicability of HOBs as reference particles was investigated by flow cytometry using HOBs and platelet-derived EVs. HOBs proved to be monodisperse with homogeneous shell thickness with mean diameters of (189±2) nm and (374±10) nm for HOB200 and HOB400, respectively, with a polydispersity below 15%. Two angle light scattering measurements showed that the scattering intensity of HOBs overlaps with the scattering intensity expected from EVs. To demonstrate that HOBs can be used independent of the light scattering collection angles of a flow cytometer, we determined the concentration of platelet-derived EVs using the FSC or SSC detector within size gates set by HOBs. The percentage difference in the gated concentration relative to the mean concentration is smallest for the gates set by HOBs compared to solid beads, suggesting that HOBs outperform solid beads to standardize EV flow cytometry. Because HOBs resemble the structure and the light scattering properties of EVs, HOBs can be used to set size gates in nanometers independent from the optical configuration of a flow cytometer. Among liposomes and low refractive index solid nanoparticles, the applicability of HOBs as synthetic reference materials for EVs will be further investigated in the METVES II project.

## 36

**Blundell Emma**

## High-Precision Isolation of Extracellular Vesicles and exRNA for Clinical Studies: A Combination Strategy of Size Exclusion Chromatography and RNA Extraction Technologies

Emma Blundell (1)

(1) Izon

Extracellular vesicles (EVs) derived from biological fluids possess extensive heterogeneity with regards to their size, number, membrane composition and cargo. Tremendous research interests lie toward development and use of EV fractions of bio-fluids as rich sources of robust diagnostic and prognostic biomarkers, more recently involving extracellular RNA (exRNA) such as micro RNA (miRNA). The ability to isolate EVs and exRNA subpopulations reproducibly from biological samples is a current challenge in EV research.
Size exclusion chromatography (SEC) is the most standardised technique for isolating EVs, already widely used in the field for biological samples. Significant improvements to SEC have been achieved through automating the process using the Automatic Fraction Collector (AFC) with advanced functionality that includes a precise mass-based measurement for the fractions. The AFC is a low-cost advanced technology that adds high precision, improves repeatability and speeds up workflow. SEC columns provide a convenient, reproducible and highly effective means of eliminating >99% of non-vesicular protein from bio-samples, effectively separating exosomal and non-exosomal volumes for further analysis, both important in biomarker research.
The qEV-AFC platform alone allows for QA, high-precision EV volume collection and minimises current reproducibility issues for clinical studies as well as retaining the functionality of the isolated molecules. Thus, a qEV-AFC-exRNA combination strategy to isolate EVs and exRNA from various biological fluids will result in reproducible high-purity samples and will be revolutionary for the EV-research demographic. The qEV-AFC-exRNA initiative allows for interlaboratory consistency and for users to complete more comprehensive analysis of a single sample. The ability to setup parallel AFC units allows for processing multiple samples simultaneously. A critical consideration for groups aiming at processing cohorts of samples as part of larger clinical trials.
qEV-AFC-exRNA workflow is currently being tested with key academic groups. One such pilot study involves evaluating plasma derived exosomal microRNA obtained from cardiovascular patients (n=40).

## 37
**Wild Stefan**

## Antibody beads for standardized analysis of extracellular vesicles

Stefan Wild (1)

(1) Miltenyi Biotec

Introduction: Extracellular vesicles (EVs) are loaded with specific sets of proteins and can be distinguished by surface marker profiling. A standardized method to analyze a range of surface markers could help to describe different EV populations and support comparability of results.
Methods: We have developed a multiplex bead-based assay comprising 39 distinct fluorescently labeled capture antibody beads. The bead populations are discriminated by flow cytometry and bound EVs can be detected by labeled antibodies. Defined blood cell populations were separated and cultivated. EVs were isolated by serial centrifugation, filtration and ultracentrifugation. The multiplex bead assay was used to compare the surface marker profile of the different EVs.
Results: We investigated surface proteins of EVs isolated from primary cultures of T cells, B cells, natural killer (NK) cells, monocytes, monocyte-derived dendritic cells (moDCs), and platelets. Hierarchical clustering of protein intensity patterns grouped EVs according to their originating cell type. As for the originating cell types, single markers or clusters of markers can be used to discriminate the different EV populations. CD2 and CD8 were detected on T cell EVs, CD19 on B cell EVs, CD14 on monocyte EVs, or CD40, CD80, CD83, and CD86 on moDC-derived EVs. The platelet markers CD41b, CD42a, CD61, and CD62P appeared broadly on many EV preparation. However, a closer look revealed platelet contaminations of the primary cell cultures giving rise to platelet EV marker signals.
Summary: A multiplex bead assay can be used to characterize EVs according to their surface marker profile. A broad range of investigated markers enables to discriminate EVs e.g. according to their originating cells. Although often underestimated, not detected markers can be informative to judge the purity of EV preparations. Detection of unexpected markers might hint to impurities or additional EV subpopulations.  A standardized assay to characterize EV preparations is expected to improve the comparability of results. In addition, diverse EV surface markers might address different target cells or instruct distinct EV tasks.

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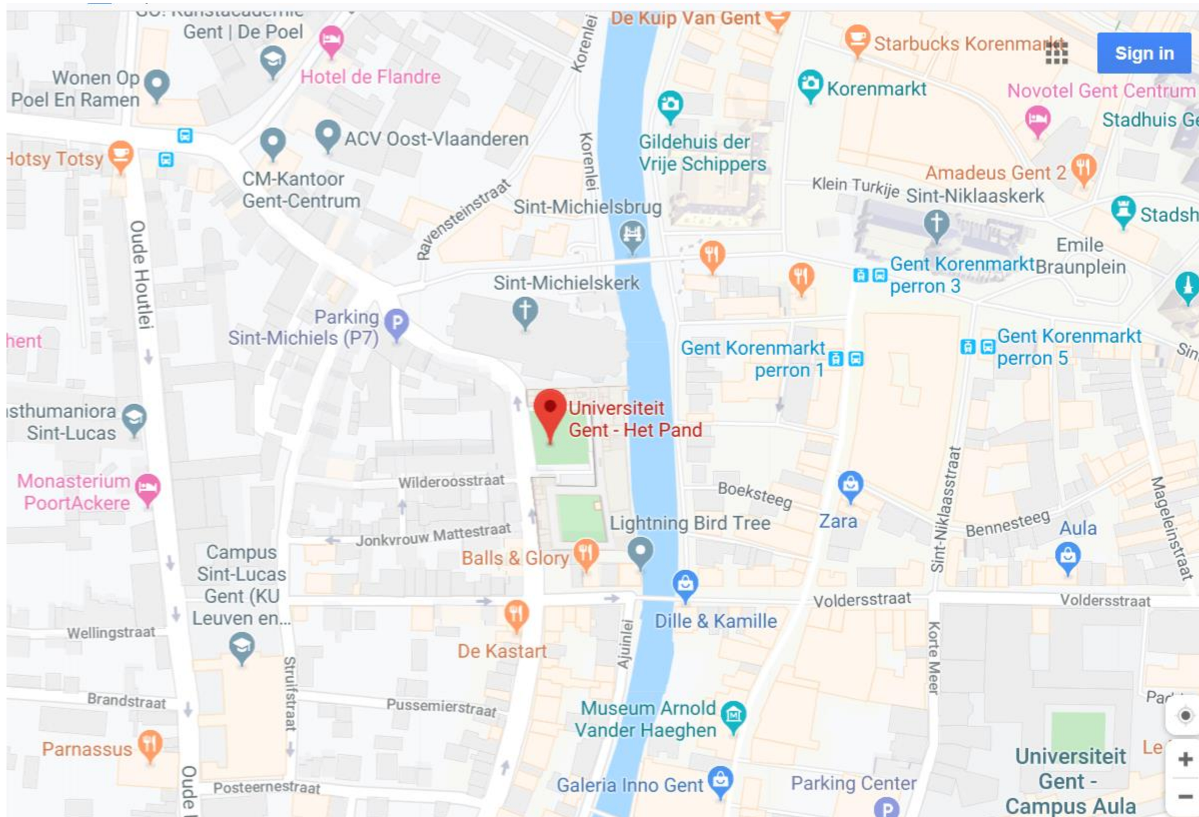


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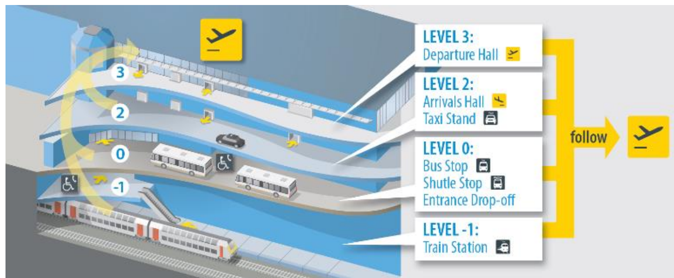


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More information: https://visit.gent.be/en/good-know/practical-information/how-get-ghent/car

**By train:**

At the main station, **Gent-Sint-Pieters**, you will find railway links to all the cities in Belgium. This city also has a direct line to Brussels International Airport.

Timetables are available at https://www.belgiantrain.be/en
More information: https://visit.gent.be/en/good-know/practical-information/how-get-ghent/train

**Local transport:**

The city has a well-organised public transport system with an extensive tram and bus network. You can get anywhere in and around Ghent by tram or bus from early in the morning to late at night. Tram 1 travels from the main railway station, Gent-Sint-Pieters, to the city centre every ten minutes.

You can buy your ticket in advance from the 'Lijnwinkel'(at the train station) or a machine at the tram or bus stop.

Tram lines 1, 2 and 4 take you from one end of Ghent to the other, passing right through the city centre. Tram 1 travels from the main railway station, Gent-Sint-Pieters, to the city centre every ten minutes.

To download tram/bus/city network :
https://visit.gent.be/en/good-know/practical-information/getting-around/public-transport-ghent

Alternatively taxis are waiting for you at the Gent-Sint-Pieters train station, and at many other strategic locations around the city:

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|---|---|
| V-TAKS | Tel: +32 9 222 22 22; Email: info@v-tax.be; Website: www.v-tax.be |
| Gentse Taxi | Tel: +32 487 53 06 31 |
| Gentse Taxi Service | Tel: +32 9 345 45 45; Email: info@gt-service.be; Website: www.gt-service.be |