GC-IMS Applications in Human Volatilomics

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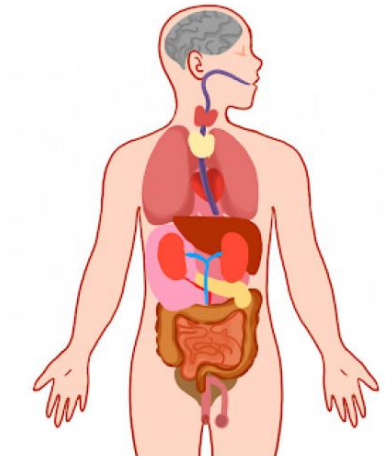
Bologna, 22nd April 2026

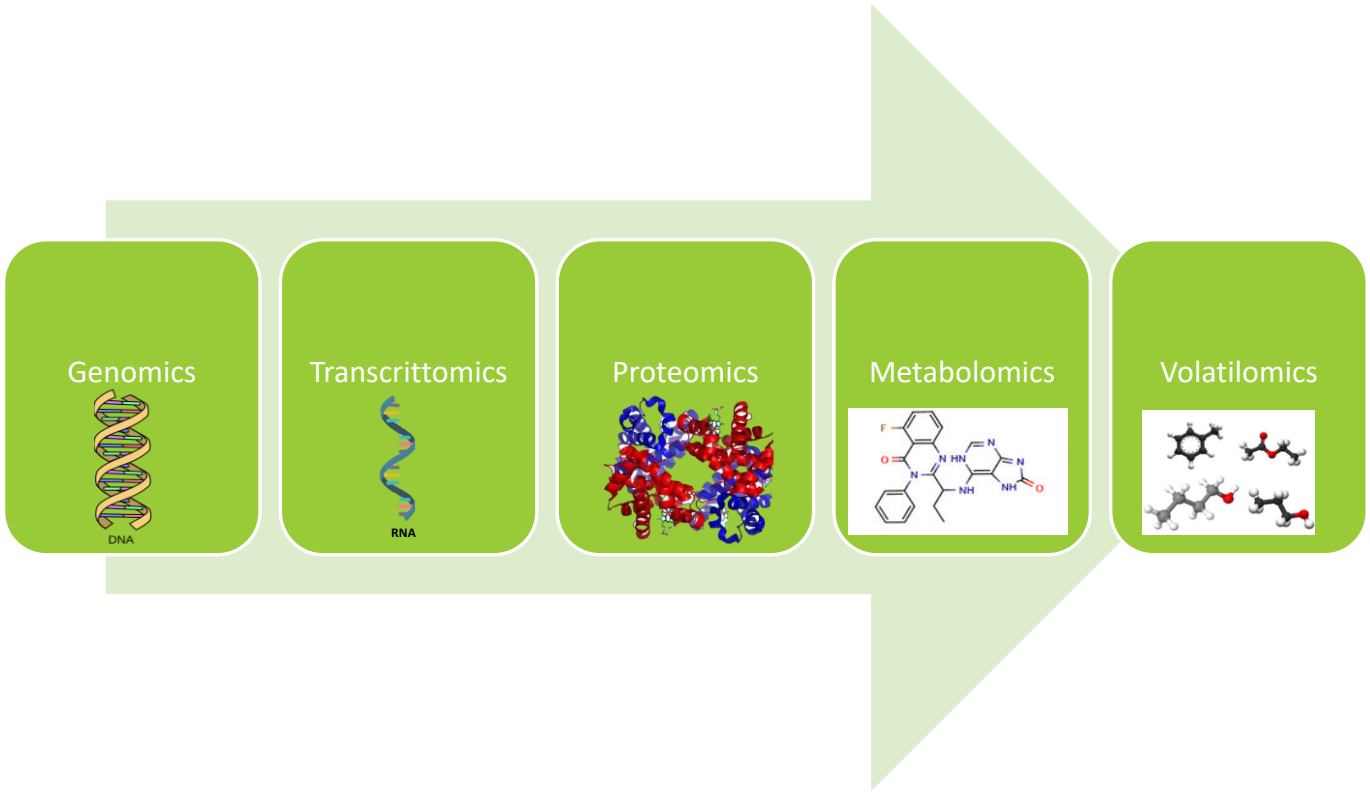
Outline

- 'Omics' technologies
- Introduction to volatolomics
- Volatilome measurement methods
- GC-IMS in volatilomics
  - Lung Cancer case
  - Pediatric eosinophilic esophagitis (EoE)
  - Extracellular vesicles (EVs) extracted from cultured cells

«Omics» Technologies

They aim at the overall detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), metabolites (metabolomics) and volatile compounds (volatilomics) in a specific biological sample.





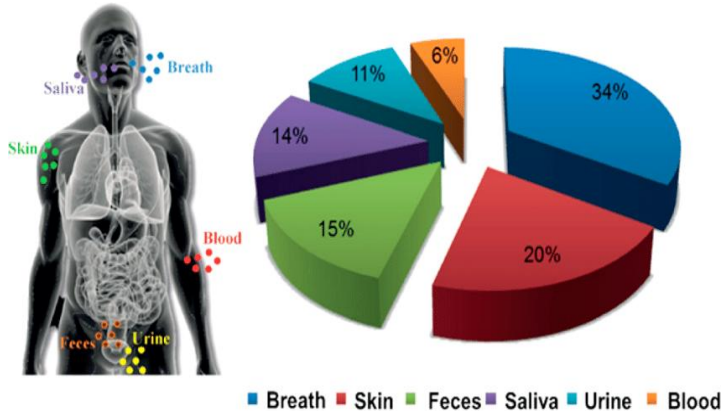
Metabolomics and volatilomics

Metabolomics:«*the systematic study of the unique chemical fingerprints that certain cellular processes leave behind*» (Bennet, The Scientist, 2005)

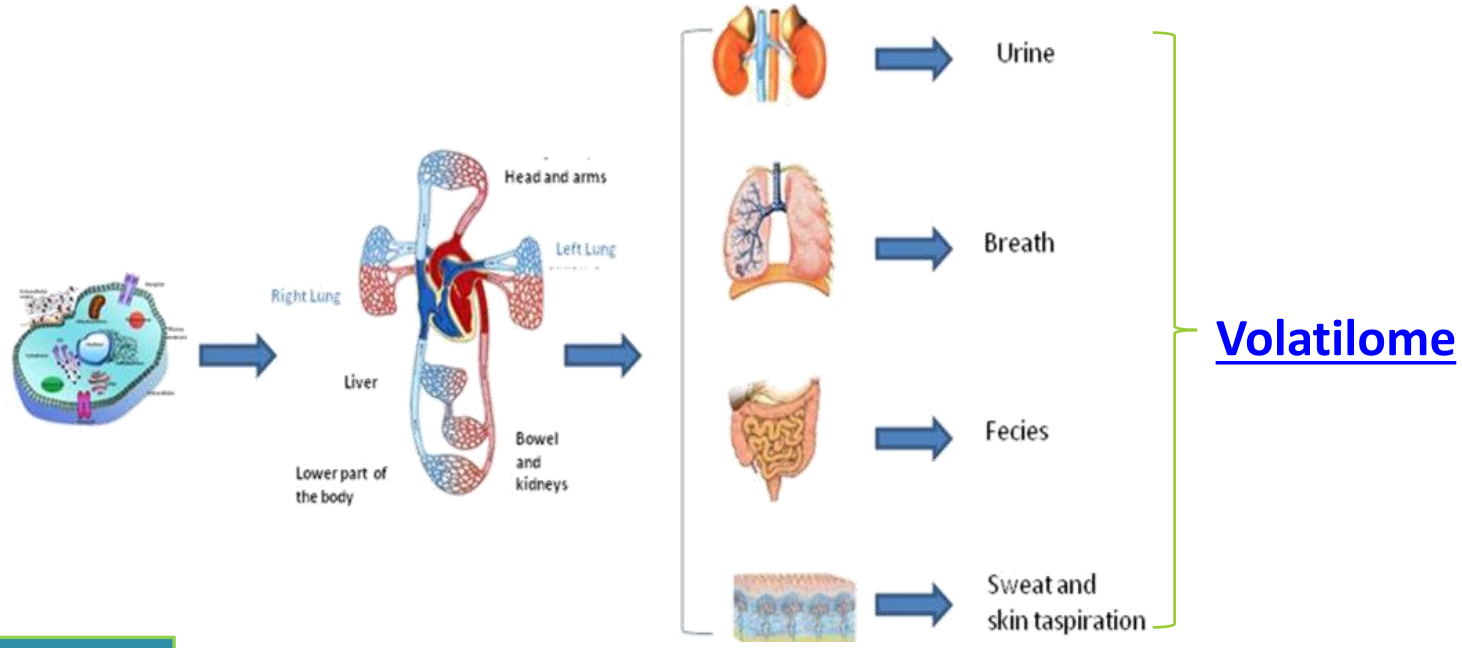
Metaboloma: collection of all metabolites in a biological cell, tissue, organ or organism, and are the end products of cellular processes.

Metabolic profiles consist of hundreds of different compounds; Specific conditions involve the variation of many of them.

Volatile metabolites excreted through different pathways form the **volatilome**



Volatilome

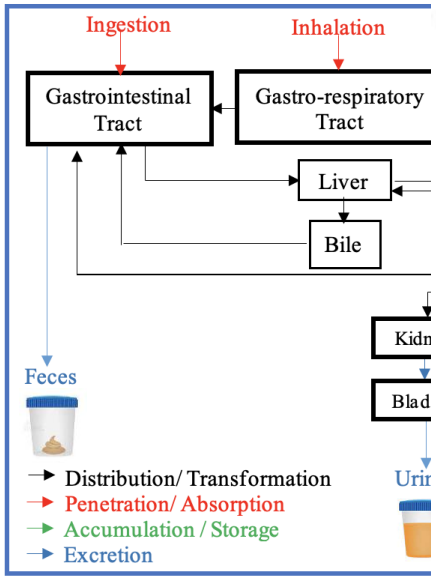


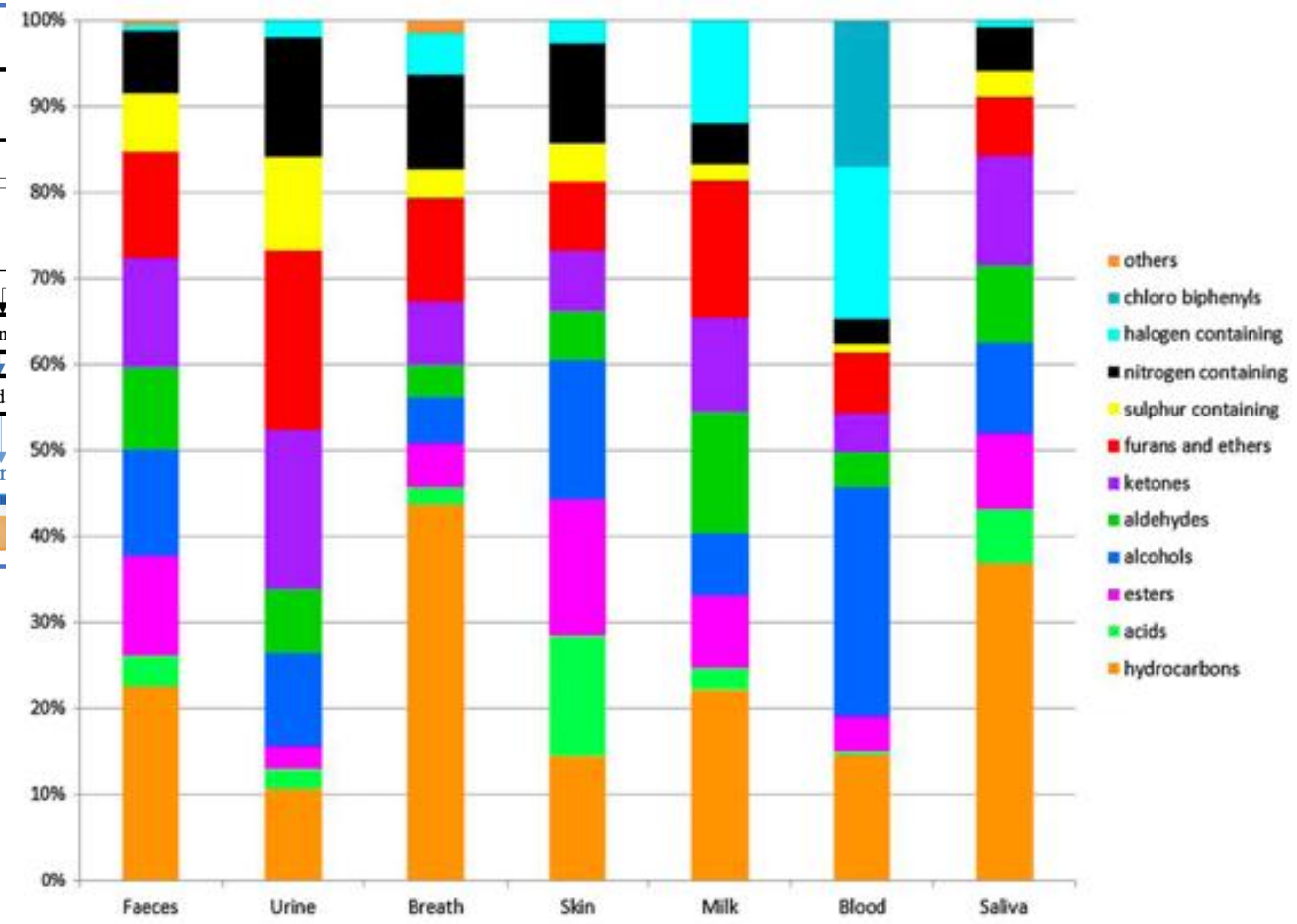
CHANGE IN BIOLOGICAL FLUID COMPOSITION

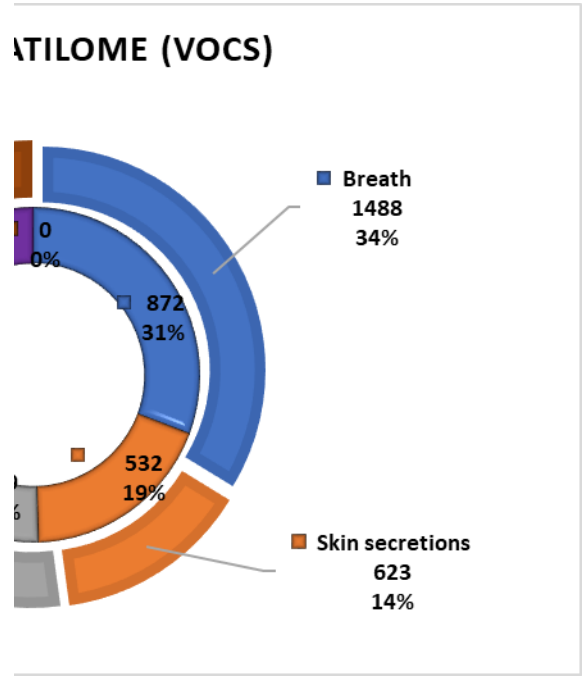
ALTERATION OF THE VOC PROFILE

VOC analysis of human body fluids may be a promising **non-invasive** tool for medical diagnosis and monitoring the effects of therapy.

Chemical aspects of the Volatilome







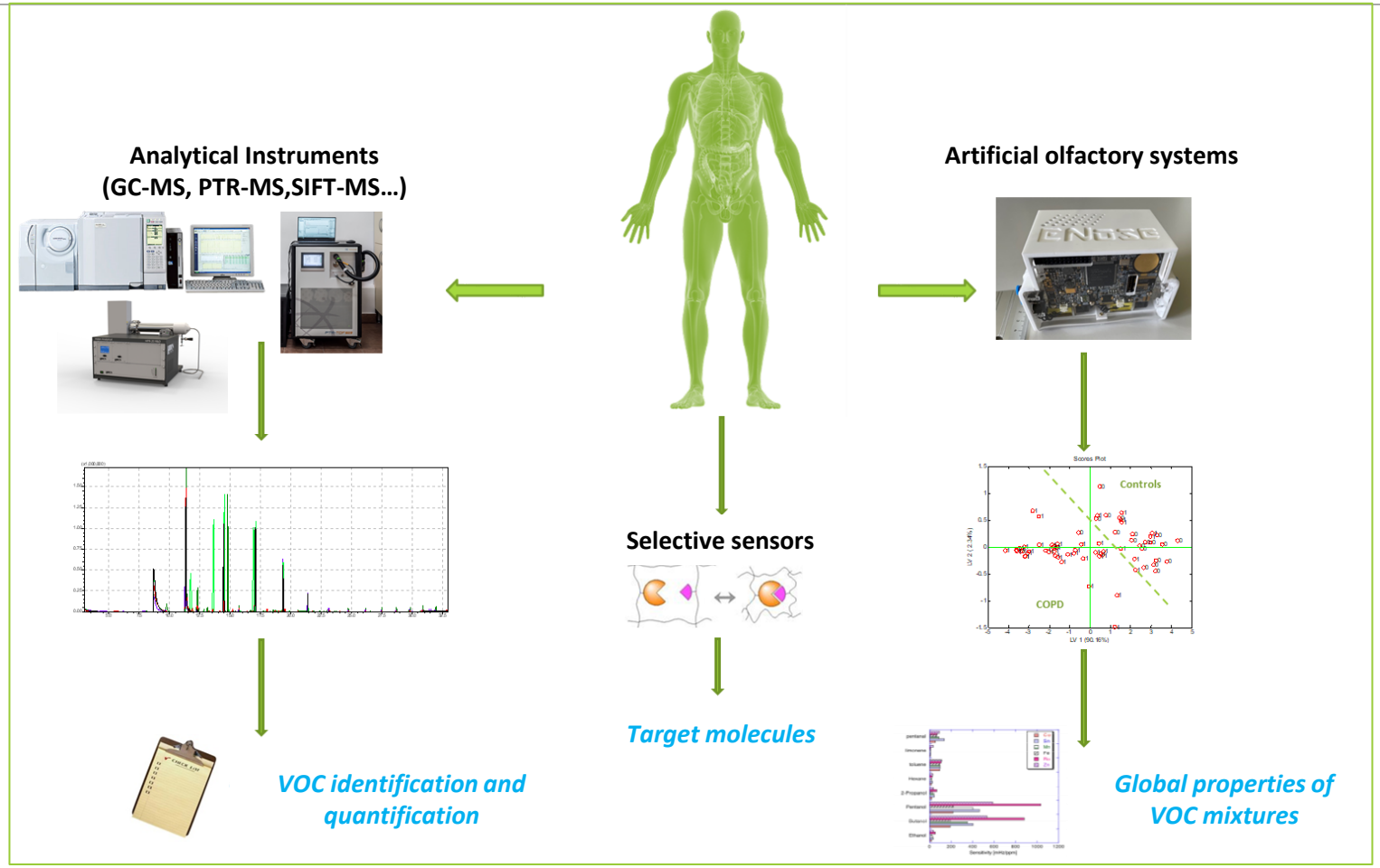
De Lacy Costello et al, J. Breath Res. 2014
Drabinska er al., J. Breath Res. 2021

Advantages and disadvantages of volatilomic analysis

- Non-invasiveness of sample collection
- Different biological fluids to be analyzed
- Ability to use low-cost technologies for analysis

- Lack of standard analysis protocols
- Difficulty in understanding the true origin of the detected VOCs (exogenous or endogenous)
- Difficulty in associating VOCs with different metabolic pathways and specific pathologies
- Interference due to comorbidities and/or environmental factors

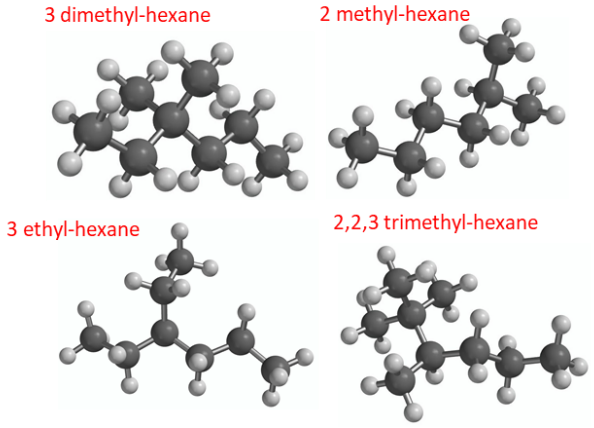
Volatilome measurement strategy

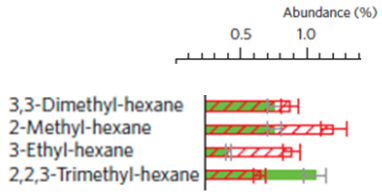


Consideration on Analytical Approach

- Except in rare exceptions, **lack of identification of volatile biomarkers**
  - *Acetone for diabetes and ammonia for liver and kidney disorders*
- It is rather the pattern of fluid VOCs that changes under the influence of a pathology
  - **Fingerprint**

Example: Lung cancer





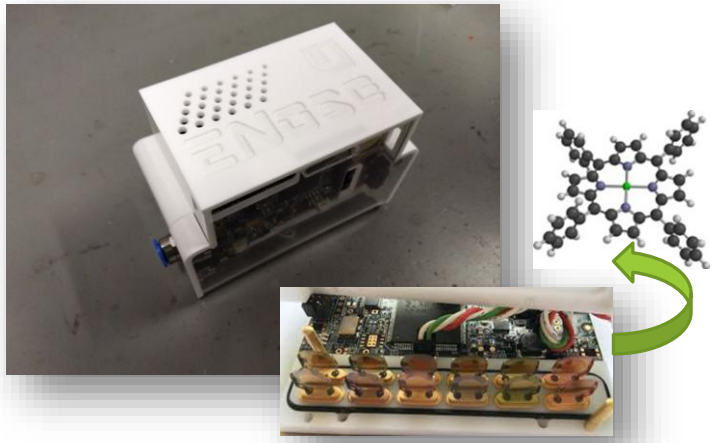
Peng et al, Nature Nanotechn; 2009

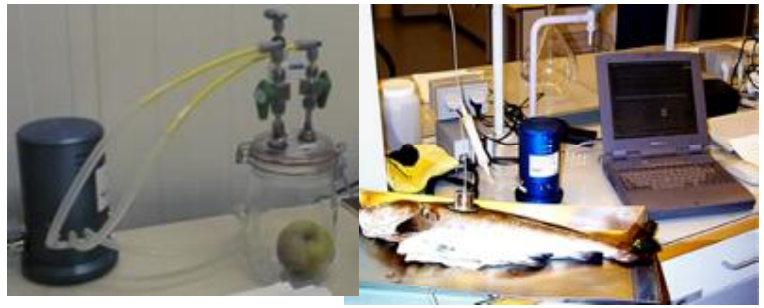
There is no direct relationship between the structure of the molecule and its abundance.

Sensors Group (UTOV) Volatilomic approach

Spacecraft air quality control
Martinelli et al. Micrograv Sci Techn. 2008
Fortezza et al. Acta Astronautica, 2006
••••••

Electronic Nose



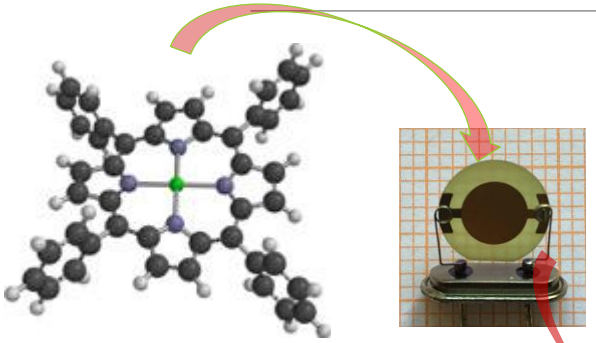


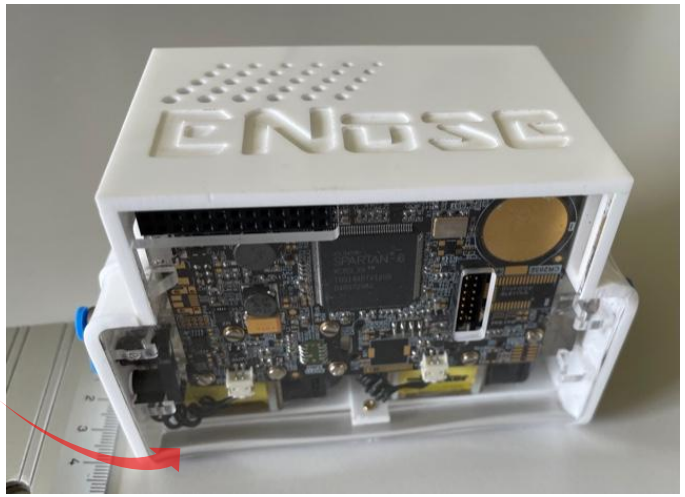
Food Quality and Control
Compagnone et al. Sens Actuators B 2015
Pizzoni et al. J Food Eng. 2015
Eifler et al. PLoS ONE, 2012
Santonico et al. Food Chem, 2010
Santonico et al. Sens Act. B 2008
Olafdsottir et al. Trends Food Sci Tech. 2004
Saevels et al. Posth. Sc. Techn. 2003
••••••

Diagnosi medica
Kethchanji et al. iScience 2021
Murdocca et l Cancers, 2021
Capuano et al. Sci Reports 2019
Capuano et al. Sci Reports 2018
Montuschi et al. Front Pharmac 2018
Capuano et al. Sci Repors 2017
Zetola et al. J Infection, 2017
Capuano et al. Sci Reports 2015
Santonico et al. Lung Cancer 2012
D'Amico et al. Lung cancer, 2010
Montuschi et al. Chest, 2010
D'Amico et al. Skin Res. Techn. 2007
Di Natale et al. Bios. Bioelec. 2003
••••••

https://www.youtube.com/watch?v=W9XkyycleUs

Volatolomics applications of porphyrinoids coated QMB electronic nose





currently in use at:

Hospital Foch, Paris	pneumology
Istituto Europeo Oncologico, Milano	Lung cancer
University of Rome La Sapienza	oro-farigenal diseases
University of Sassari	Head-neck tumors
University of Teramo	sensors development

breath:	Lung cancer tuberculosis asthma

urines:	Lung cancer kidney cancer

serum:	Covid-19

animal models:	Malaria (*Pl. Berghei*) differentiation staminal cells melanoma colon cancer

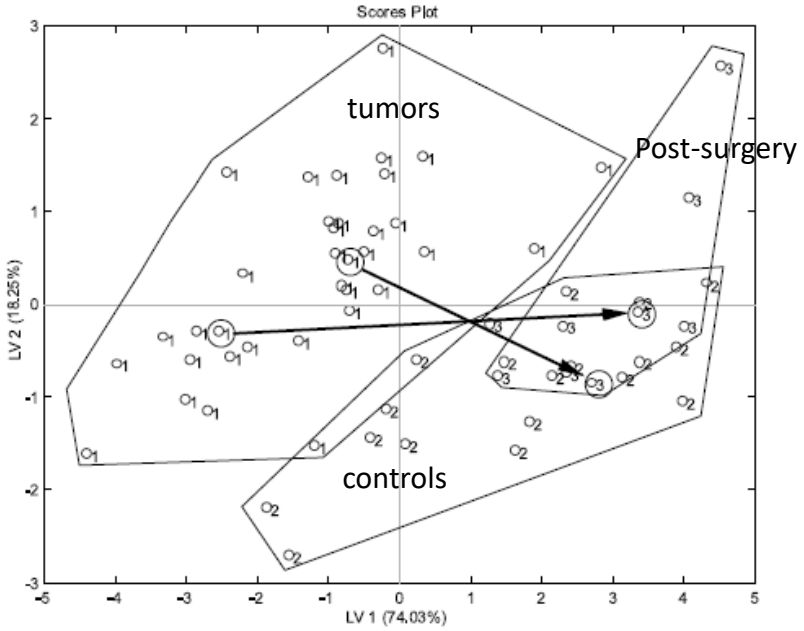
In vitro:	Malaria (*Pl. Falciparum*) differentiation of staminal cells identification of microorganisms

Lung cancer detection with porphyrins coated QMB arrays

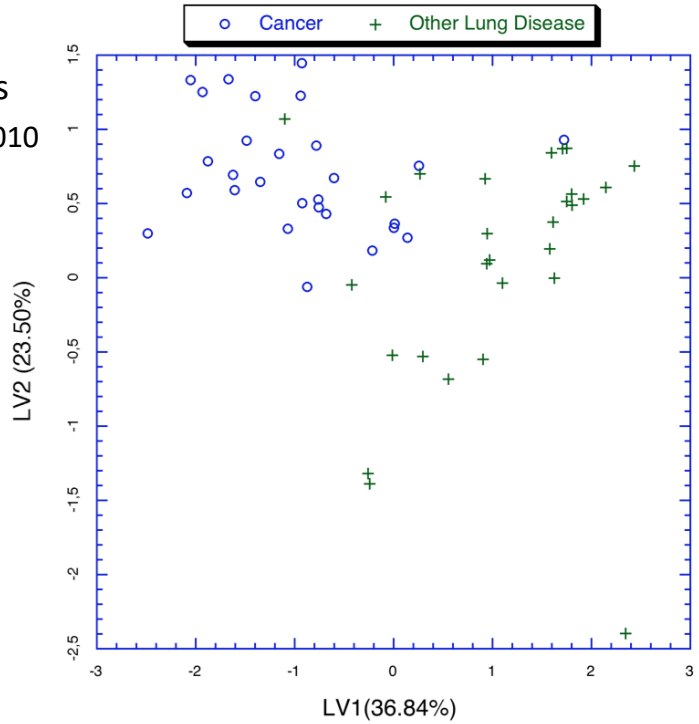
LC vs. controls

- Di Natale et al, Biosens and Bioelectronics, 2003

first paper in literature about the use of gas sensors for lung cancer diagnosis



- LC vs. pulmonary pathologies
  - D'Amico et al, Lung Cancer 2010

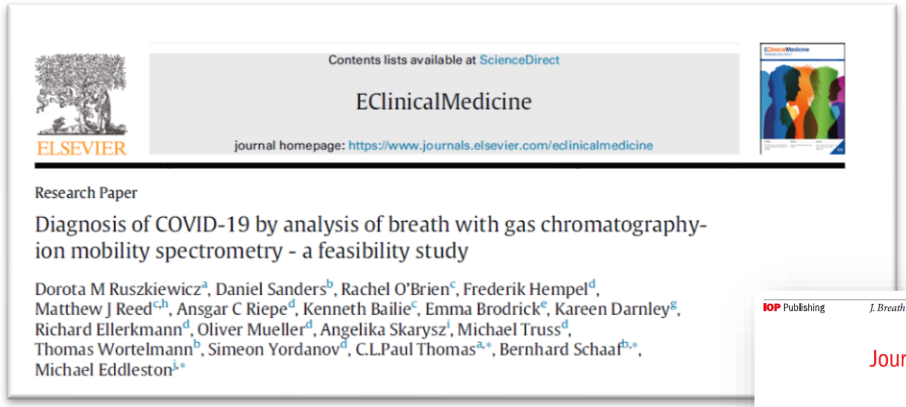


- LC vs. Metabolic disorders
  - Gasparri et al., J Breath Anal. 2016

Table 4. Sensitivity and specificity for metabolic and non-metabolic diseased populations.

	Groups compared (*n*)		Sensitivity (%)	Specificity (%)
Metabolic diseased group	Lung cancer (41)	Controls (41)	85	88
	Stage I (21)	—	90	—
	Stage II/III/IV (14)	—	57	—
Non-metabolic diseased group	Lung cancer (29)	Controls (35)	76	94
	Stage I (19)	—	94	—
	Stage II/III/IV (10)	—	60	—

GC-IMS application in volatilomics



Contents lists available at ScienceDirect

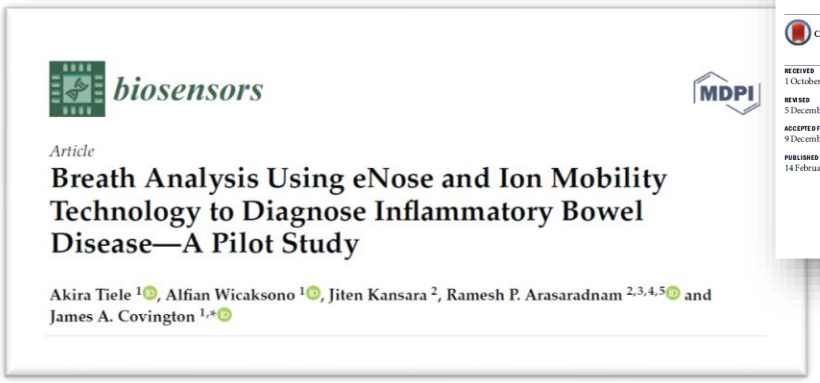
EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Research Paper

Diagnosis of COVID-19 by analysis of breath with gas chromatography-ion mobility spectrometry - a feasibility study

Dorota M Ruszkiewicz[a], Daniel Sanders[b], Rachel O'Brien[c], Frederik Hempel[d], Matthew J Reed[c,h], Ansgar C Riepe[d], Kenneth Bailie[c], Emma Brodrick[e], Kareen Darnley[g], Richard Ellerkmann[d], Oliver Mueller[d], Angelika Skarysz[i], Michael Truss[d], Thomas Wortelmann[b], Simeon Yordanov[d], C.L.Paul Thomas[a,*], Bernhard Schaaf[b,*], Michael Eddleston[j,*]



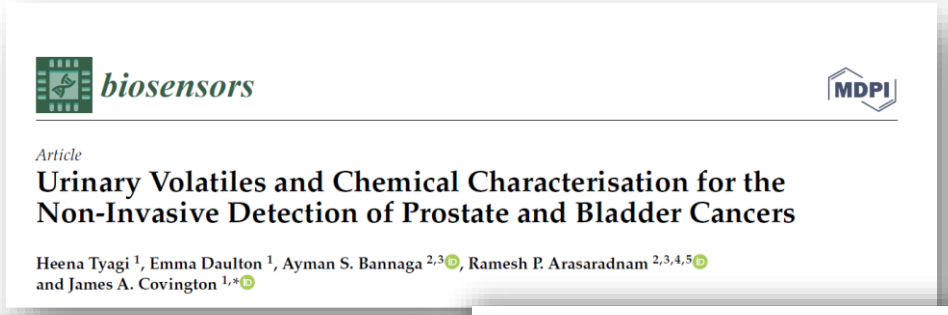
biosensors

MDPI

Article

Breath Analysis Using eNose and Ion Mobility Technology to Diagnose Inflammatory Bowel Disease—A Pilot Study

Akira Tiele [1], Alfian Wicaksono [1], Jiten Kansara [2], Ramesh P. Arasaradnam [2,3,4,5] and James A. Covington [1,*]



biosensors

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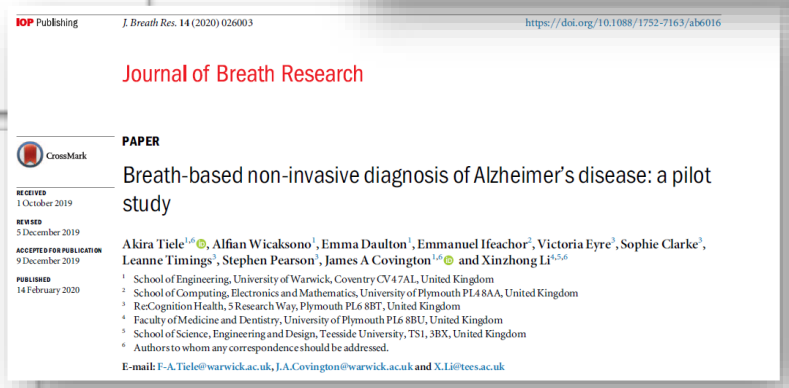
Article

Urinary Volatiles and Chemical Characterisation for the Non-Invasive Detection of Prostate and Bladder Cancers

Heena Tyagi [1], Emma Daulton [1], Ayman S. Bannaga [2,3], Ramesh P. Arasaradnam [2,3,4,5] and James A. Covington [1,*]



IOP Publishing J. Breath Res. 14 (2020) 026003 https://doi.org/10.1088/1752-7163/ab6016

Journal of Breath Research

CrossMark

PAPER

Breath-based non-invasive diagnosis of Alzheimer's disease: a pilot study

RECEIVED 1 October 2019

REVISED 5 December 2019

ACCEPTED FOR PUBLICATION 9 December 2019

PUBLISHED 14 February 2020

Akira Tiele[1,6], Alfian Wicaksono[1], Emma Daulton[1], Emmanuel Ifeachor[2], Victoria Eyre[3], Sophie Clarke[3], Leanne Timings[3], Stephen Pearson[3], James A Covington[1,6] and Xinzhong Li[4,5,6]

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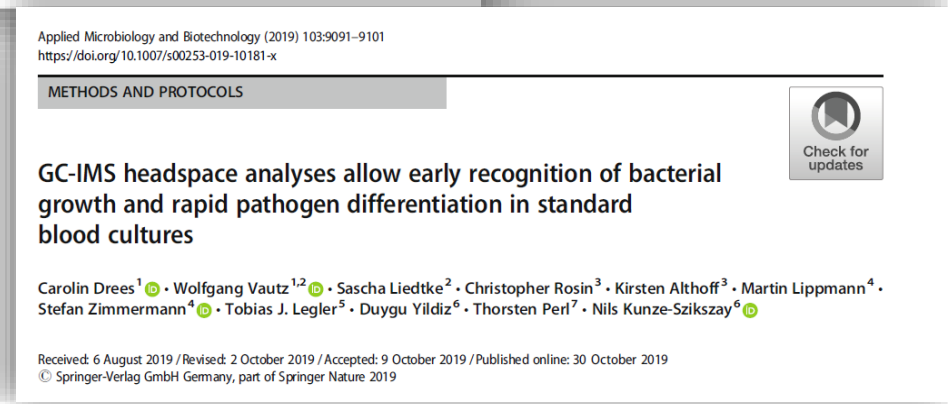


Applied Microbiology and Biotechnology (2019) 103:9091–9101
https://doi.org/10.1007/s00253-019-10181-x

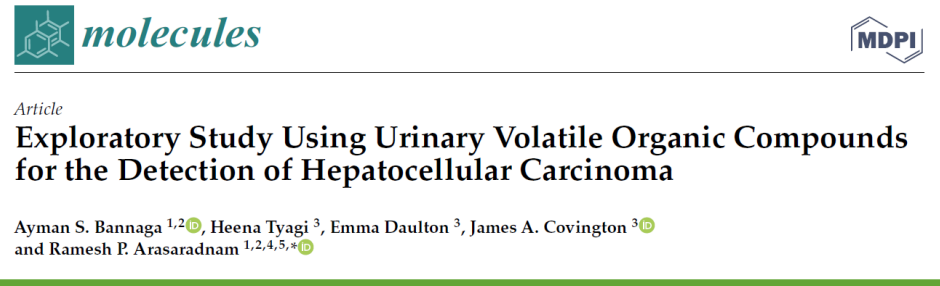
METHODS AND PROTOCOLS

Check for updates

GC-IMS headspace analyses allow early recognition of bacterial growth and rapid pathogen differentiation in standard blood cultures

Carolin Drees[1] · Wolfgang Vautz[1,2] · Sascha Liedtke[2] · Christopher Rosin[3] · Kirsten Althoff[3] · Martin Lippmann[4] · Stefan Zimmermann[4] · Tobias J. Legler[5] · Duygu Yildiz[6] · Thorsten Perl[7] · Nils Kunze-Szikszay[6]

Received: 6 August 2019 / Revised: 2 October 2019 / Accepted: 9 October 2019 / Published online: 30 October 2019



molecules

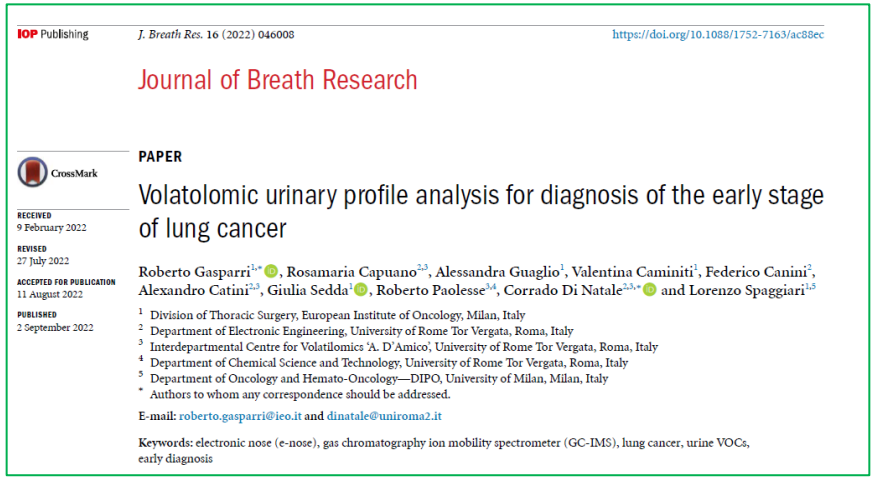
MDPI

Article

Exploratory Study Using Urinary Volatile Organic Compounds for the Detection of Hepatocellular Carcinoma

Ayman S. Bannaga [1,2], Heena Tyagi [3], Emma Daulton [3], James A. Covington [3] and Ramesh P. Arasaradnam [1,2,4,5,*]

GC-IMS: The case of Lung Cancer



IOP Publishing J. Breath Res. 16 (2022) 046008 https://doi.org/10.1088/1752-7163/ac88ec

Journal of Breath Research

PAPER

RECEIVED 9 February 2022
REVISED 27 July 2022
ACCEPTED FOR PUBLICATION 11 August 2022
PUBLISHED 2 September 2022

Volatolomic urinary profile analysis for diagnosis of the early stage of lung cancer

Roberto Gasparri[1,*], Rosamaria Capuano[2,3], Alessandra Guaglio[1], Valentina Caminiti[1], Federico Canini[2], Alexandro Catini[2,3], Giulia Sedda[1], Roberto Paolesse[3,4], Corrado Di Natale[2,3,*] and Lorenzo Spaggiari[1,5]

[1] Division of Thoracic Surgery, European Institute of Oncology, Milan, Italy
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[3] Interdepartmental Centre for Volatilomics 'A. D'Amico', University of Rome Tor Vergata, Roma, Italy
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[5] Department of Oncology and Hemato-Oncology—DIPO, University of Milan, Milan, Italy
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Keywords: electronic nose (e-nose), gas chromatography ion mobility spectrometer (GC-IMS), lung cancer, urine VOCs, early diagnosis

Cohort

Features	Lung cancer group	Control group
Subjects (n)	46	81
Sex	Females: 21 Males: 25	Females: 34 Males: 47
Age (mean ± standard deviation)	65 ± 8	62 ± 9
Smokers	20	33
Ex-smokers	21	38
Pulmonary arterial hypertension	26	39
Cardiac diseases	16	8
Metabolic diseases (diabetes and/or dyslipidemia)	14	24
Chronic Obstructive Pulmonary Disease (COPD)	3	11
Asthma	0	3

Lung cancer patients

Classifiers	Type	Subjects (n)
Histology	Adenocarcinoma	33
	Adenosquamous	1
	Neuroendocrine carcinoma	3
	Squamous cell carcinoma	9
Grading	I	7
	II	21
	III	18
Stage	IA1	2
	IA2	9
	IA3	7
	IB	10
	IIA	2
	IIB	10
	IIIA	6

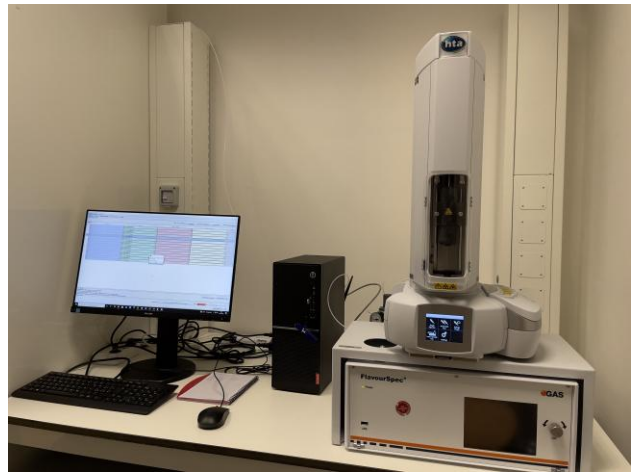
Urinary Volatilome analysis by GC-IMS

GC column:a 30 m × 0.53 mm I.D. × 1 µm MXT-5 column (Restek Corporation,Bellefonte, Pennsylvania)

Urine sample conditioning (HTA autosampler, HT2000H)

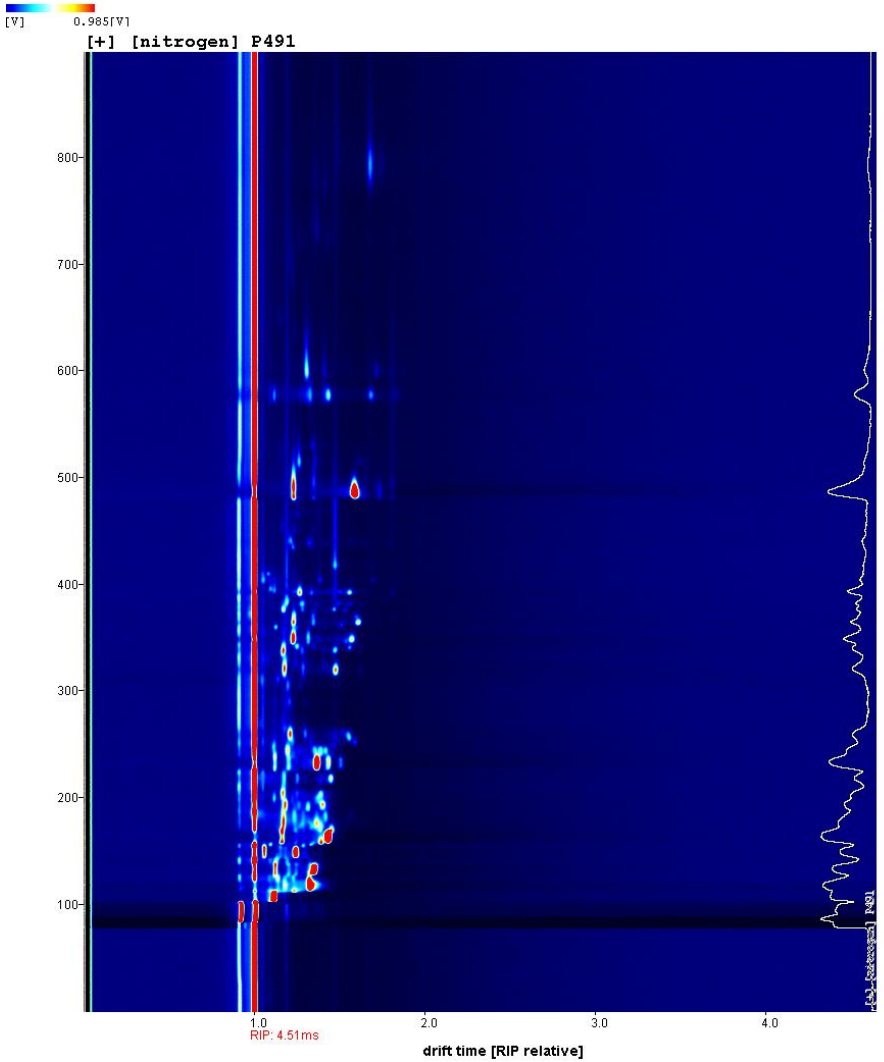
@50°C for 10 min

700 µl of urine headspace were automatically injected in the GC-IMS by means of a 2.5 ml syringe (70 ◦C).



GC and IMS temperature @45 ◦C
Inlet injector temperature @ 80 ◦C.
GC carrier gas flow :@ 5 ml/min for 5 min ; increased to 50 ml/min in 5 min and held for a further 5 min.
TOT run time of 15 min for each sample.

- IMS analysis was conducted in positive ion mode.
- GC-IMS data were extracted using VOCal software platform (G.A.S. Dortmund, Germany).



Characteristic Urine Map

GC-IMS Results

A total of 66 areas was obtained

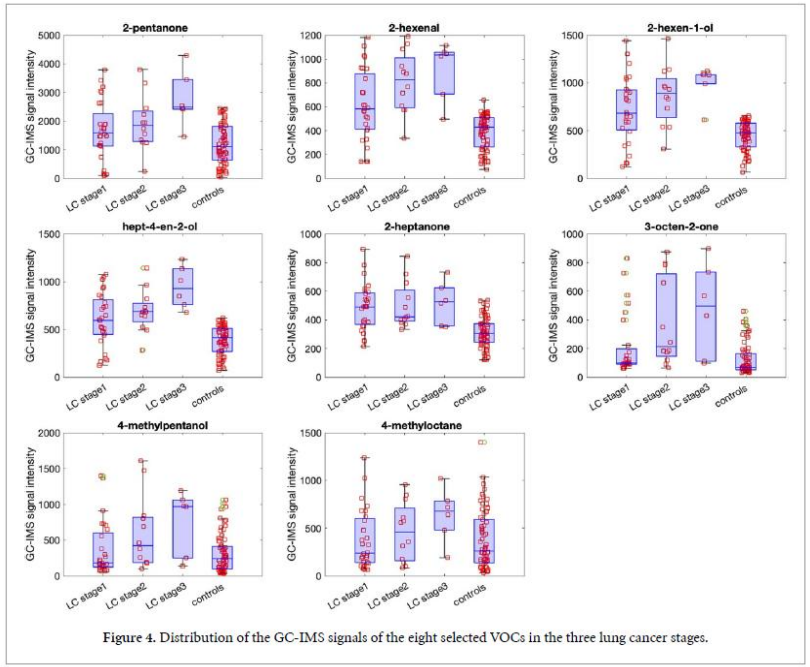


Figure 4. Distribution of the GC-IMS signals of the eight selected VOCs in the three lung cancer stages.

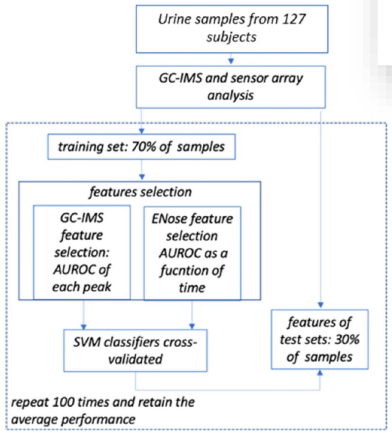
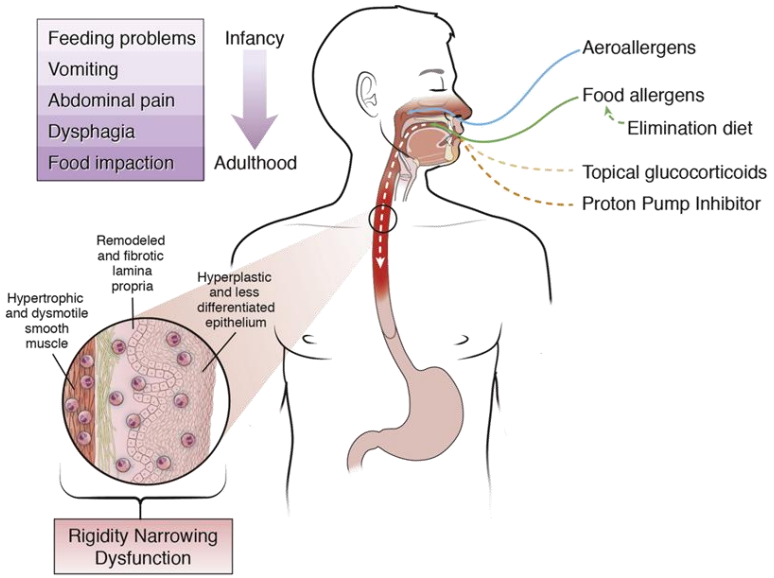


Figure 1. Observational design and data analysis flow.

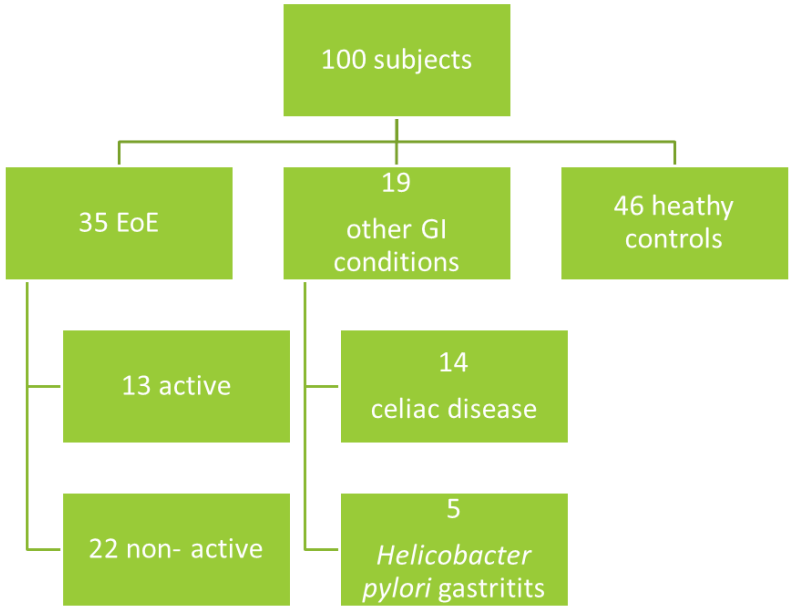
Table 3. GC-IMS data classification. Results of SVM classifier. All cancer vs. controls.

Number of selected features	AUROC	Accuracy	True Positive Rate	True Negative Rate
8	Training: 0.99 Test: 0.91	Training: 97% Test: 91%	Training: 100% Test: 89%	Training: 96% Test: 92%

Salivary volatilome profiling in pediatric eosinophilic esophagitis(EoE)



EoE: Chronic immune disease requiring repeated endoscopies for diagnosis and monitoring in children

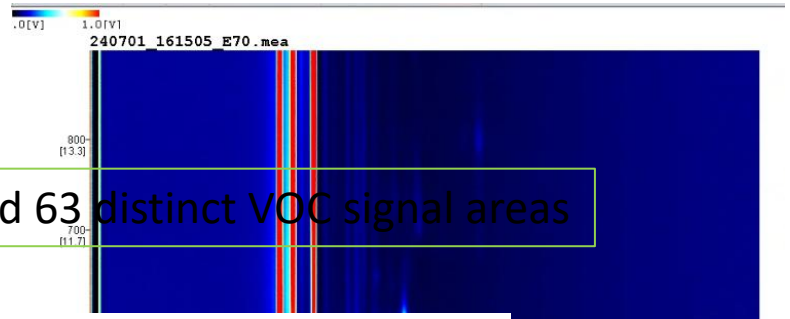


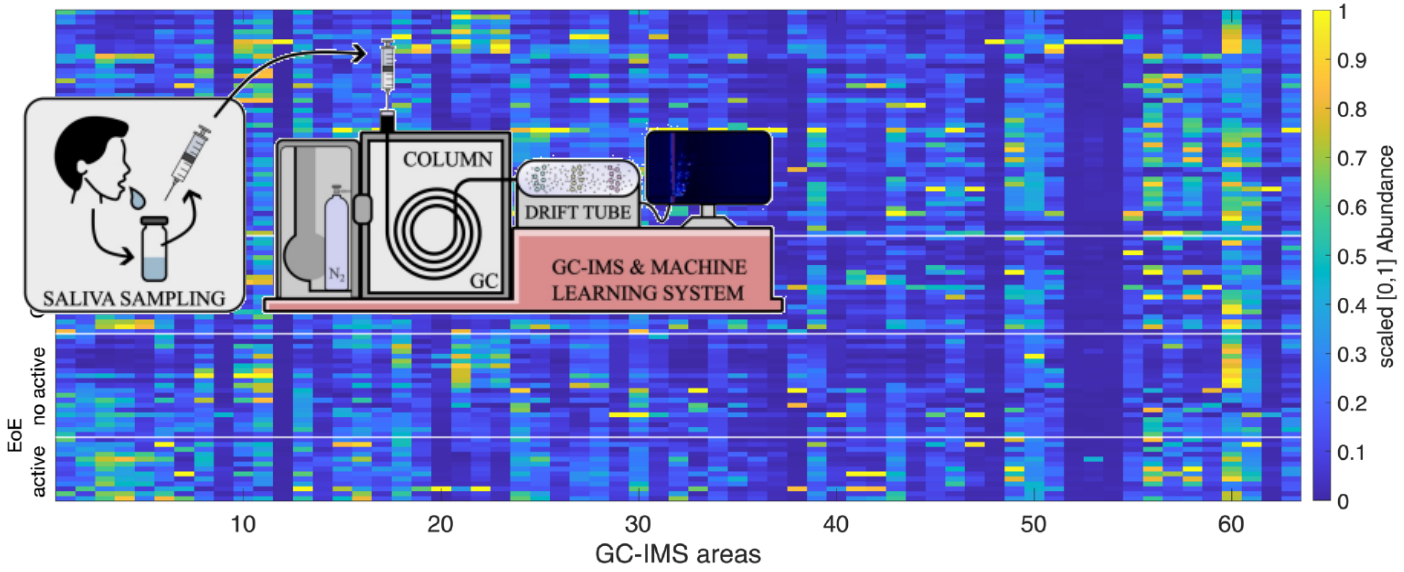
Characteristics of the population	EoE (n= 35)	CD (n = 14)	HP gastritis (n = 5)	healthy controls (n = 46)
Age, median (IQR)	14 (9-17)	10.5 (6.7-13)	13 (8.5-15)	11 (8-14.5)
Disease duration, median (IQR)	36 (19-75)	-	-	-
Sex M, N (%)	27 (77)	7 (50)	3 (60)	17 (37)
Eosinophil count (x10^3/mL), median (IQR)	0.31 (0.2-0.53)	0.13 (0.11-0.48)	0.4 (0.1-1.7)	0.16 (0.14-0.26)
CRP (mg/dL), median (IQR)	0.07 (0.06-0.1)	0.06 (0.06-0.23)	0.06 (0.06-1.6)	0.06 (0.06-0.1)
ESR (mm/h), median (IQR)	10 (4-17)	6.5 (2.7-12)	15 (4-37)	7 (4-16)
Endoscopically active (EREFS ≥ 3), n (%)	7 (20)	-	-	-
Histologically active (>15 Eos/HPF)	13 (37)	-	-	-
Therapy at the time of EGDS, n (%)				
PPI	13 (37)			
Oral viscous Budesonide	18 (51.4)			
Budesonide dispersable tablet	5 (14)			
Symptoms at the time of EGDS, n (%)				
Food impaction	6 (17)	-	0	-
Epigastric pain	5 (14)	1 (7)	3 (60)	11 (24)
Heartburn	8 (23)	-	2 (40)	10 (22)
Dysphagia	6 (17)	1 (7)	0	7 (15)

EoE: eosinophilic esophagitis; CD: celiac disease, HP: helicobacter pylori; IQR: interquartile range; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Eos: eosinophils; HPF: high power field; EGDS: esophagogastroduodenoscopy; PPI: proton pump inhibitors.

Salivary volatilome profiling in pediatric eosinophilic esophagitis(EoE)

GC–IMS identified 63 distinct VOC signal areas





VOC	EoE *vs.* controls	EoE *vs.* CD and HP	Active *vs.* non active EoE	Biological matrices	Associated disease
cis-3-hexen-1-ol	p<0.02	p<0.007	p<0.05	-	-
1,8-cineole	p<0.0005	-	p<0.05	Plasma, urine	Inflammation
2-Hexanone	p<0.06	-	-	Breath	Asthma
2-methylpropanoic acid	p<0.01	p<0.08	-	Saliva	Periodontitis
2-Phenylethanol	p<0.0003	p<0.07	p<0.0005	Feces	Ulcerative colitis
3-Methyl-3-buten-1-ol	p<0.06	-	-	Breath	Emphysema
Acetoin	-	-	p<0.06	Saliva	Periodontitis
Benzaldehyde	p<0.01	-	p<0.05	Urines Breath	Kidney cancer; Idiopathic pulmonary fibrosis
Benzyl alcohol	-	-	p<0.08	Breath	Hypoxia
Ethyl 2-methylpropanoate	-	p<0.08	-	Feces	Autism
Heptan-2-one	p<0.07	-	-	Saliva	Ulcerative colitis CD Oral cancer
Methyl isobutyl ketone	p<0.07	p<0.1	-	Feces	GI disease
Methylpyrazine	-	-	p<0.04	Urines	Lymphoma
n-Hexanol	-	-	p<0.05	Breath	Colon cancer
Octanal	-	p<0.004	p<0.0002	Breath	COVID-19
pentan-1-ol	-	-	p<0.005	Breath	CD

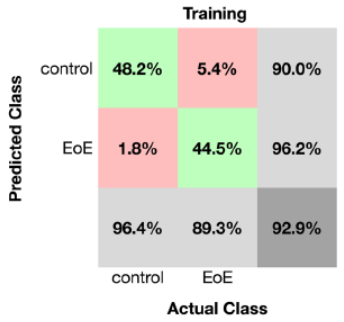
Salivary volatilome profiling in pediatric eosinophilic esophagitis(EoE)

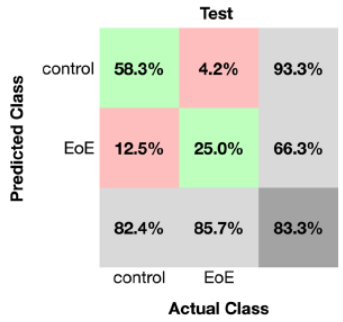
Life Medicine
JOURNAL ARTICLE ACCEPTED MANUSCRIPT

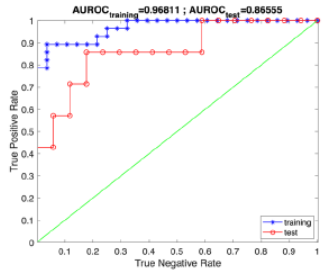
Salivary volatilome profiling in pediatric eosinophilic esophagitis: a pilot study on a non-invasive approach in clinical practice

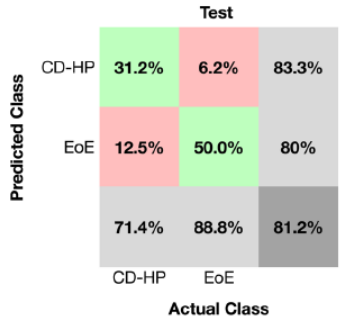
Rosamaria Capuano, Carla Petrella, Christian Barbato, Giulia D'Arcangelo, Giusy Russo, Alexandro Catini, Antonio Minni ✉, Corrado Di Natale ✉, Salvatore Oliva ✉
Author Notes

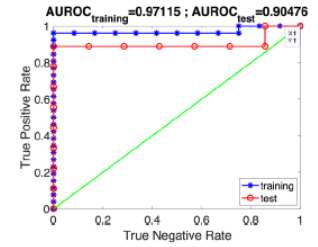
Life Medicine, lnag012, https://doi.org/10.1093/lifemedi/lnag012
Published: 07 April 2026 **Article history**

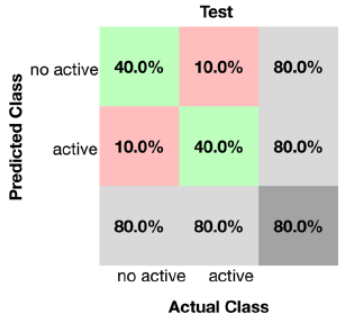


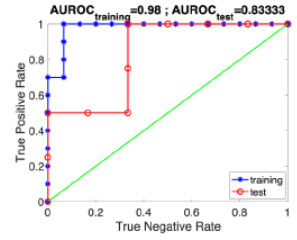












Linear Discriminant Analysis (LDA)

		Accuracy	True positive rate	True negative rate	AUROC
EoE vs. controls	Training	92.9%	89.3% ± 10%	96.4 ± 11%	0.96
	Test	83.3%	85.7% ± 17%	82.4 ± 18%	0.86
EoE vs CD-HP	Training	92.1%	96.2% ± 10%	83.3 ± 11%	0.97
	Test	81.2%	88.8% ± 17%	71.4 ± 18%	0.90
EoE active vs. non-active	Training	96.0%	88.9% ± 10%	100% ± 11%	0.98
	Test	80.0%	80.0% ± 17%	80.0 ± 18%	0.83

Volatolomic analysis of extracellular vesicles extracted from cultured cells

Talanta 307 (2026) 129776

Contents lists available at ScienceDirect

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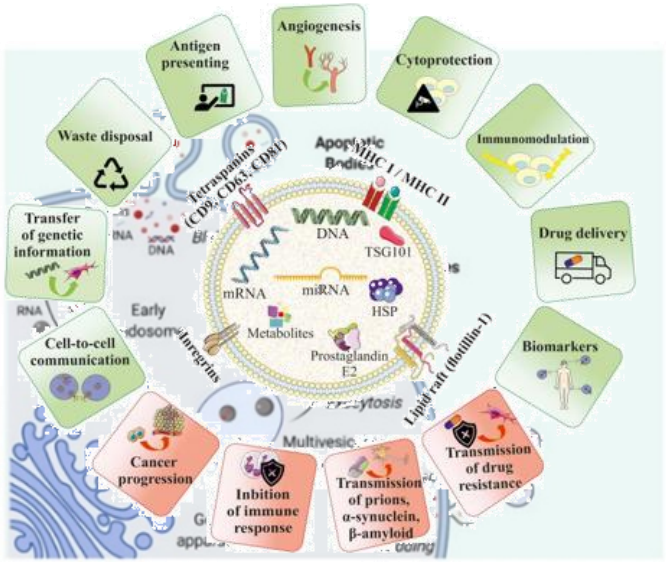
Rosamaria Capuano [a], Siong Fong Sim [b], Mohammad Mehedi Hasan [c,d], Pascale V. Guillot [e], Corrado di Natale [a,h], Alexandro Catini [a], Carmenza Spadafora [f], Mihai Brebu [g], Radu Ionescu [d,*]

Membrane-bound lipid nanovesicles secreted by various cells



Biological role of EVs in homeostasis and pathophysiology. (Karnas E, *et al.*, 2023)

Significance of EVs in **information transfer** and **cell-to-cell communication**, such as tumor development, immune responses, and cell apoptosis.

They can also **pass through the blood–brain barrier**, which characterizes their value in initiation and development of neurological diseases

NEUROLOGICAL DISEASES

SUBDIVIDED INTO **MICROVESICLES (MVS)**, **EXOSOMES**, AND **APOPTOTIC BODIES**, AND THEY DIFFER FROM EACH OTHER IN **SIZE, SHAPE, FORMATION PROCESS,** AND **BIOLOGICAL FUNCTIONS**

EV DIAMETER REANGES FROM 30nm to [illegible]m

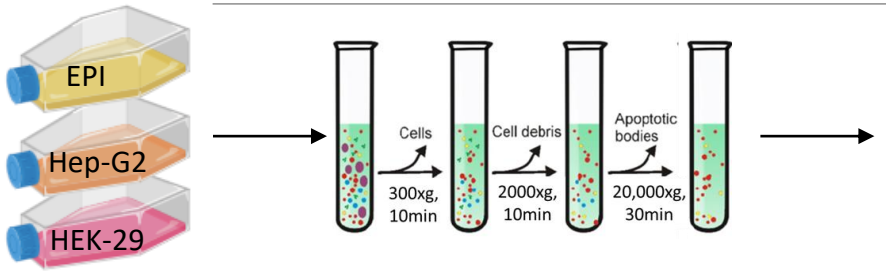
PRODUCED BY BOTH **PROKARYOTIC** AND **EUKARYOTIC** CELLS

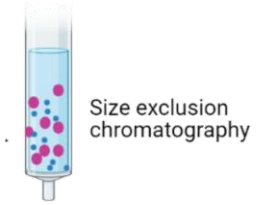
PRESENT IN ALL BIOLOGICAL FLUIDS (SERUM, URINE, SALIVA, CEREBROSPINAL FLUID, AMNIOTIC FLUID, BREAST MILK, ETC)

They can be systemically disseminated in the body, transferring their content/signals to cells physically separated from the secreting cell

They represent a powerful frontier for diagnostic innovation and therapeutic breakthroughs

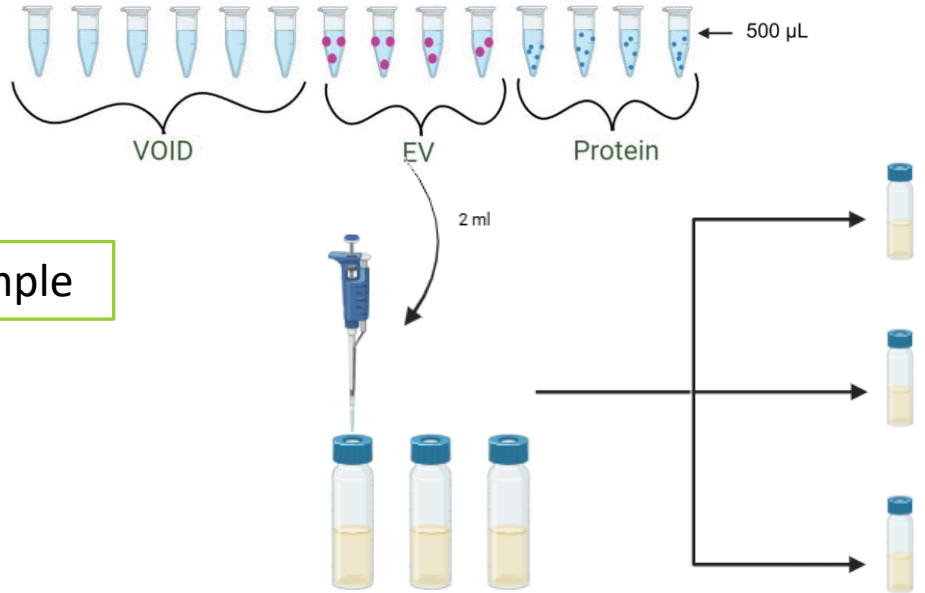
Volatolomic analysis of extracellular vesicles extracted from cultured cells

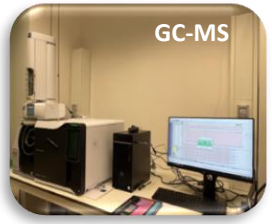


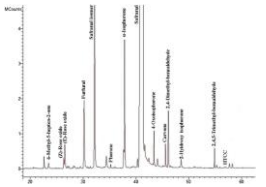


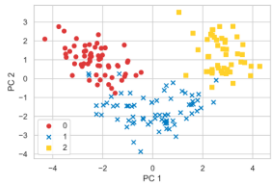
- EPI : Human epithelial cells
- Hep-G2 :Human hepatocellular cancer cells
- HEK-293: Human embryotic kidney cells

PBS used as reference sample









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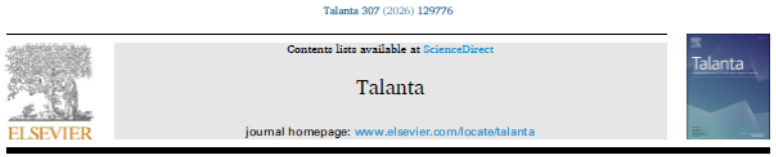
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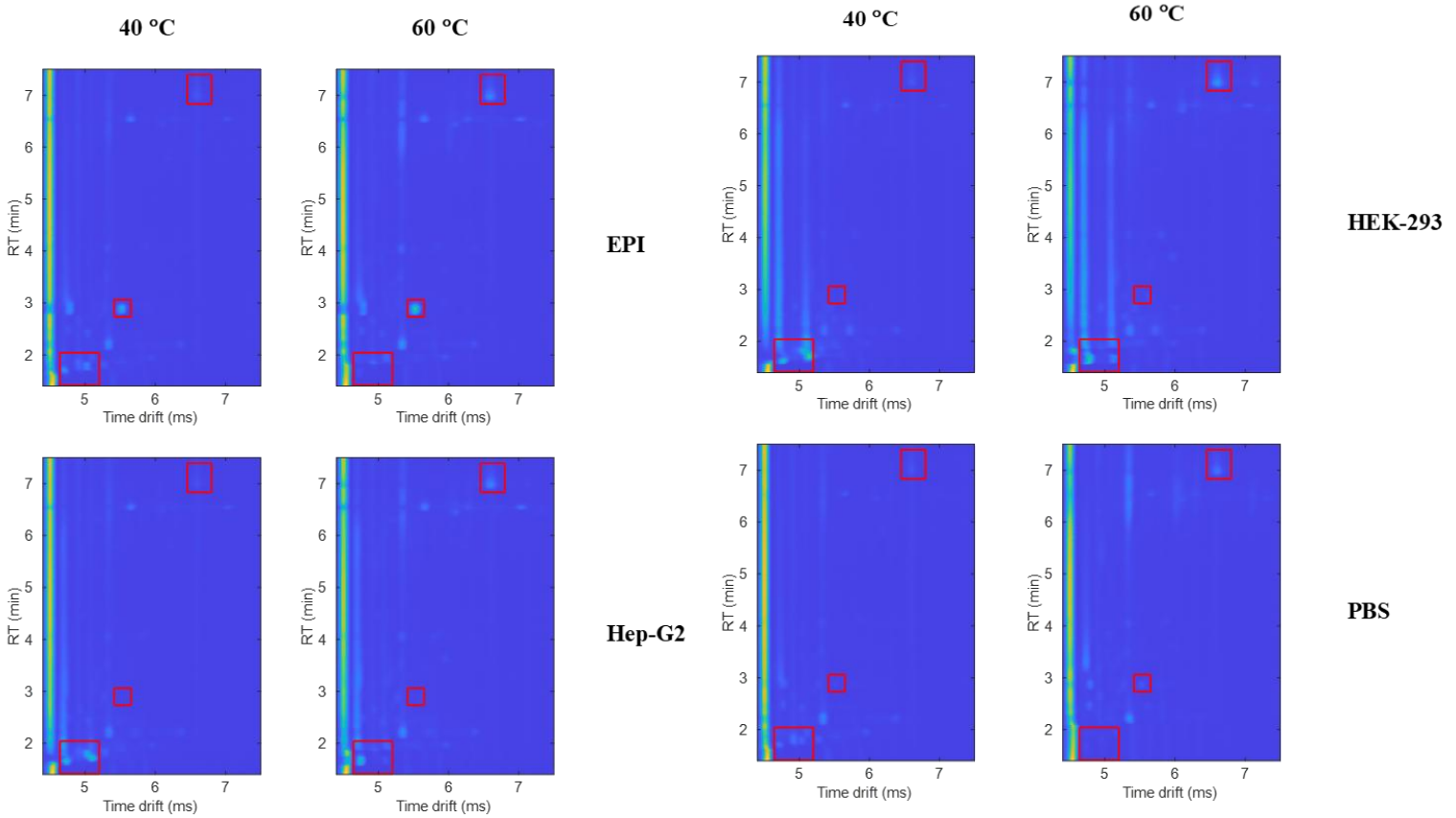
- Region differentiating Hep-G2 and HEK-293 EVs from the other groups:
  - Retention time: between 1.41 and 2.04 min
  - Drift Time: between 4.7 and 5.20 ms
- Region characteristic of the EPI-derived Evs:
  - Retention time: between 2.72 and 3.06 min
  - Drift Time: between 5.41 and 5.65 ms



Ongoing studies

- **Urine** for gynecological disease detection (Uni. Rome Tor Vergata)- CRC diagnosis (EasyCRCscreen Project)

- **Saliva** for pathologies of the oral cavity diagnosis (Uni. La Sapienza)

Interdepartmental Centre for Volatilomics ‘A. D’Amico’ (CIVAD)

Directed by: Prof. Corrado Di Natale

Department of Electronic Engineering

Department of Chemical Science and Technology

Department of Experimental Medicine

Department of Biomedicine and Prevention