LIÈGE université
Arlon Campus
Environnement

Sensing of
Atmospheres and
Monitoring

From Environmental Monitoring to Breath Analysis: Leveraging Sensor arrays for Healthcare – Lessons from the PATHACOV project –

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Responsable Labo SAM
(Sensing of Atmospheres and Monitoring)

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Sensing of
Atmospheres and
Monitoring

Outline

1. Lung cancer and AOS (briefly)
2. PATHACOV project
3. Methodology to develop AOS without/with Patient breath samples?
4. Sensor and AOS performances for LC
5. From Environmental Monitoring to Breath Analysis
6. Take-home messages

1. Lung cancer and AOS (briefly)

- Lung cancer (LC) is one of the most common and deadly forms of cancer.
- Screenings are carried out late (asymptomatic disease): the later the screening, the lower the chances of survival.
- Diagnosis equipment is expensive, not portable, requires trained personnel and

  (screening campaign) Doubts on scans on the entire at-risk population:

  undesirable effects, management of abnormalities, insufficiently defined frequency of checks!

→ **early diagnosis, simple and non-invasive are requested by pulmonologists.**

- New approaches:
  - Hospital-based solutions
  - Screening solutions: dogs and… **Artificial Olfactory System (AOS or IOMS or E-NOSE)**

Volatilome for Lung cancer: VOC biomarkers? no yet a consensus on a list of specific compounds/the efficiency

- **"Breathprint"? → AOS?**
  - numerous studies but samples size too low (exceptionally above 100 for non-healthy patients)
  - results not convincing

  The timeless story of electronic nose !!!

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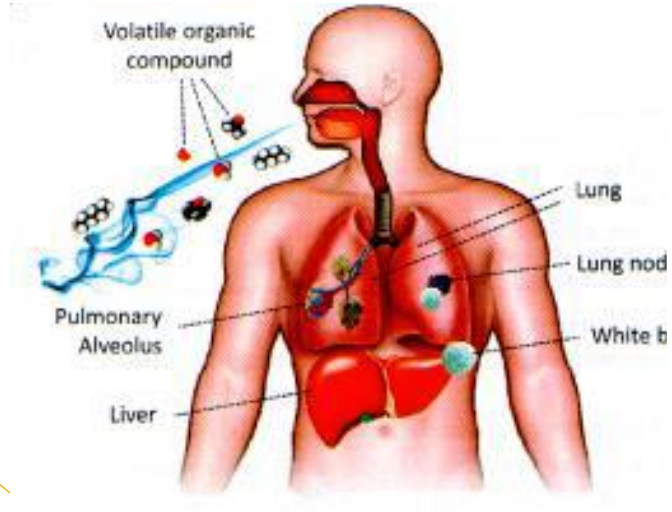
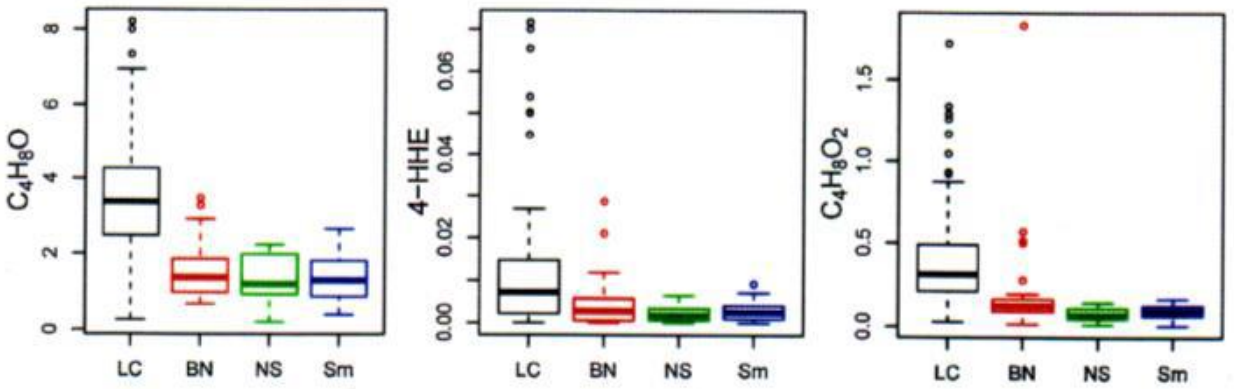
1. Lung cancer and AOS (briefly)

VOC biomarkers

Volatilome





LC : lung cancer, BN : benign nodule, NS : No Smoker Sm: smoker

Antonini JM et al., J Breath Res. 2016

More than 1000 exhaled VOCs

- endogenous and exogenous chemicals
- cellul/organ specific
- organ pathology specific
- + environmental contaminants/life conditions (dtrugs, smoke, food,…)

Courtesy from Prof. Régis Matran, pneumologue, CHU Lille

PATHACOV

HUMAN DISEASES DIAGNOSIS
BY VOC ANALYSIS IN BREATH

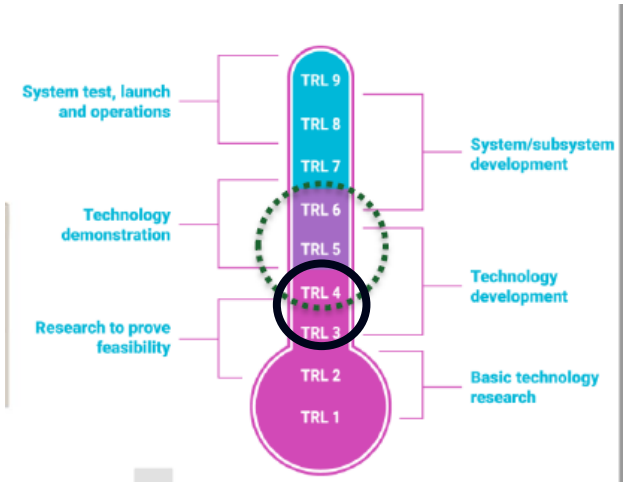
2018 - (end of) 2022
11 partners
doctors, mathematicians, chemists, physicists, electronic engineers, computer scientists…

Large-scale study in various French and Belgian hospitals

- Determining a VOC signature marker for LC (“breathprint”)
- Developing an artificial olfaction system

Interreg France-Wallonie-Vlaanderen
Wallonie
West-Vlaanderen
Door mensen gedreven

CHU Amiens Picardie
CHU Lille
CHU de Reims
MateriaNova Materials R&D Centre
Liège université
Université de Lille
eurasanté Entreprendre et Réussir
IMT Lille Douai École Mines-Télécom IMT-Université de Lille
KU Leuven
UMONS Université de Mons
Université de Reims Champagne-Ardenne



https://pathacov-project.com/

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❑ **Clinical study**: 1 400 subjects (650 patients and 750 control people (control: smoker, non-smoker)

→ VOC markers of bronchopulmonary cancer (ReCIVA® mask, GC-MS, data analysis by Machine learning – confidential-)

✓ VOC signature (breathprint) obtained (confidential)

(not yet for AO development)

❑ **New sensors**: Synthesis and study of materials, manufacture of sensors and ability to detect markers

metal oxide sensor (ZnO) ; polymer ; polymer and FET transistor (confidential)

❑ **AO system development**

1. with commercial sensors
2. integration of new sensors

❑ **Communication system**

Exchanging the data collected, via Bluetooth, with a server linked to a database-Secure data exchange between the device and the server-Requires minimal interaction with the doctor

PATHACOV
HUMAN DISEASES DIAGNOSIS BY VOC ANALYSIS IN BREATH

2 years between
the Authorisation of the study protocol by the « Commission de Protection des Personnes (CPP) » and authorising inclusion of the first patient
(due to time to acquire equipment and administrative concerns)

Why clinical study was not used for AO development?

In addition, new sensors cannot be developed in few weeks without knowing the target compounds and their concentration

Essential prerequisites for starting the study:

- Obtaining all "administrative" authorisations
- Sufficient supplies of equipment
- Setting up the data collection system (tested on the Lille CHU team for Reciva)
- Consideration of patient targeting, the patient's journey after the diagnosis (psychologically difficult!) and the proposal to take part in the study (explanations, consent, data collection, patient follow-up).

Before the other recruiting hospital centres can begin the study, the principal investigator (Lille University Hospital) must:

- Integrate all recruiting centres into the study protocol
- Initiate patient recruitment
- Draw conclusions / Improve operating procedures
- Obtain sufficient supplies of the necessary equipment for all recruiting centres (electronic nose, RECIVA masks, gloves, etc.)
- Set up each centre: training, equipment, etc.

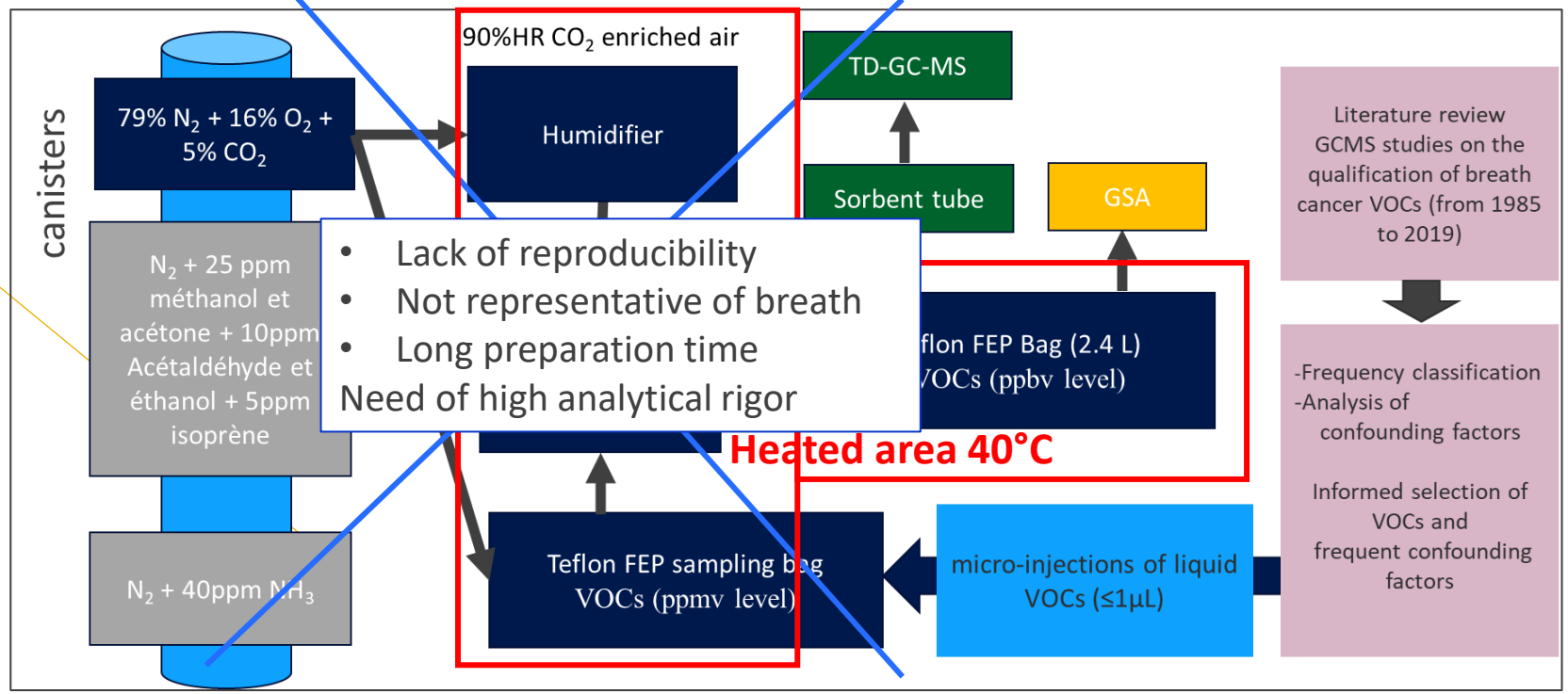
3. Methodology to develop AOS without Patient breath samples? (1/5)

- **Artificial breath?**

  → Potential biomarkers and respective chemical concentration: Literature survey (under publication)

  → VOC signature (breathprint) obtained at the end of the Pathacov clinical study (confidential-Patent)

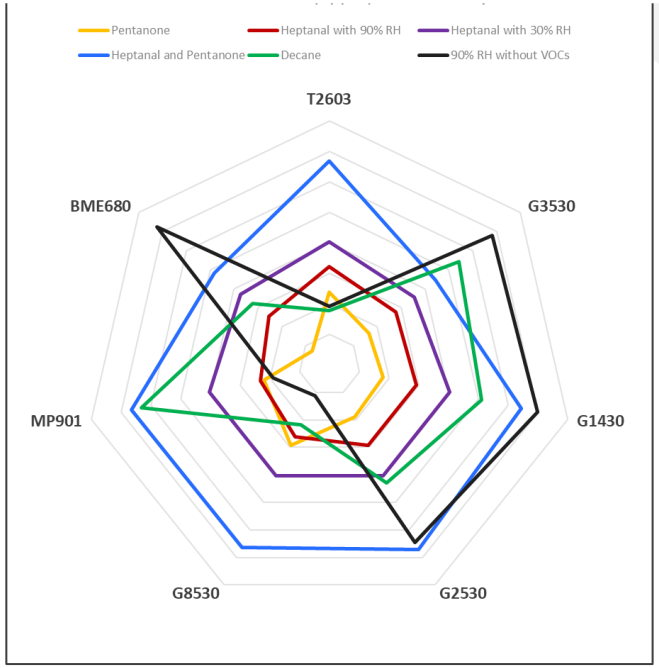
  ➢ Sample? first trial: **breathless mixture = CO_2 enriched air + VOC + Humidity**



- Lack of reproducibility
- Not representative of breath
- Long preparation time

Need of high analytical rigor

Could be helpful for sensor selection



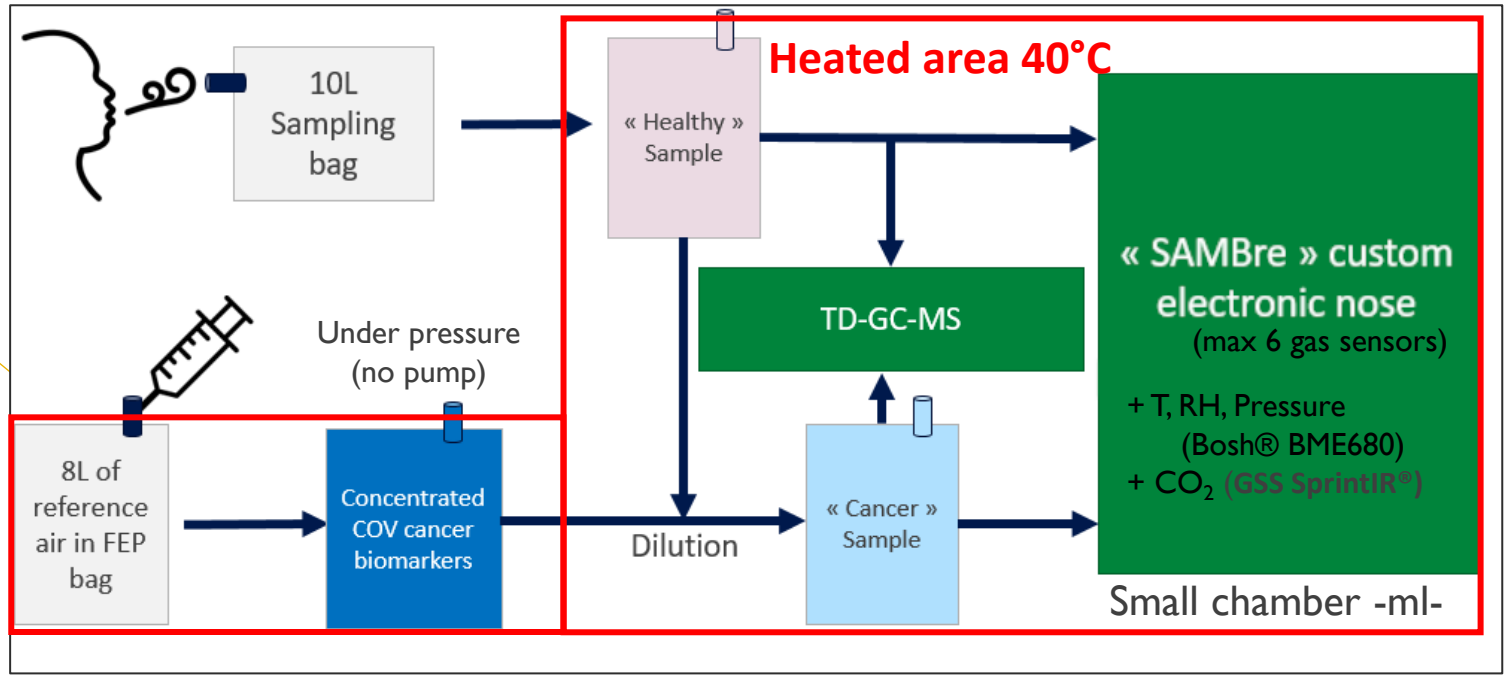
4 replicates, 4 concentrations, several weeks, randomly

3. Methodology to develop AOS without Patient breath samples? (2/5)

- **Artificial breath?**

→ Potential biomarkers and respective chemical concentration: Literature survey (under publication)
→ VOC signature (breathprint) obtained at the end of the Pathacov clinical study (confidential)

➢ Sample, second trial: **Real breath** + 9 VOC*



Some sensors…

- G **1430**T, **2530T, 3530T**, 8530T, (Umwelt Sensor Technik™)
- MP901 (Winsen™),
- TGS2603 (Figaro Engineering™)
- Experimental 1,3 and 5% Fe-doped ZnO **

Biomarkers (VOCs) additions to breath

2-pentanone 5 ppbv	2-butanone 5 ppbv
Acetone 295 ppbv	Dodecane 5 ppbv
Hexanal 5 ppbv	1-propanol 60 ppbv
Toluene 10 ppbv	2-propanol 245ppbv
	Ethanol 310 ppbv

**Martin, J.D.M.; Romain,A.-C. Building a Sensor Benchmark for E-Nose Based Lung Cancer Detection: Methodological Considerations. Chemosensors 2022,10, 444. https://doi.org/10.3390/chemosensors10110444*

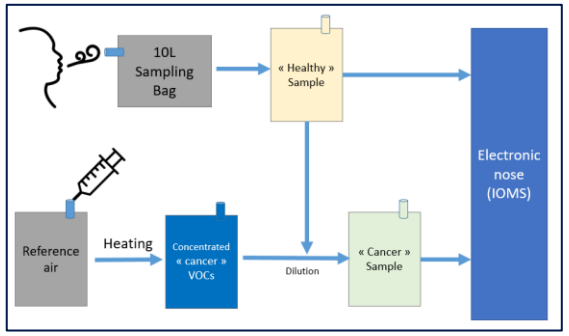
***Y Luo, A Ly, D Lahem, J D.M. Martin, AC Romain, C Zhang, M Debliquy, Role of cobalt in Co-ZnO nanoflower gas sensors for the detection of low concentration of VOCs, Sensors and Actuators B: Chemical,Volume 360,2022,131674, https://doi.org/10.1016/j.snb.2022.131674.*

3. Methodology to develop AOS without Patient breath samples? (3/5)

- "Artificial" Real breath

protocol validated by the ULiège-CHU ethics committee (Pr. Schleich)

Participant Breath sampling



best methodology we found to reduce the intersubject variability

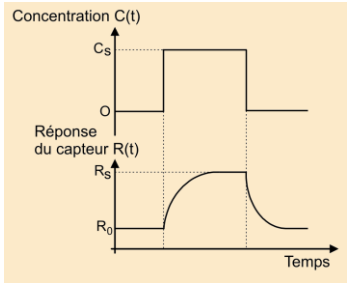
- same "environment" (local, T, RH and air exchange); same operator
- Time: no restrictions, "nothing by mouth" 12 hours before sampling
  (i.e., no smoking, teeth brushing, chewing, or eating. Drinking water was allowed).
- 5 minutes to acclimatise and rest (seated position). Fill in the questionnaire
- They were given water for mouth rinsing before blowing in a bag
- Inhale to full capacity then blow into bag.
- Successive exhalations until +/- 8L of exhalation is obtained (whole breath)
- Storage: max 03 hours before measurement

FEP sampling bag through a spirometry saliva filter in a Polytetrafluoroethylene (PTFE) holder

AOS measurement

- cycle: reference air (humidity-saturated air – synthetic air or filtered ambient air –); 5min/5min
- thermostated
- Feature: one/sensor = raw signal difference (stable conductance)

- not in-line sampling (if direct blowing = no stable signal)
- "Off-line" sampling: 2 steps in the same device
  1. Breath stored in a medium by sampler
  2. Medium is connected to AOS



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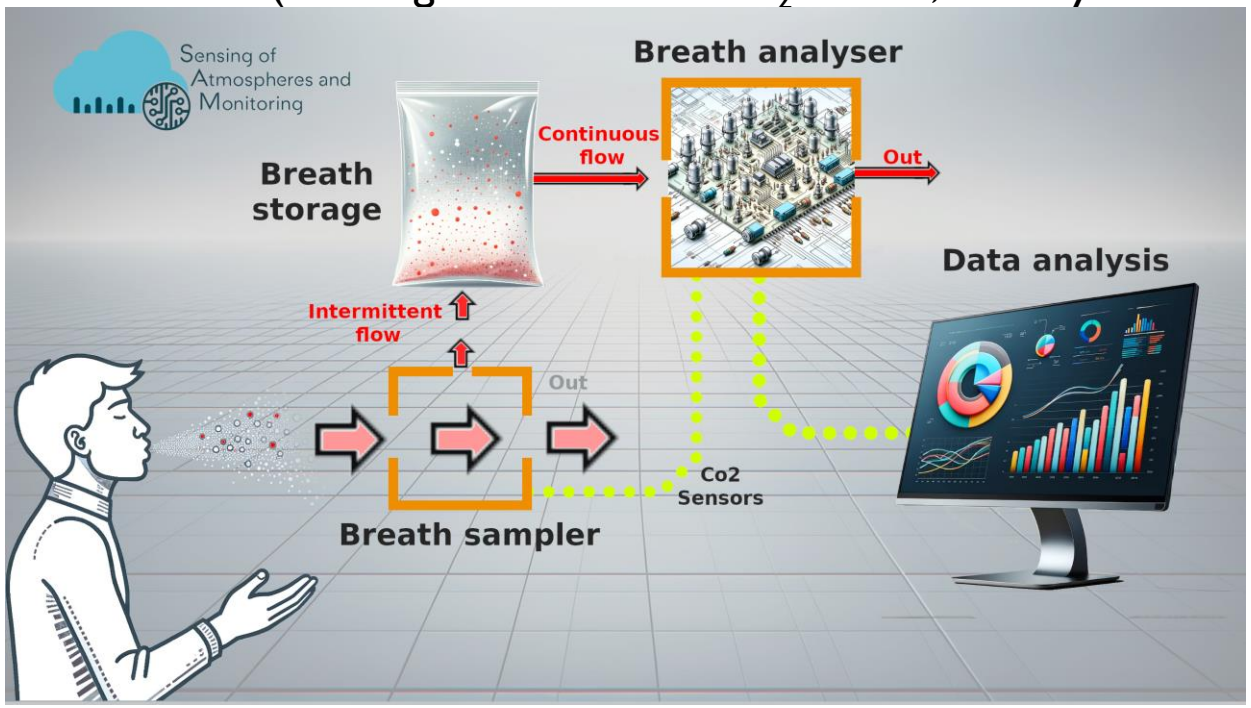
Methodology to develop AOS with Patient breath samples? (4/5)

- **Patient breath sampling for clinical study**

Same conditions but blowing in a « specific sampler »

- reduced bag volume: max one liter
- very low-pressure resistance to help patients suffering from respiratory diseases
- several blowing; a wait of few seconds before sending the breath through the analyser (blowing control with CO_2 sensor, actually not really "capnography")



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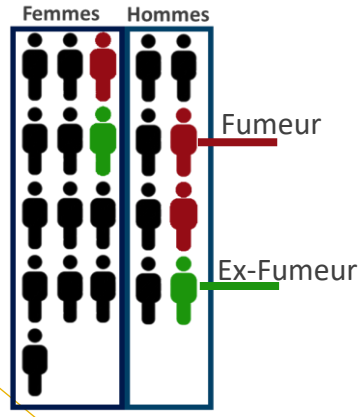
3. Methodology to develop AOS without Patient breath samples? (5/5)

protocol validated by the ULiège-CHU ethics committee (Pr. Schleich)

Illustration "Real" breath + 9 VOC



- 21 participants
- 26 sampling days across four months (August 2022 and January to March 2023)
- 127 unique breath samples (average of 6 samples/participants)
- 236 measurements
  - 117 « healthy »
  - 119 « sick »

Male (%)	40 (31.7%)	BMI – Average	24.0
Age – Average	36.6	BMI – Median	26.0
Age – Median	34.0	BMI – Range	18.4 – 29.4
Age – Range	21 - 62	Sport – Daily (%)	15 (11.9%)
Smokers (%)	7 (5.6%)	Sport – Weekly (%)	61 (48.4%)
Ex-smokers (%)	18 (14.2%)	Sport – Monthly (%)	32 (25.4%)
Never-smokers (%)	101 (80.2%)	Sport – Rarely (%)	18 (14.3%)

(Some data are presenting in the next slides)

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4. Sensor and AO system performances for LC (1/5)

Metrological performances

- operating conditions (e.g. working temperature, energy consumption): Easy to consider
- sensitivity to temperature and humidity variations: Easy to keep constant conditions
- sensor response time and recovery time: Not important for this application
- stabilisation period: Not so important for this application
- sensor lifetime: Should be better
- linear or non-linear response : not a real problem but essential to consider for algorithm development
- …
- stability over time (drift): Important to correct but "rather" easy to do

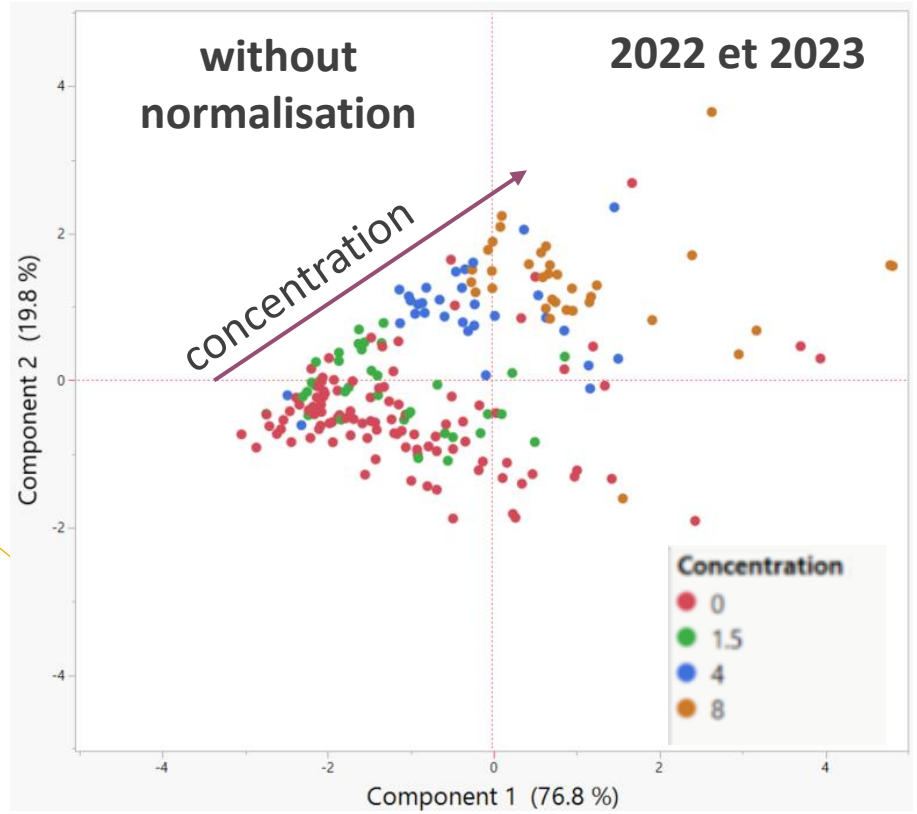
- **Sensitivity**
- **LOD**
- **Selectivity**

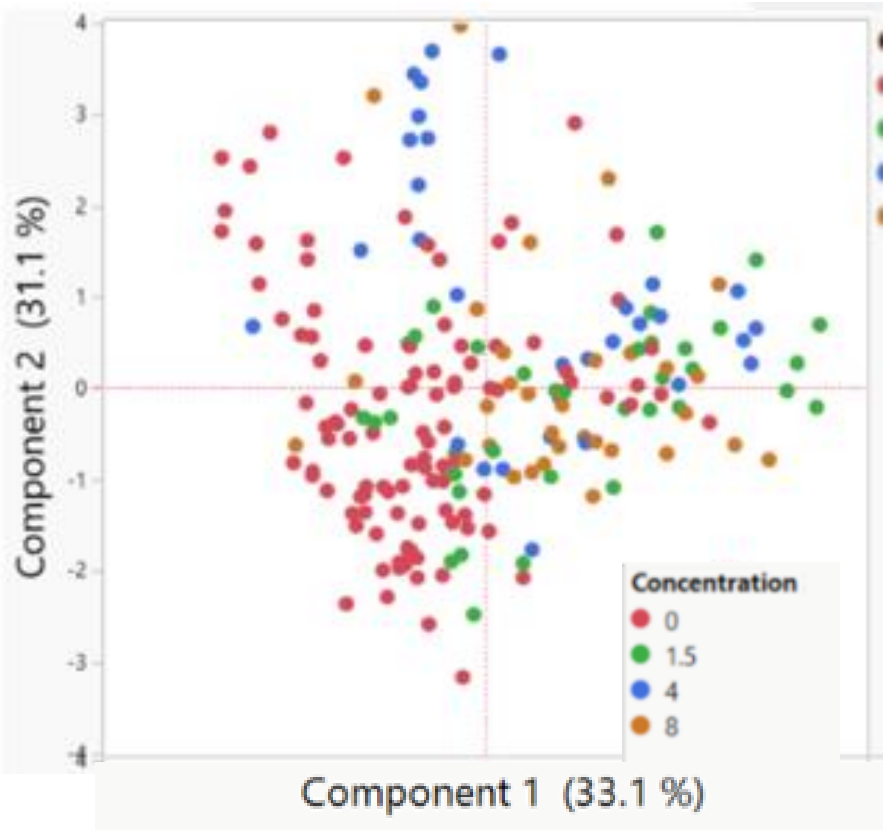
~~Important to know before data analysis!~~
(and do not forget pre-processing)

4. Sensor and AO performances for LC (2/5)

Normalisation?

$$x'_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{n} x_{ij}^2}}$$





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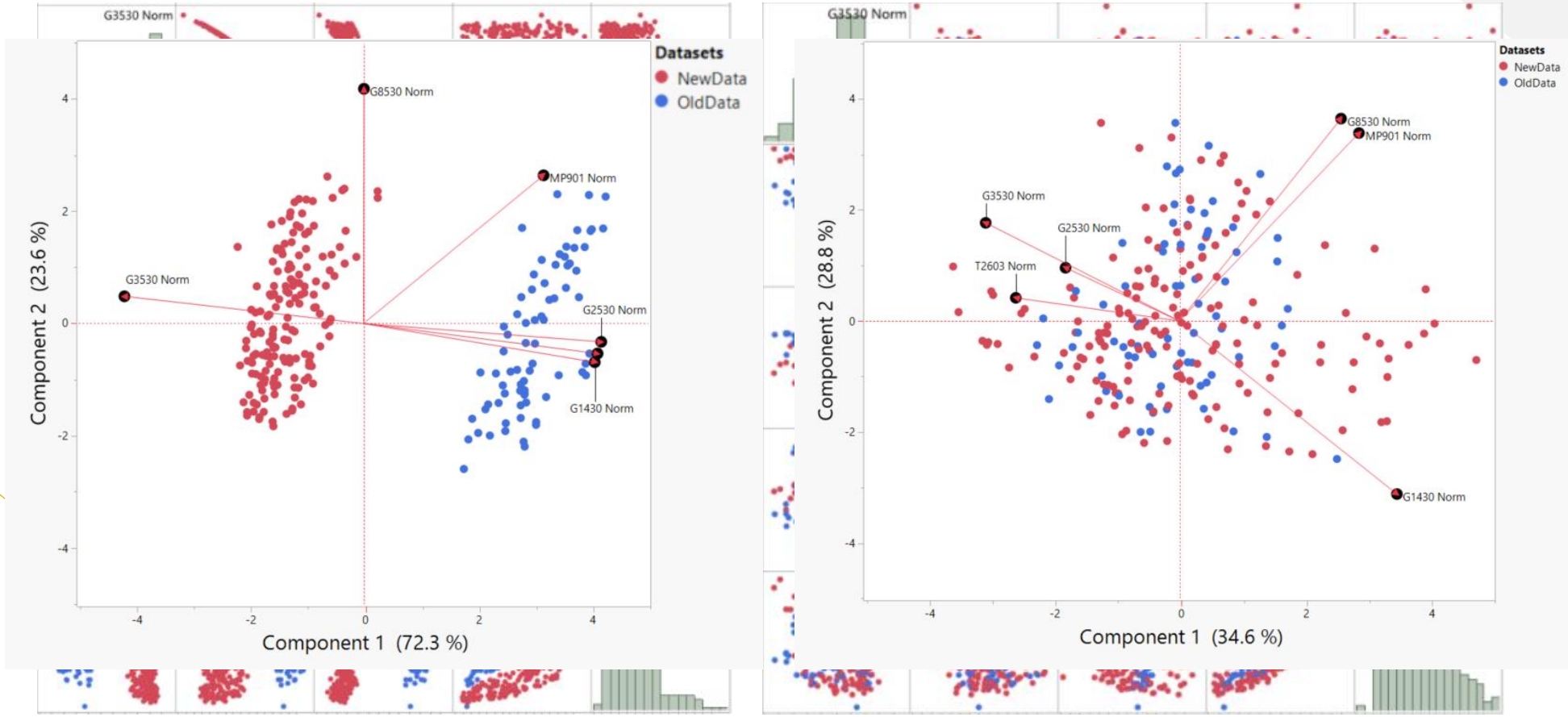
4. Sensor and AO performances for LC (3/5)

Drift: 2022 and 2023

Drift correction



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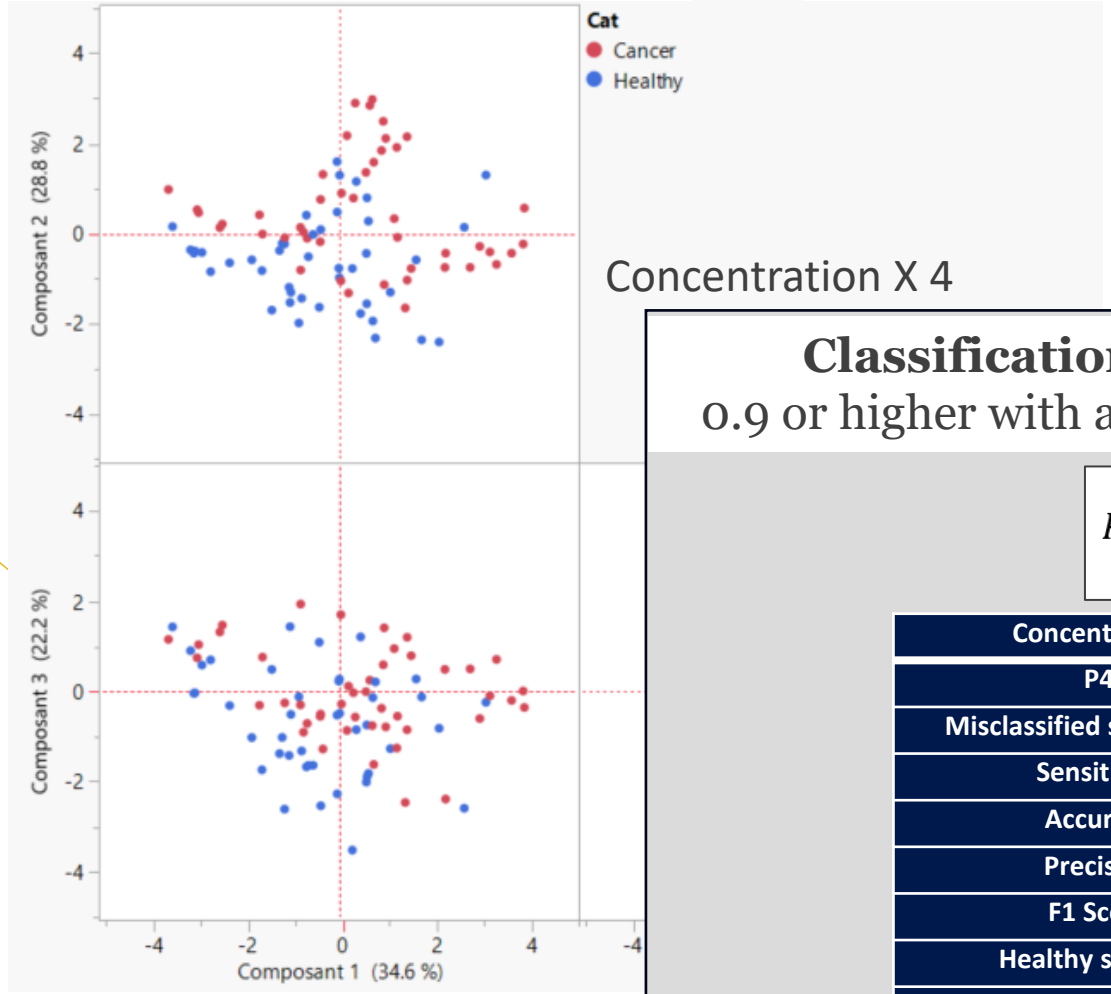
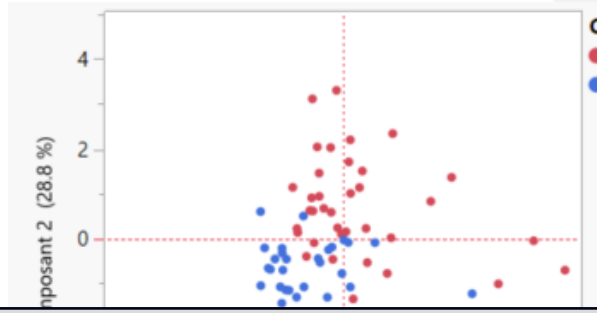
4. Sensor and AO performances for LC (4/5)

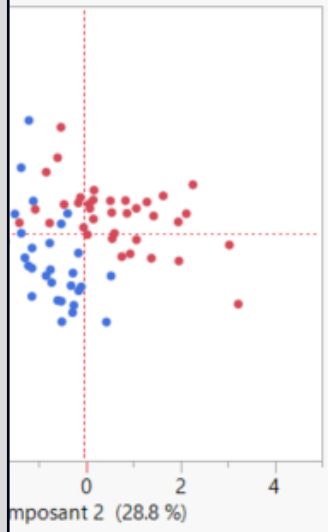
LOD - critical point for breath analysis → limit of classification



Concentration X 4



Concentration X 8



Classification Performance Criterion (CPC) :
0.9 or higher with a minimum of 35 samples from each group

$$P_4 = \frac{4 \cdot TP \cdot TN4}{TP \cdot TN + (TP+TN) \cdot (FP+FN)}$$

Concentration	1.5x	4x	8x
P4	*0.709*	*0.879*	*0.932*
Misclassified samples (%)	*28.76%*	12.08%	6.75%
Sensitivity	*0.806*	0.867	0.946
Accuracy	*0.712*	0.879	0.932
Precision	*0.674*	0.886	0.921
F1 Score	*0.734*	0.876	0.933
Healthy samples	*37*	46	36
Artificial cancer samples	*36*	44	37

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4. Sensor and AO performances for LC (5/5)

LOD - critical point for breath analysis → limit of classification

Discrimination Performance Score (DPS) :

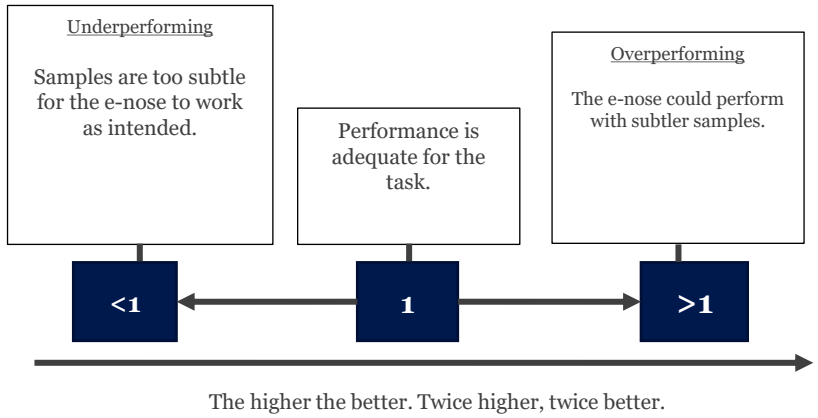


Fig. 2. Diagram illustrating the DPS interpretation for three cases.

J. Martin, C. Falzone and AC. Romain 2024 ***How well does your E-nose detect cancer? Application of artificial breath analysis for performance assessment*** *J. Breath Res.* *https://doi.org/10.1088/1752-7163/ad1d64*

Procedure:

- "healthy" breath collected **on participants** (not patients)
- artificial cancer breath: by adding biomarkers to the "healthy" breath at concentrations "realistic" (low values, ppb-ppt)

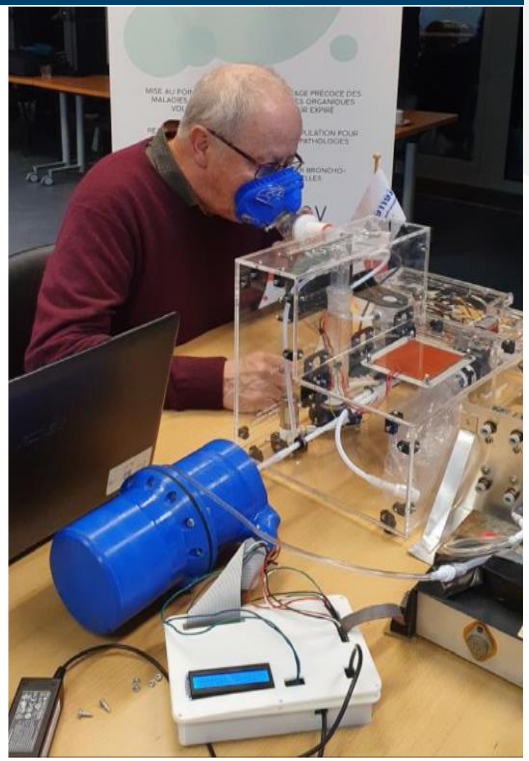
→ Evaluation of the AOS performance in classifying

If the AOS fails to meet the CPC at these initial concentrations,
→ concentration doubled for a repeated test and so on until success is achieved.
The point at which the success is achieved gives the DPS

5. From Environmental Monitoring to Breath Analysis (1/5)

Environmental (odour monitoring)
long experience (30 years)

Breath analysis
short experience (5 years)



ation	
-	- Lab - Patient
uncontrollable	certain freedom
external parameters easy to control	medical constraints

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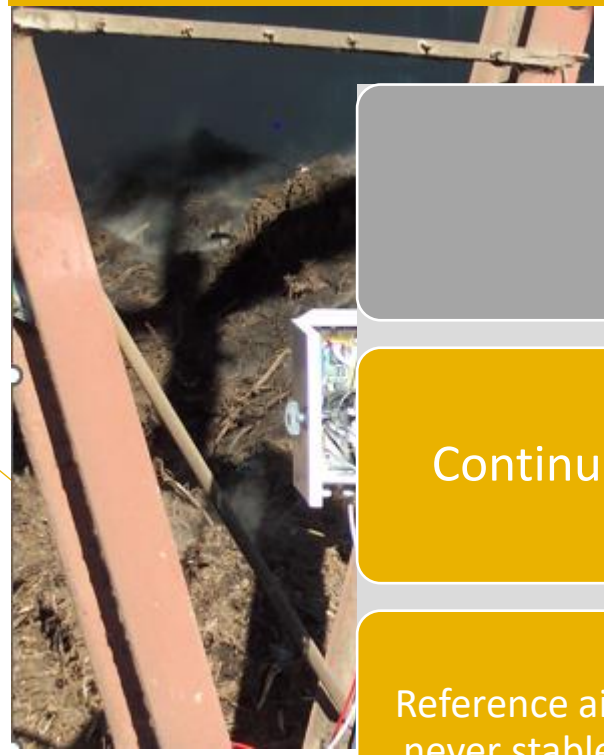
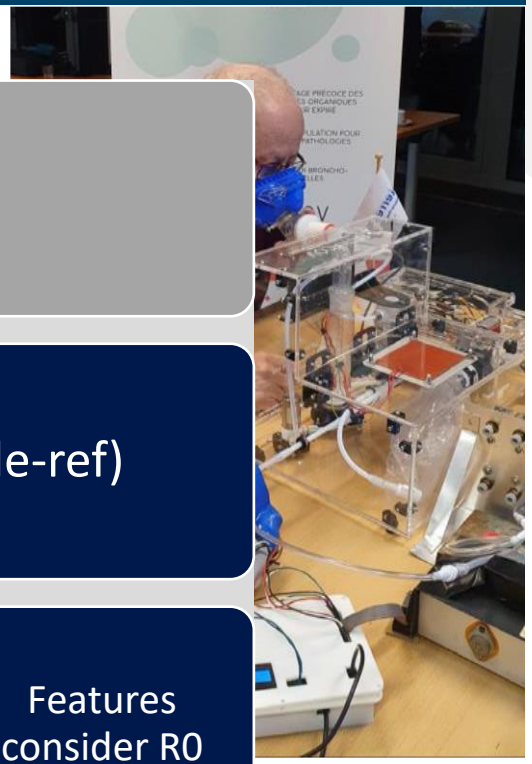
5. From Environmental Monitoring to Breath Analysis (2/5)

Environmental (odour monitoring)
long experience (30 years)



Breath analysis
short experience (5 years)



Measurement

Continuous/Real time	Punctual/Cycle (ref-sample-ref)
Reference air never stable	Feature: raw signal
Reference air constant	Reference air easy to generate
Features consider R0	

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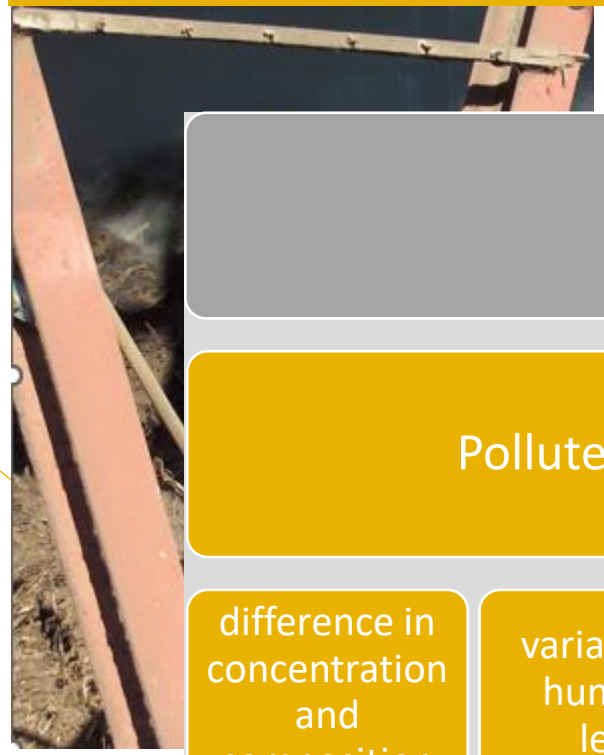
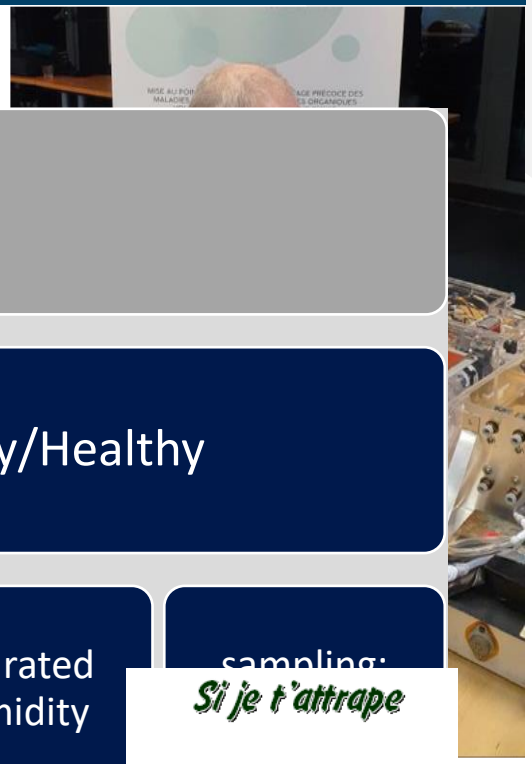
5. From Environmental Monitoring to Breath Analysis (3/5)

Environmental (odour monitoring)
long experience (30 years)



Breath analysis
short experience (5 years)



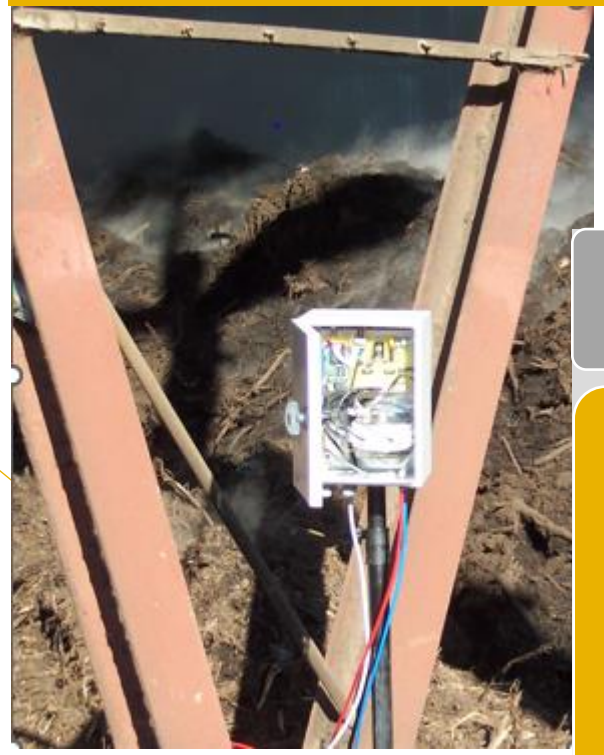
Real sample

Polluted/Unpolluted			Non-Healthy/Healthy		
difference in concentration and composition	variation of humidity level	« relatively » easy to collect (several samples for the same « class »)	same compounds, low concentration: question of ratios	saturated humidity	sampling:

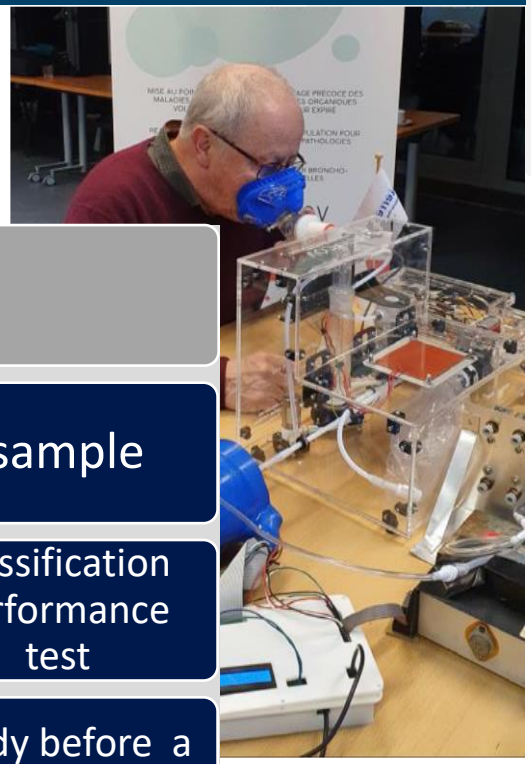
cfr Santi: controlled (we know), not observationnal

5. From Environmental Monitoring to Breath Analysis (4/5)

Environmental (odour monitoring)
long experience (30 years)



Breath analysis
short experience (5 years)



Synthetic sample

Not necessary

If possible, avoid

Essential alternative to the real sample

Sensors pre-selection	Instrumentation test	Classification performance test
Ethic aspects	Staff teaching	Ready before a first clinical trial

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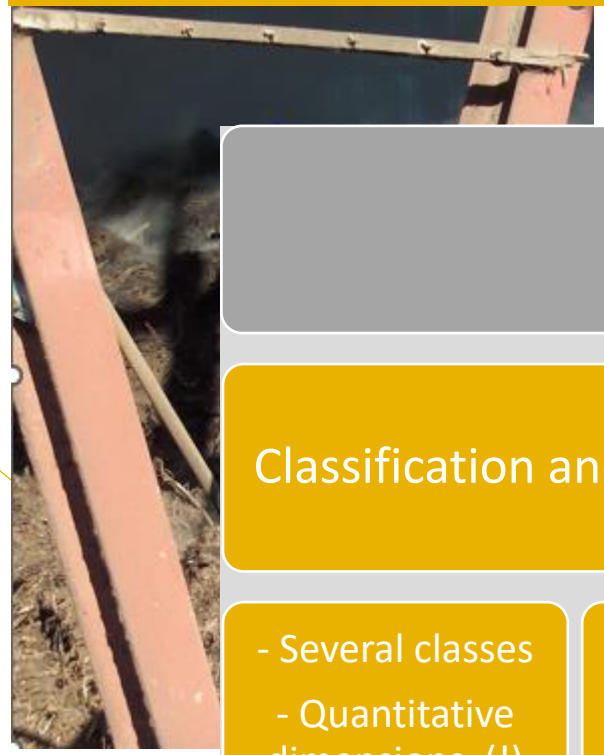
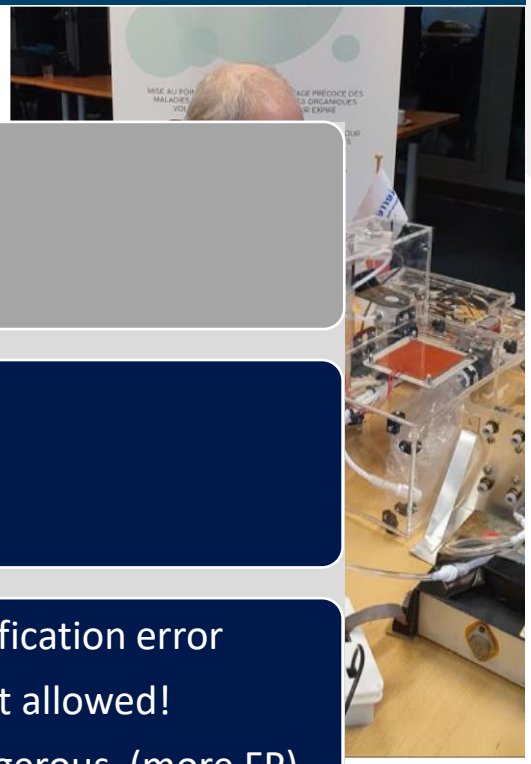
5. From Environmental Monitoring to Breath Analysis (5/5)

Environmental (odour monitoring)
long experience (30 years)



Breath analysis
short experience (5 years)



Data analysis

Classification and quantification		Classification	
- Several classes - Quantitative dimensions (!)	Classification error (FP and FN) Low impact	Only 2 classes Healthy-Not Healthy	Classification error not allowed! FN is dangerous (more FP)

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6. Take home messages

- Comparing to Environmental applications, Medical ones are more
  - ✓ humanly satisfying
  - ✓ subject to numerous project calls
  - ✓ funded
    - ➢ but also constraining (ethical aspects)

Medical research projects:
- ➢ several years
- ➢ several clinical centers,
- ➢ a lot of different resources

- Scientifically, easier for certain aspects (in lab, cycle,…) and interesting
  but less performing (due to breath composition and "lack of knowledge on biomarkers (volatilome))

  chemical analysis (individual biomarkers) ↔ **sensor array-AOS- (BreathPrint)**

- **AI**: Machine learning and
  Deep learning (big big neural network, Danger of black box)

YES BUT DO NOT <u>NEVER NEVER</u> FORGET THE QUALITY OF THE DATA (YOUR SENSOR SIGNAL) !
(large volume of data)

« Medical Applications: The notion of risk is so important…

Media like it!

Given the performance of current chemical sensors, I must admit that I'm not comfortable with medical projects. ISOCS scientist community need to be careful about the message we send to medical partners who are expecting a lot from this technology" AC Romain.


Interreg
France-Wallonie-Vlaanderen
Colloque 2022 - 9h50 - Dépistage : du collectif au ciblage
Copier le lien
Dépistage : du collectif au ciblage
2012-2022
10 ANS DE PRÉVENTION :
ET MAINTENANT
COMMENT
ON ACCÉLÈRE...
YouTube
TRL 1 2 3

New European project submission

Development of eleven AO devices; Distributed in 5 clinic centers

- **Clinic study on 246 LC patients** (eligible or not to surgery)
  and 246 healthy « risky » subjects
- **17 partners**
- **9 hospitals**
- **one partner for technology transfer toward industrial world**

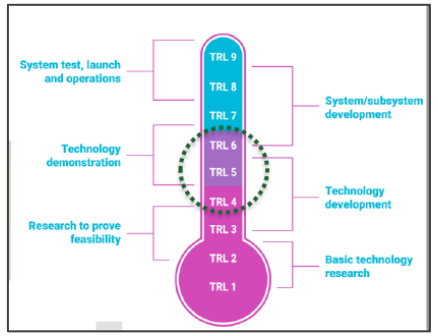
Clinical study

First step

- Analysis of breath sample simultaneously by ReCIVA® /GC-MS analysis and AOS (white box) linked with Pathacov VOC biomarkers
- Accuracy rate of classification (percentage of correct classification) 80 % ± 5 % (min. 75 %) : 246 patients statistically needed

Second step (only if accuracy criteria reached)

- Validation of the « real » performance of the AOS
- Only AOS (no RECIVA)
- Same population than step 1 but with more ration of early LC (70%)



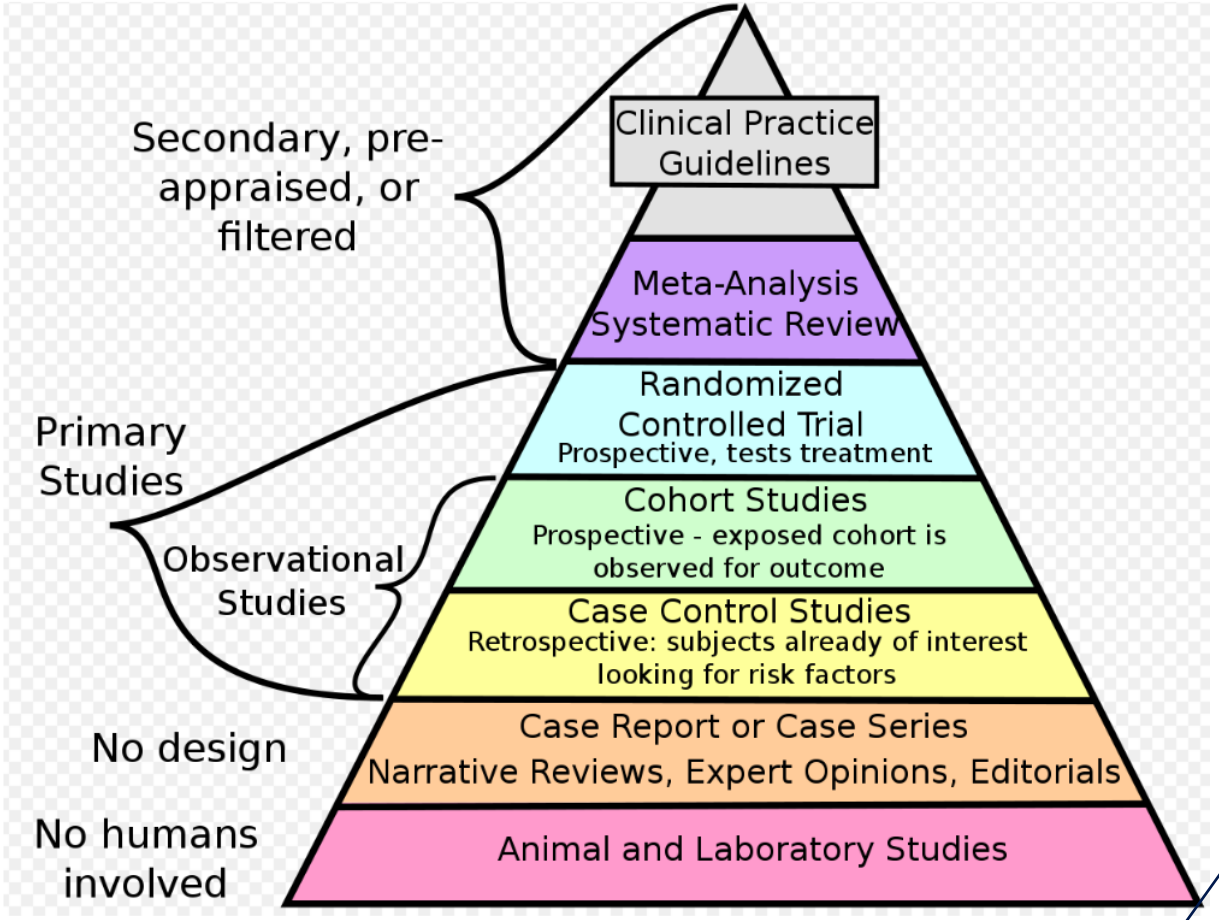
Avec le soutien du Fonds européen de développement régional

According to the PATHACOV projet leader and for clinicians:

PATHACOV

« *Pathacov projet was:*
between ***control studies*** *(yellow/green)*
and ***randomised trial*** *(blue) »*

« *Pathacov has been useful: in a case-control study, to identify the VOCs of interest and the research interest »*



(AC Romain) AOS was in the pink area

NEW: ALCOVE

« *second phase close to « big population » study and maybe, after, in a randomised situation (blue) »*

« *AOS will be between green and blue »*

(AC Romain)
AOS will be in the yellow
?

LIÈGE université
Arlon Campus Environnement

Sensing of Atmospheres and Monitoring

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Thanks for your attention

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Opened Post-doc position in April 2024
ALCOVE project

https://www.campusarlon.uliege.be/cms/c_3973705/en/arlon