ENSURING THE MACHINE LEARNER GETS THE RIGHT TUITION AND AI GETS ITS NOSE;

STANDARDISATION AND BENCHMARKING IN DIAGNOSTIC MARKER DISCOVERY IN EXHALED BREATH.

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Acknowledge and thank

Alison Monpetit, Mike Davis, Tony Davies, Jim Reynolds, Emma Brodrick, Jonathan Beauchamp, Claire Turner, Simona Cristescu

Breath Free, Billy Boyle, Marc P vd Schee, Simon Kitchen and Duncan Apthorp

Note: This PowerPoint presentation contains some slide animations and builds that may not be rendered clearly in powerpoint.
Please contact c.l.p.thomas@lboro.ac.uk for more details

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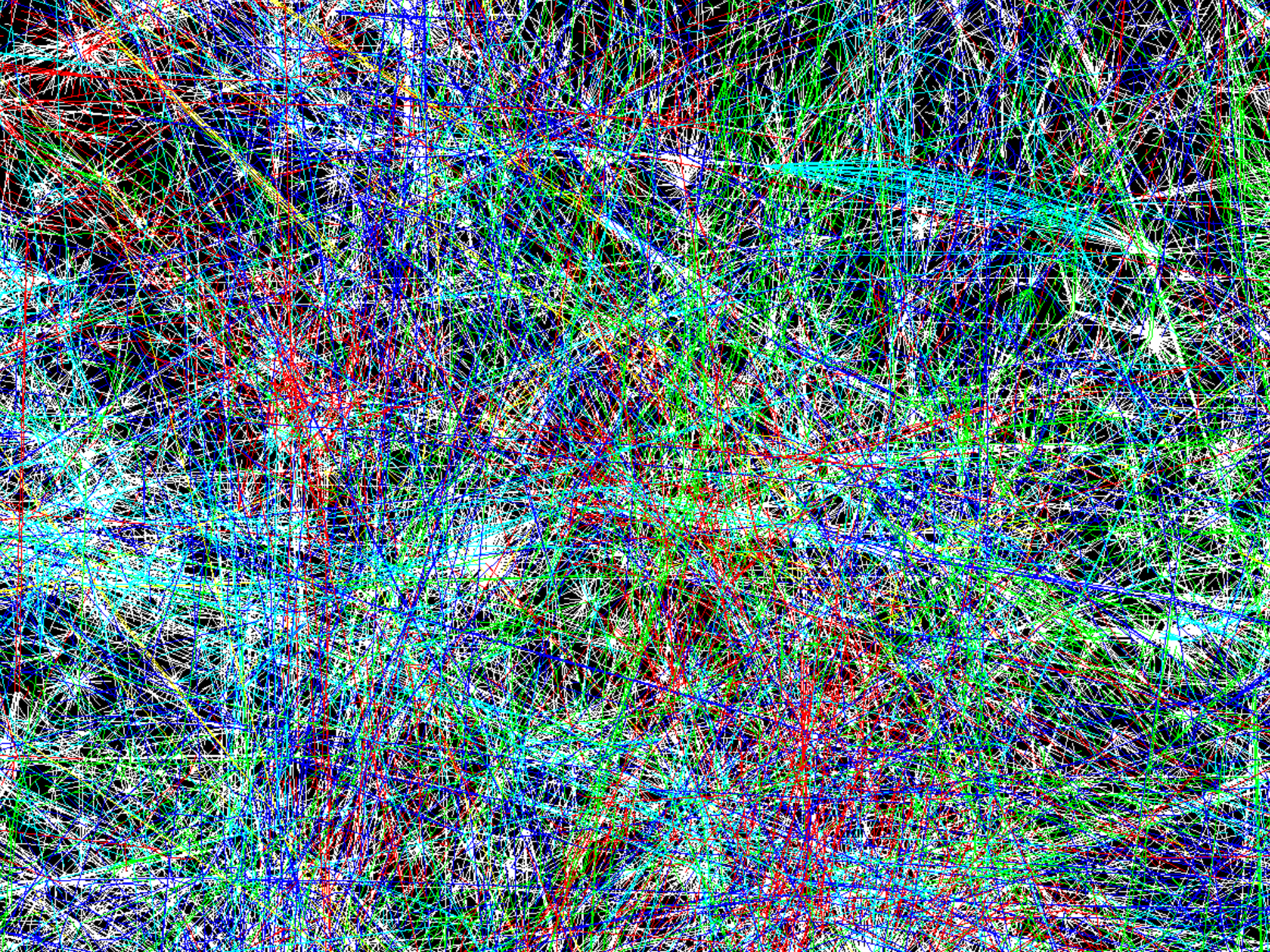
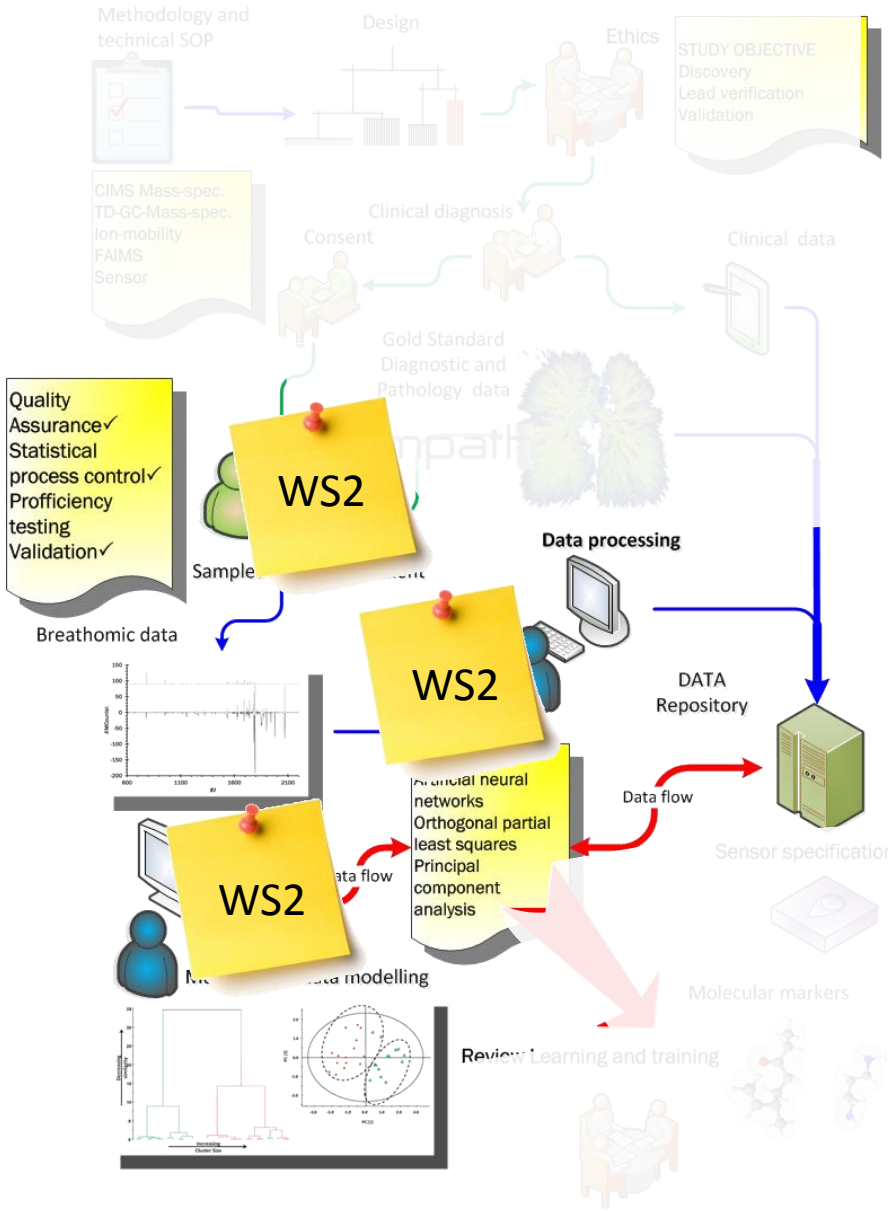


EMBER

Work Flow

- Knowledge Platforms
  - transSMART system
  - Double-blind proficiency testing
- Bio-informatics
  - Storage
    - EBI metabolite data repository, EPA COSMOS
  - Analytical systems
    - Embedded Statistical process control (SPC)
    - Data Semantics
      - COTS
        - SIMPCA; ANN/Palisade
    - Data Models
      - Verification and validation



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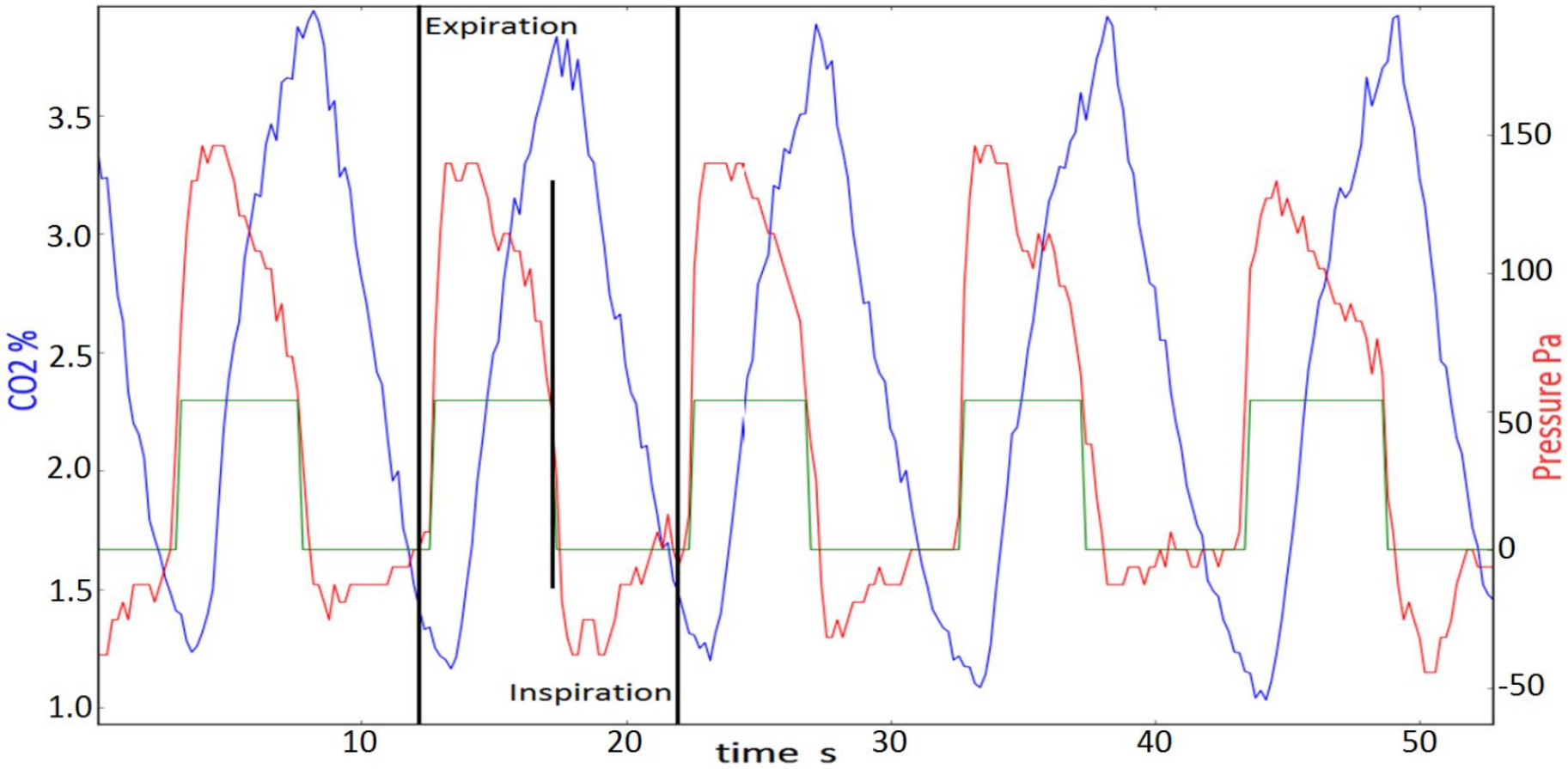
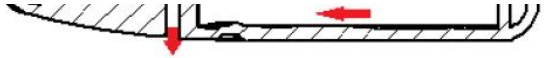
Big wins for WS2

- MVA systems approach
  - Orthogonal reinforcement
  - Semi-automated data mining and extraction for marker discovery
  - “EMBER marker discovery protocol”
- Received methods for breath sampling and analysis.
  - EMBER protocols
    - with embedded validation
    - High patient and clinical acceptance
- Breathomic data base
  - Building control data to accelerate future studies





tubes.

- Sorbent tubes containing samples can be analysed offline on a variety of platforms, currently testing with GC-MS and GC-FAIMS in progress.
- Alternatively, with modification the sampler could be directly connected to analytical instrument.

a broad range of biomedical applications.

- Clinical studies in the consortium are currently implementing the sampler.
- All designs are freely available on www.breathe-free.org.

Further information
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References
1.Schee et al, Chest, 2015
2. ERS taskforce TF-2012-11

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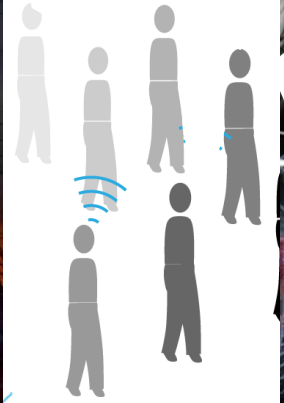

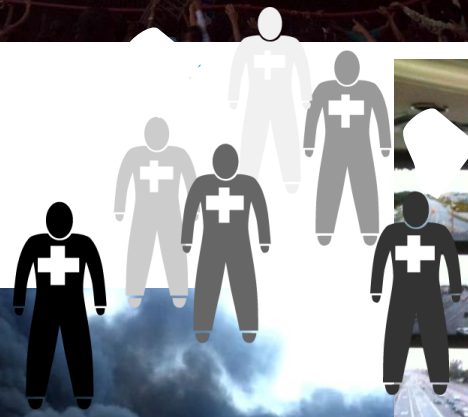

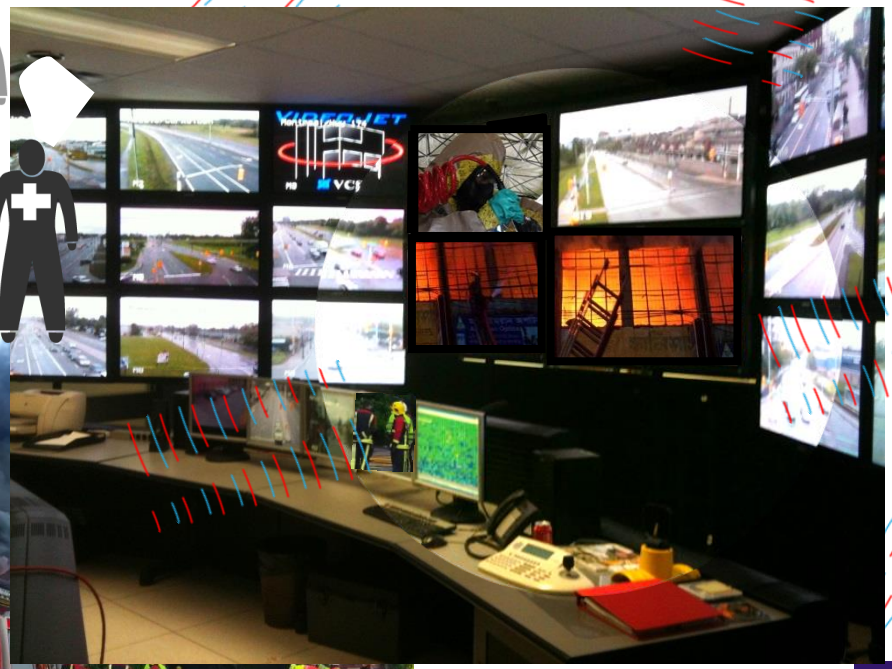

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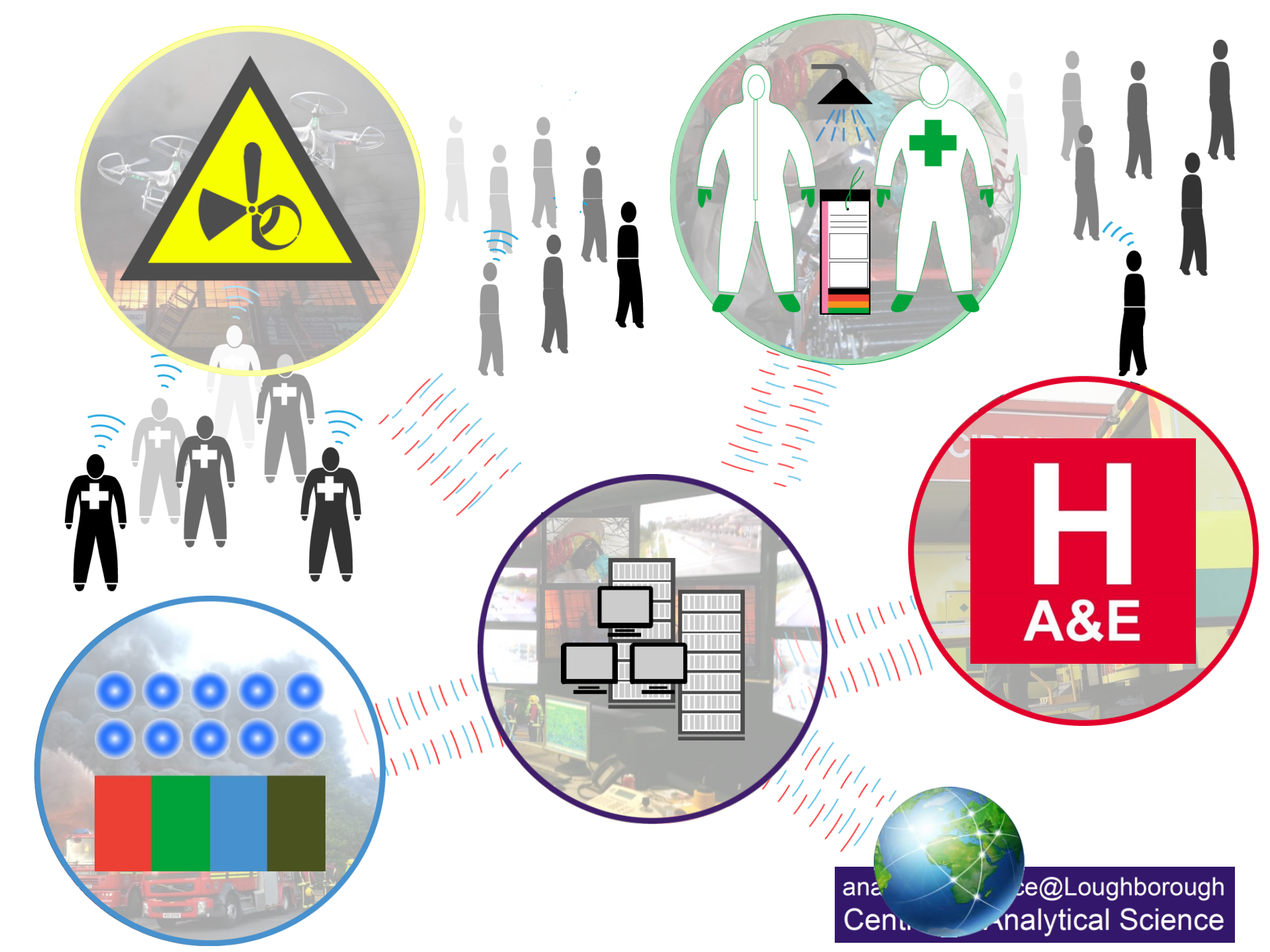
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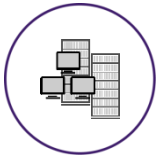
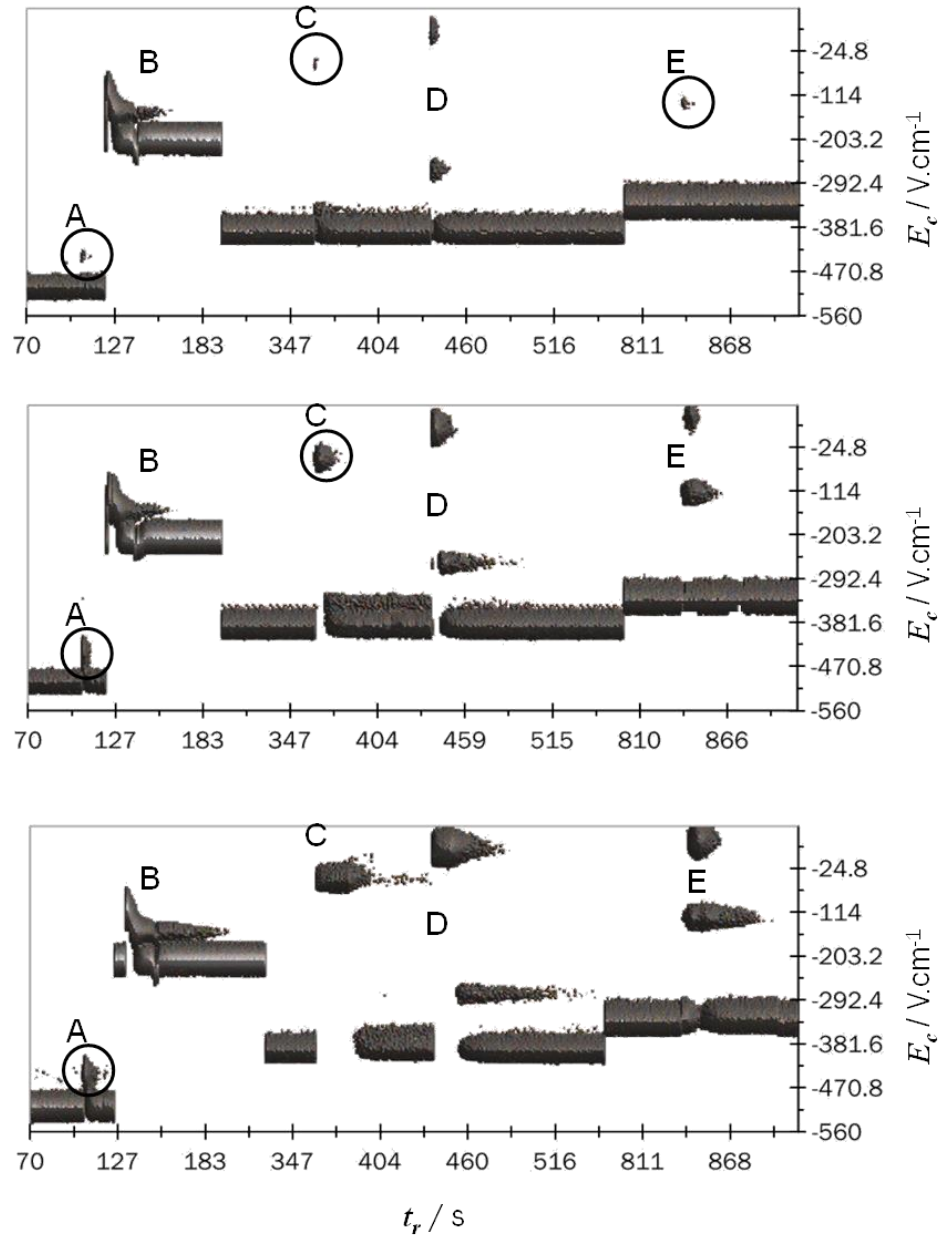
SOME PROJECT HEADLINES

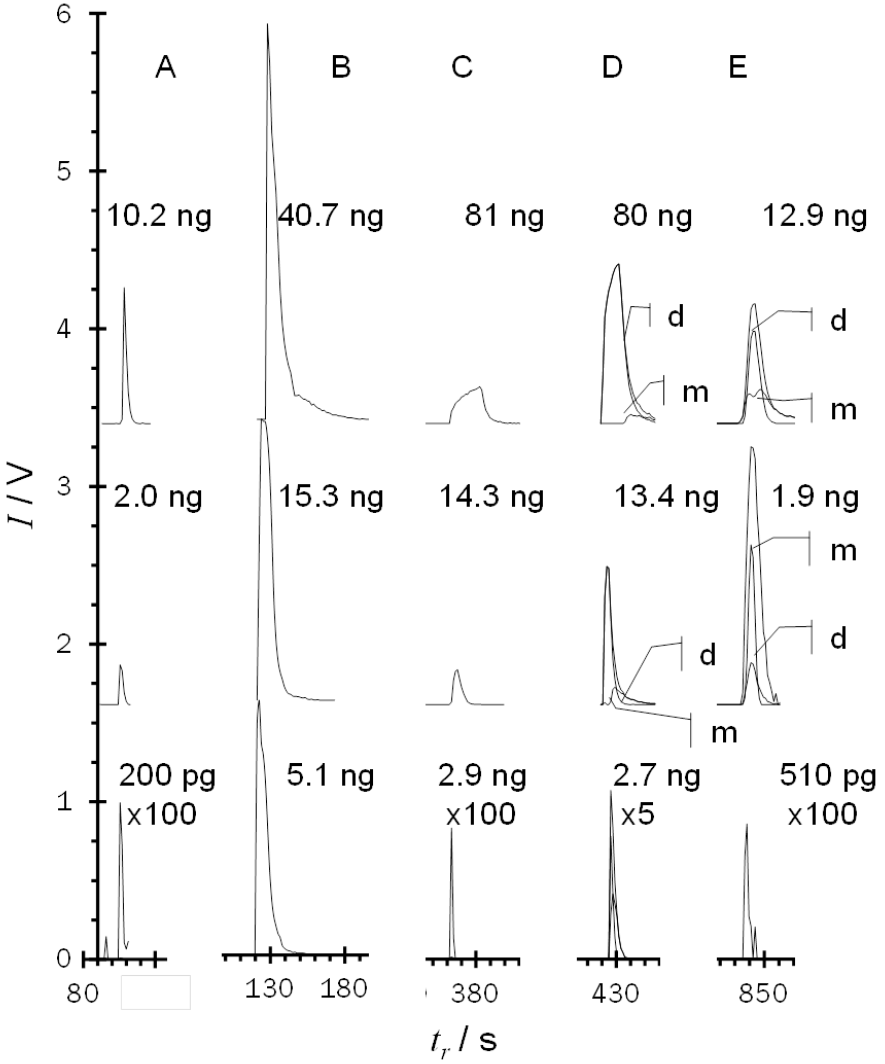
- Clinical trials
  - Metabalomic markers of agent and injury
    - organo-phosphorous
    - toxic alcohol
    - and radiation injuries
  - GC-IMS
- Field trials: “Focus” and “Disperse”
- € Pathways to economic impact through multi-use technologies





Saliva, TD-GC-DMS





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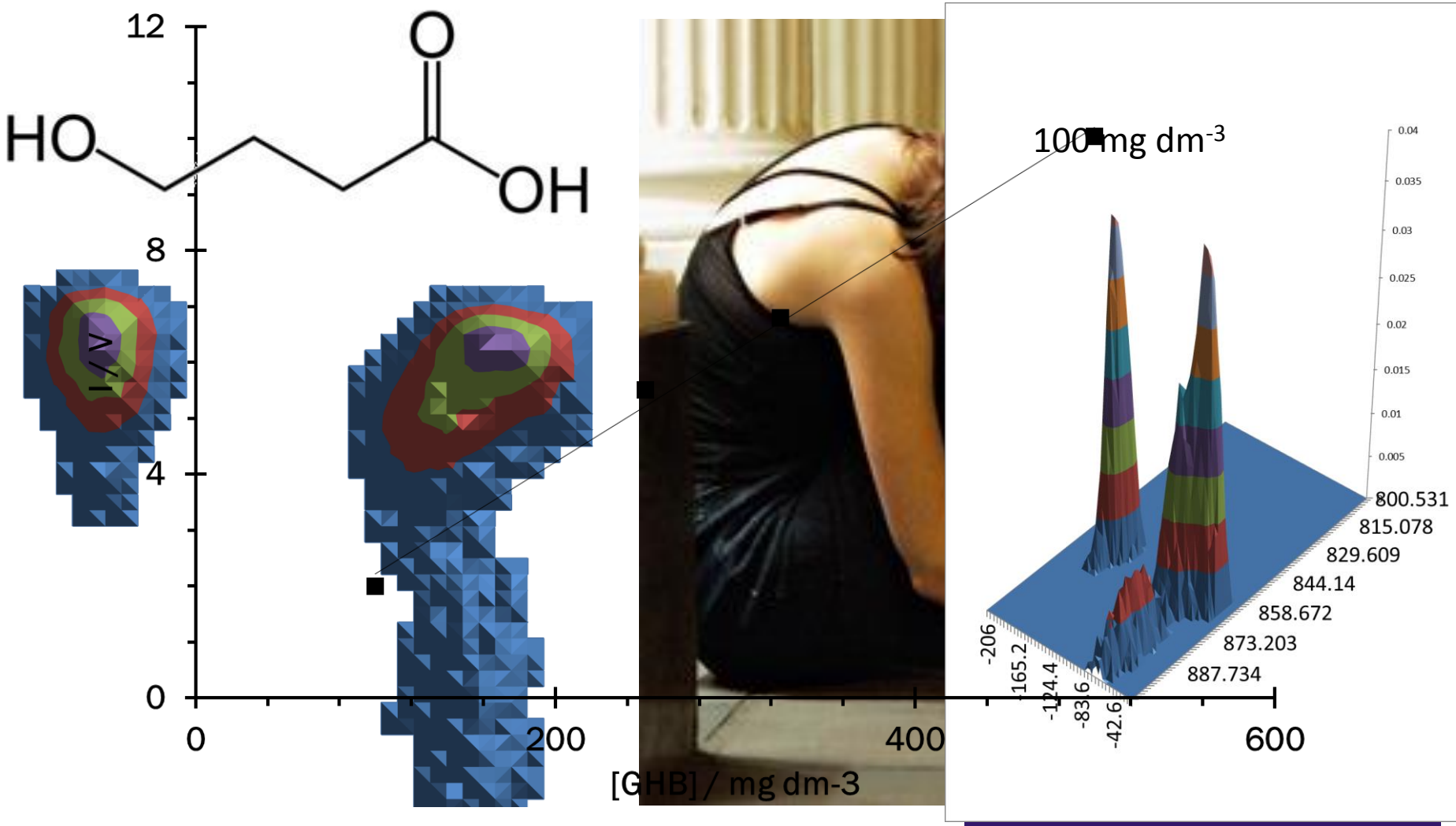
Emergency medicine: adulterated / counterfeit drinks and products are a problem



I need standardisation, and all help gratefully received.

- Multiple partners
- Different locations, countries, and techniques.

'Cos we're only as strong
'Cos we're only as strong
As the weakest link in the chain”

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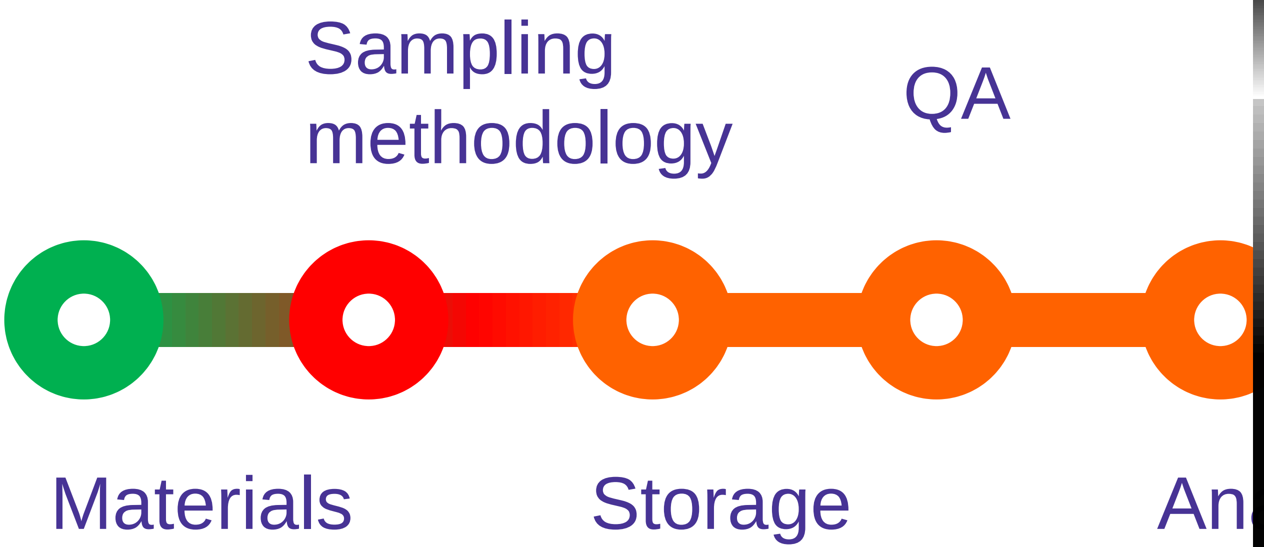
The Chain

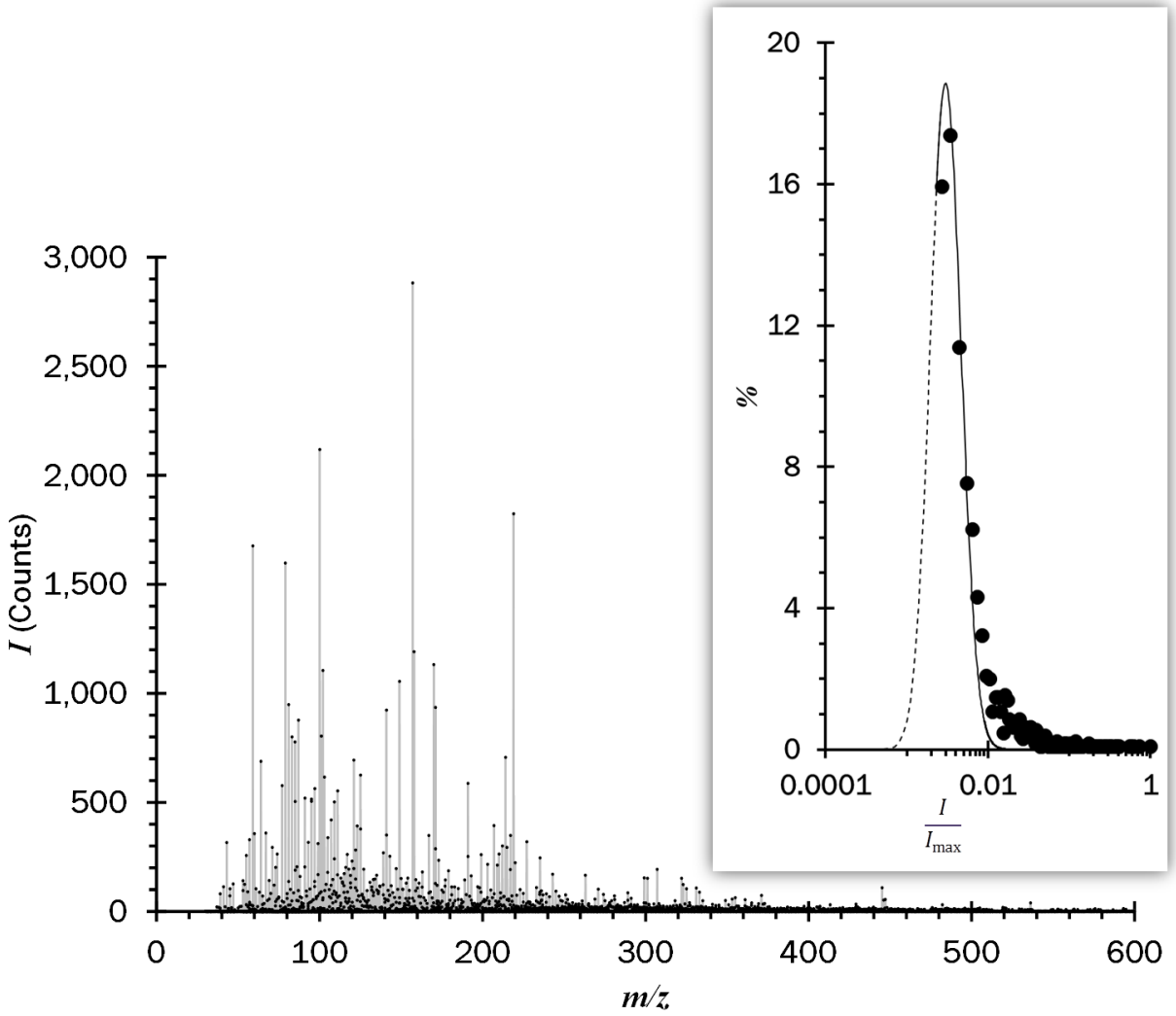


The list of factors awaiting standardisation is likely to include:

- Bags/canisters/gas-tight
- Extraction
- SPME
- Residence times / dead-volumes / construction materials / and surface treatments.
- Control: pressure /flow / CO2
- Volume of fixed-volume sample-loops
- Sample flow for continuous inlet capillary aspiration

Data evaluation,

too early to go for total standardisation



- Log_{10} normal distribution of components in positive mode SESI thermal desorption breath analysis..
- Looks like two orders of magnitude still to go.
- Checking every sample distribution identifies anomalies

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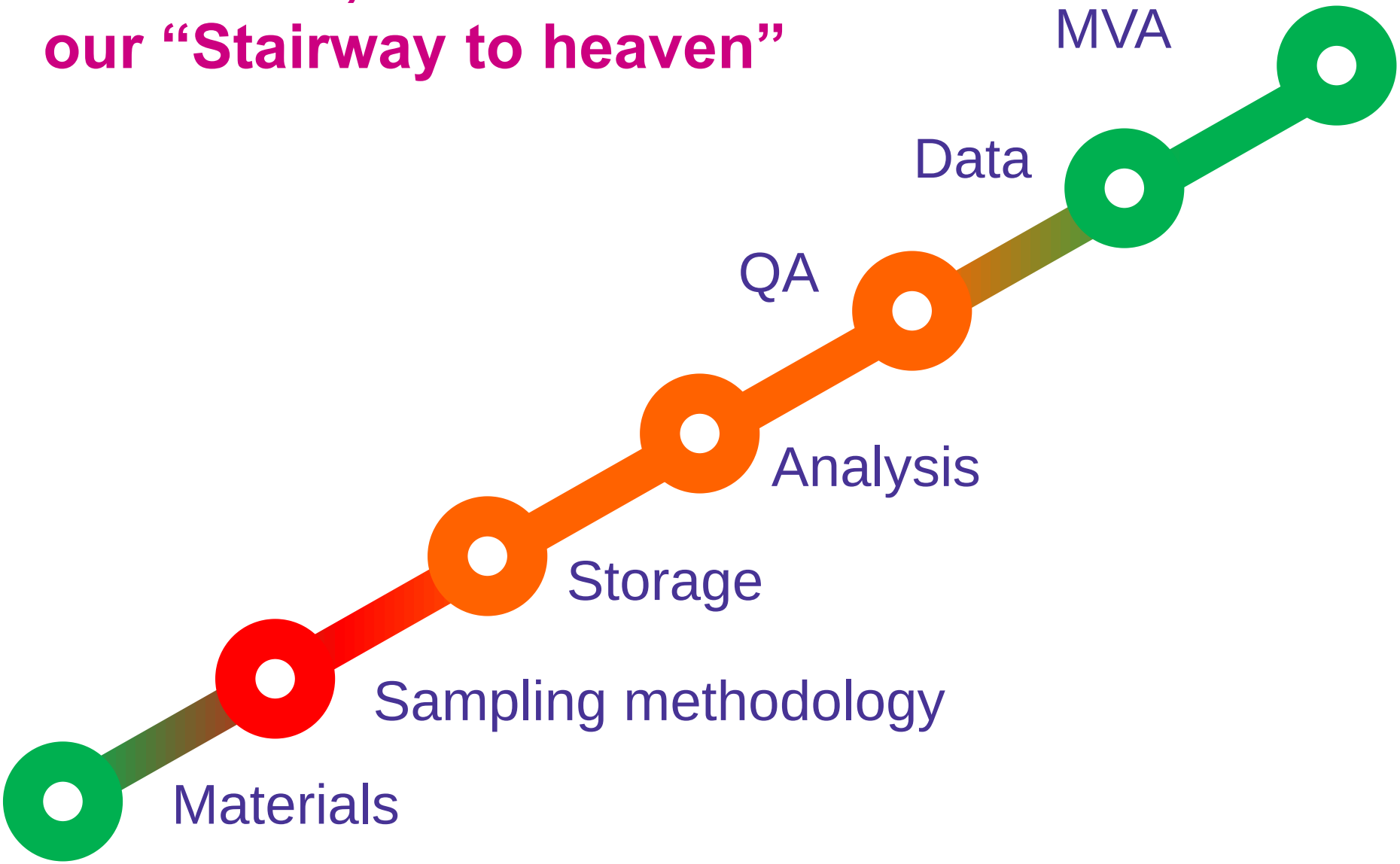
The Chain,
our “Stairway to heaven”



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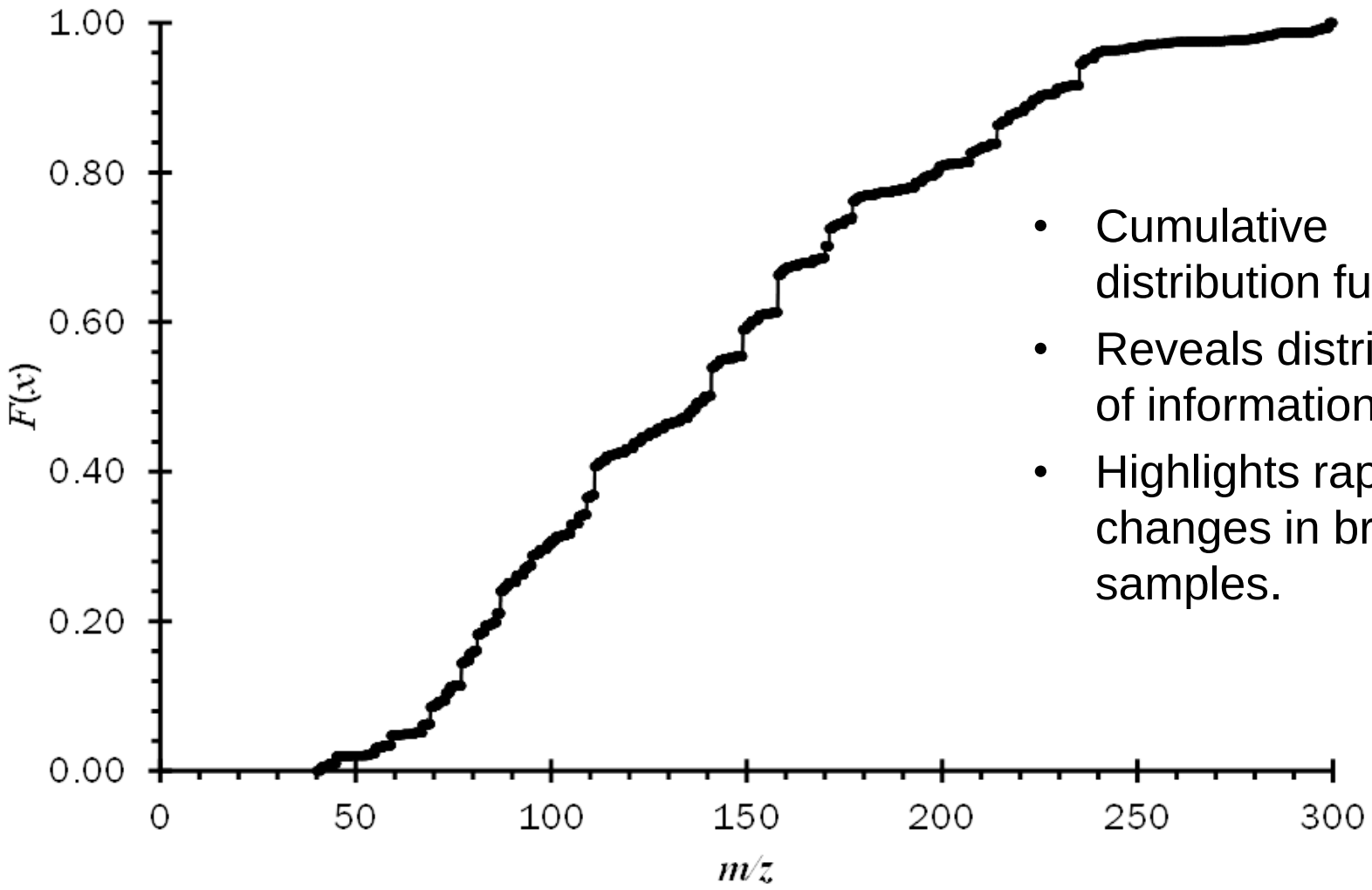
QA: Data evaluation.



- Cumulative distribution function.
- Reveals distribution of information.
- Highlights rapidly, changes in breath samples.

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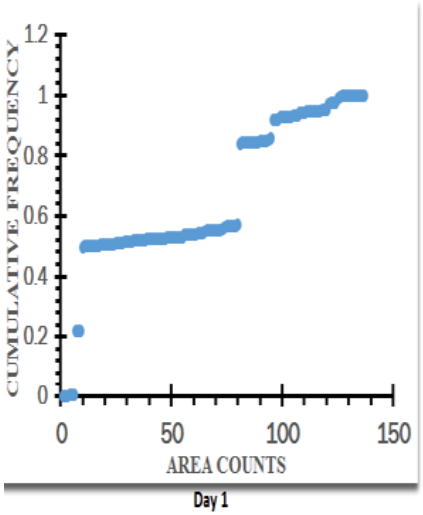
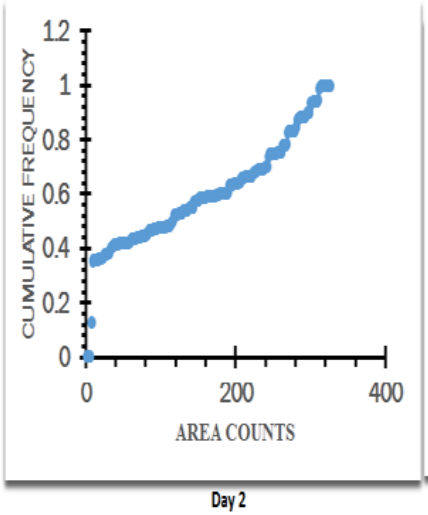
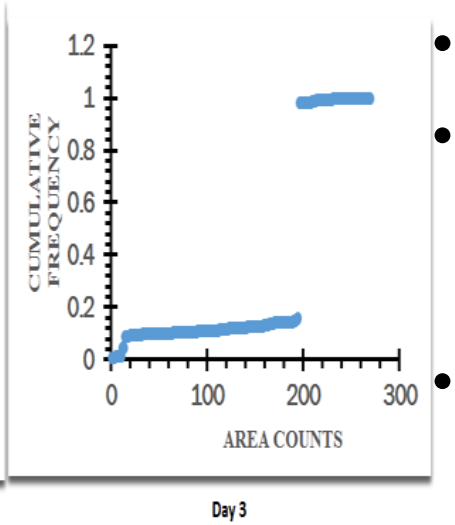
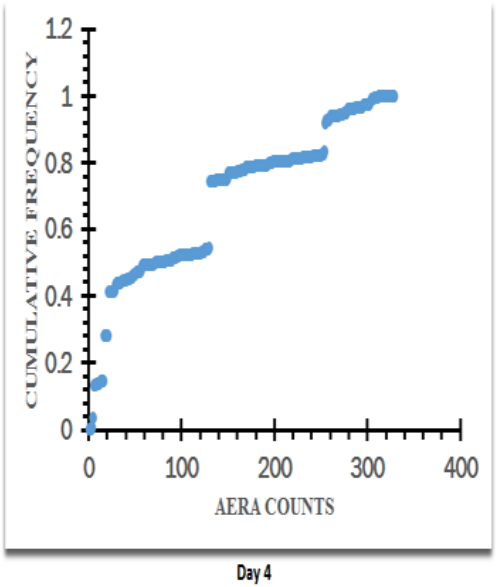
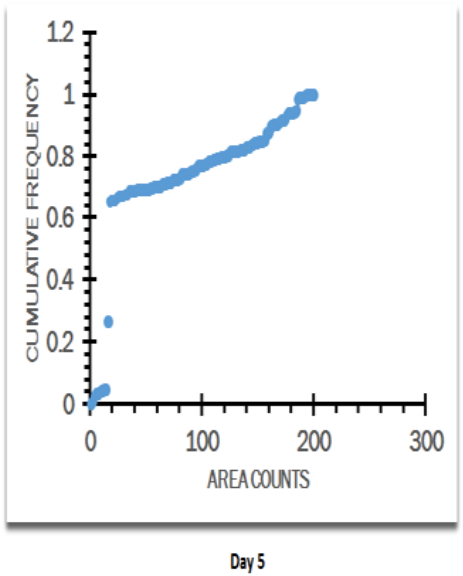
QA: Assessing cfd for breath profiles?







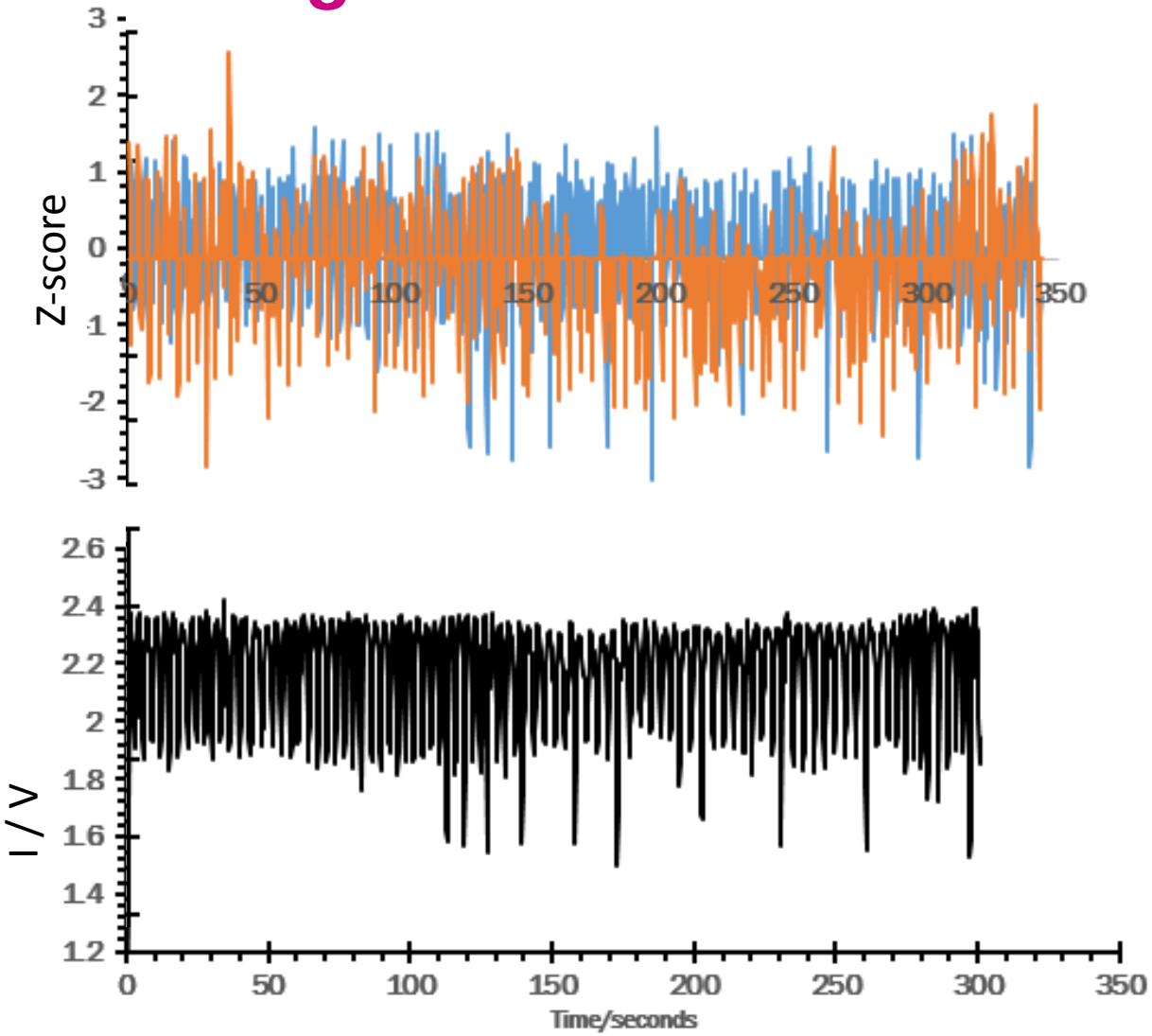




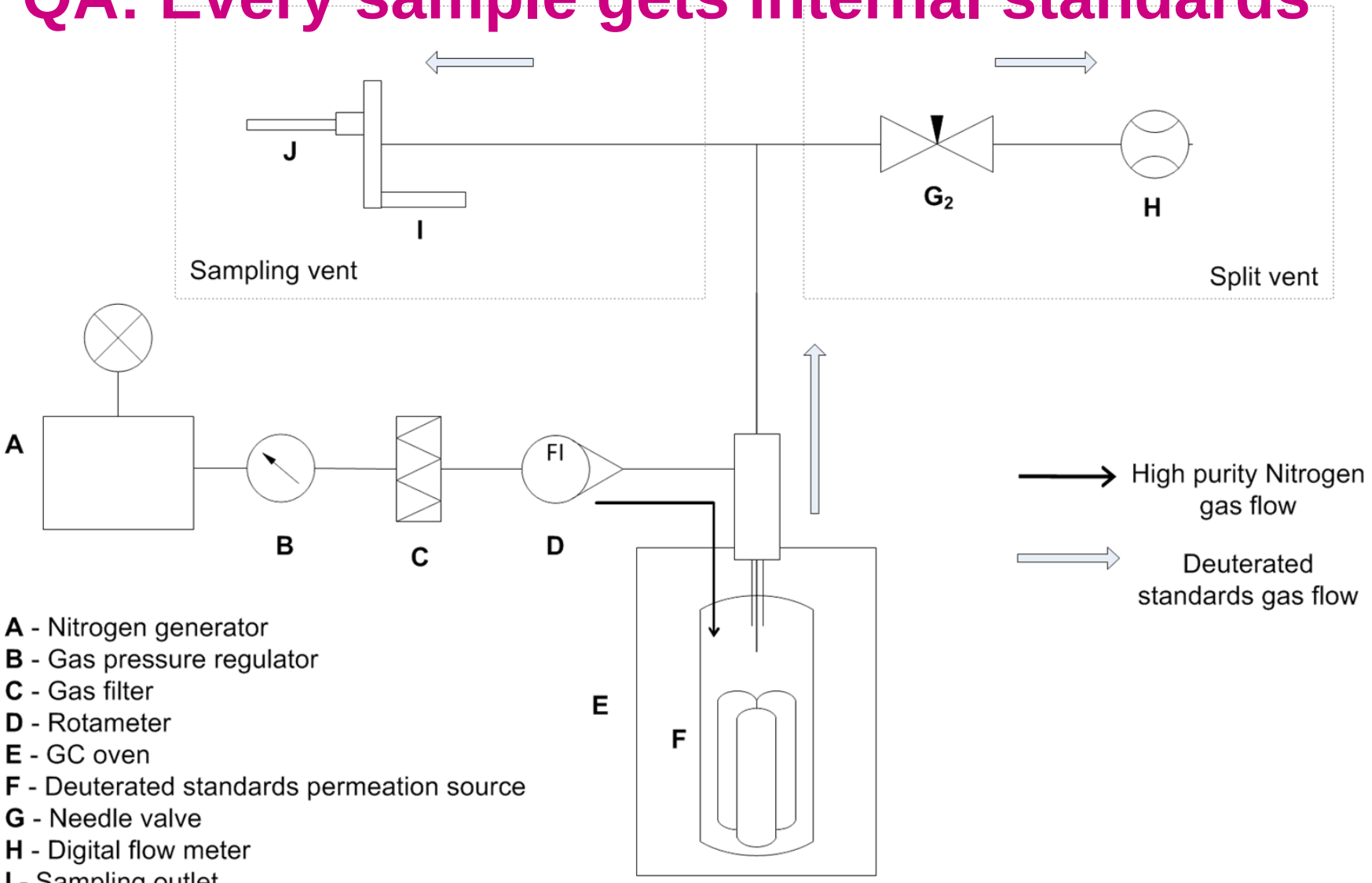
- Same participant
- Across an asthma exacerbation.
- Variable cfd profiles day-to-day.
- Difficult to discern this from the chromatography
- Highlighting this early on is helpful

QA: every breath is monitored and evaluated. Reproducible breathing?

- Every breath monitored and assessed.
- Too many abnormal breaths and the sample may reflect breathing and ventilation.
- Same participant before and after asthma exacerbation.
- Better sampling algorithms to manage abnormal breathing paterns.



QA: Every sample gets internal standards



A - Nitrogen generator
B - Gas pressure regulator
C - Gas filter
D - Rotameter
E - GC oven
F - Deuterated standards permeation source
G - Needle valve
H - Digital flow meter
I - Sampling outlet
J - Sorbent tube

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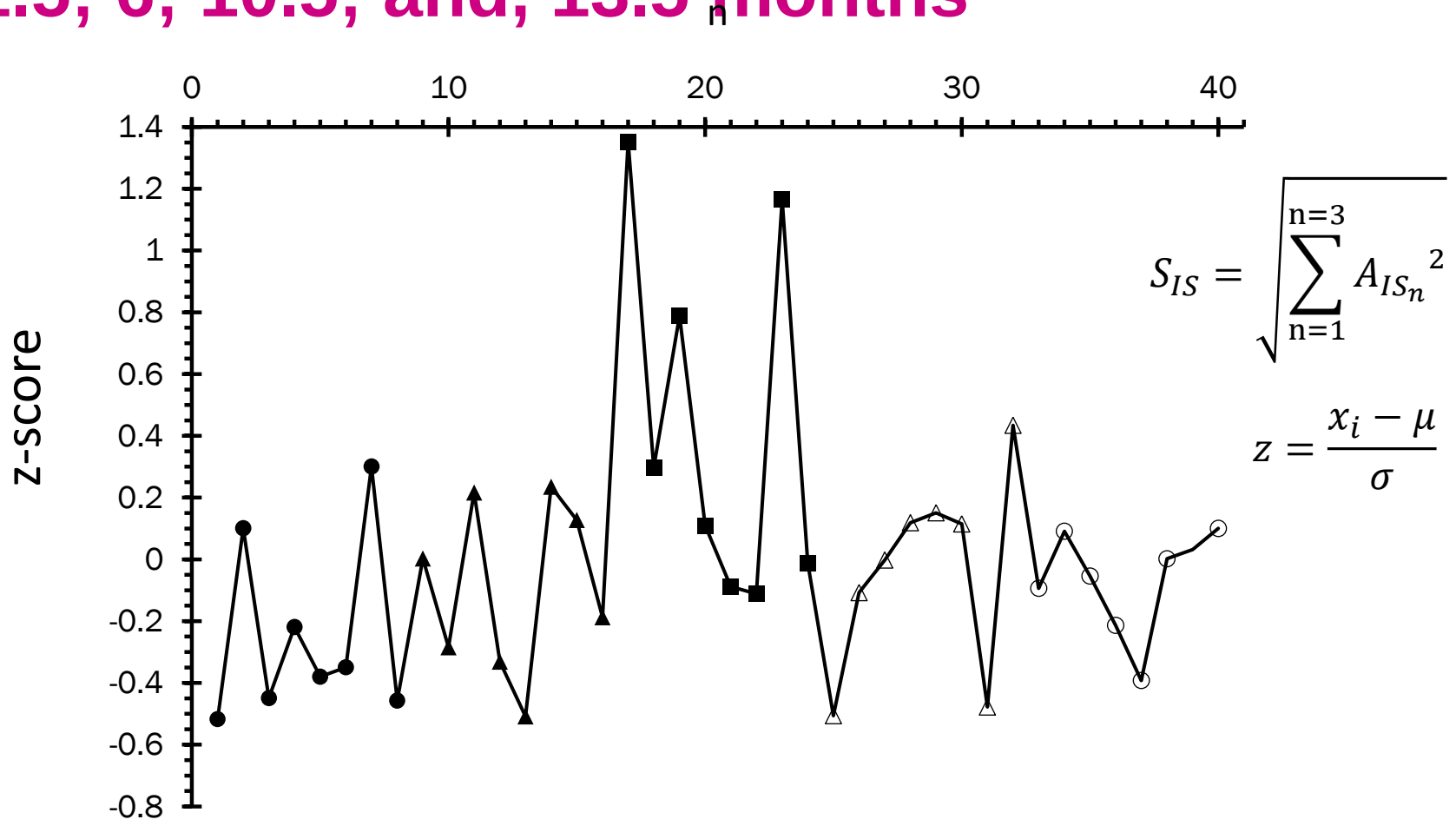
QA: Internal standard z-scores for a combined marker score across a year at: 0; 1.5; 6; 10.5; and, 13.5 months



$$S_{IS} = \sqrt{\sum_{n=1}^{n=3} {A_{IS_n}}^2}$$

$$z = \frac{x_i - \mu}{\sigma}$$

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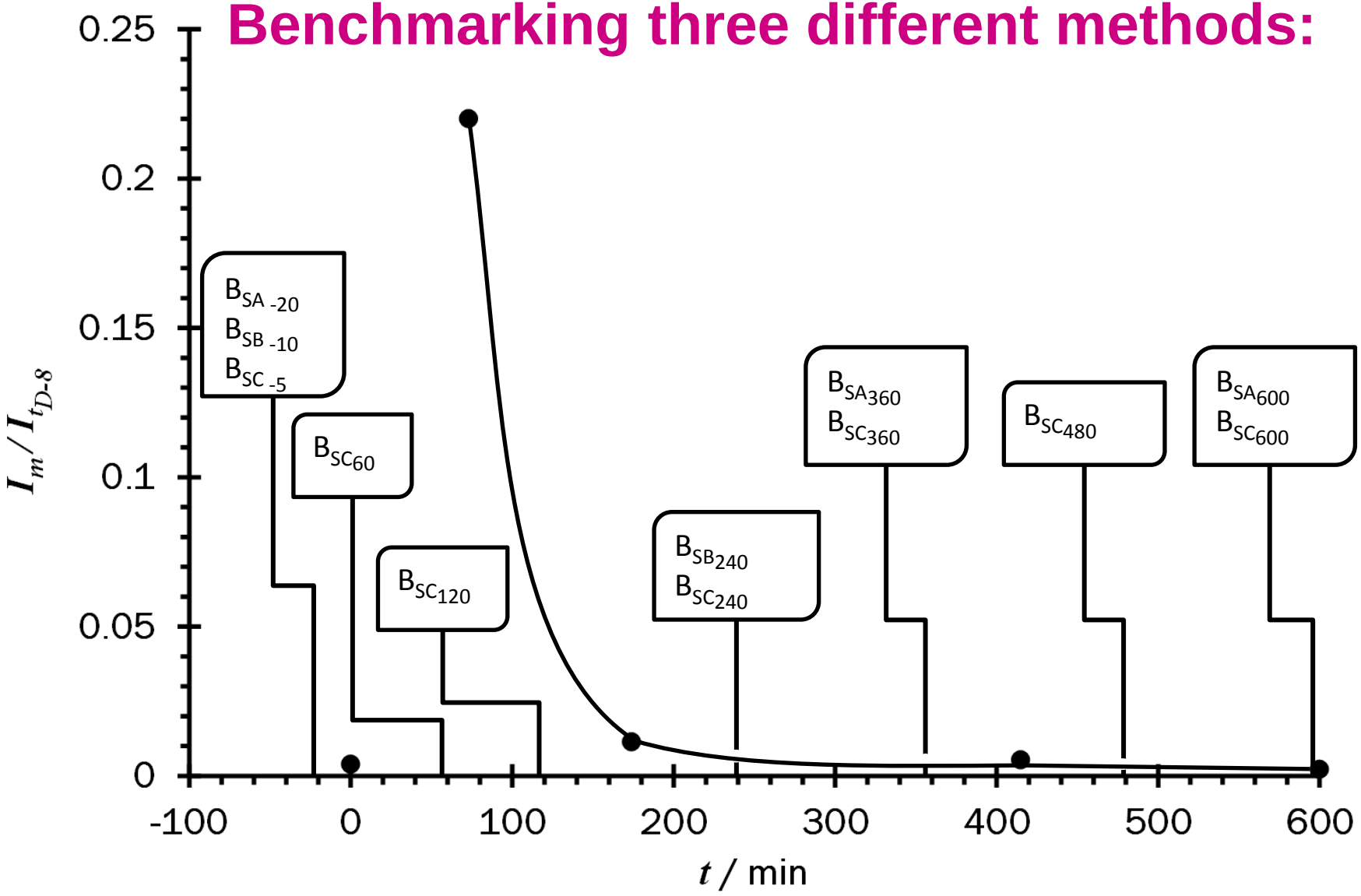
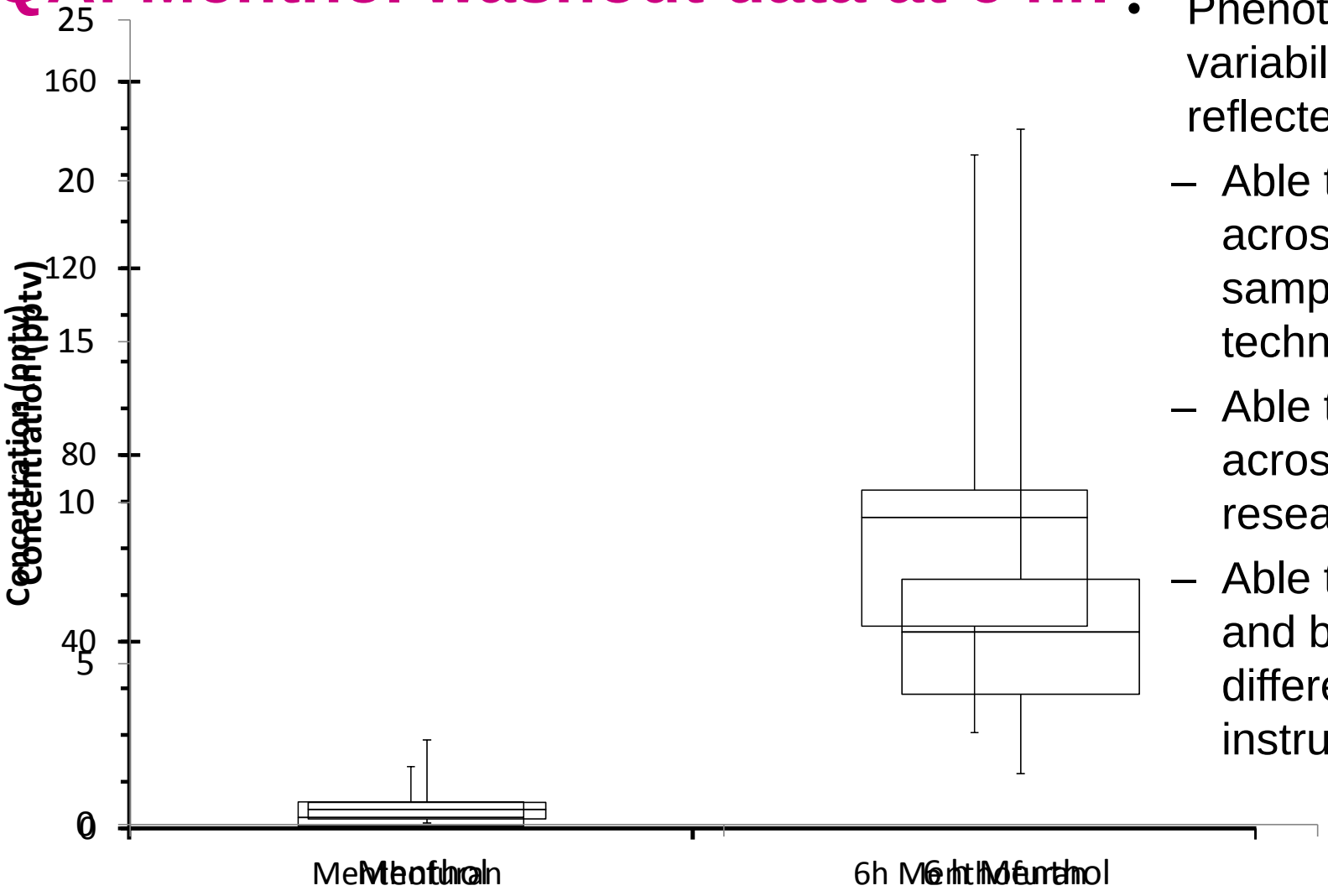
QA: menthol washout. Benchmarking three different methods:

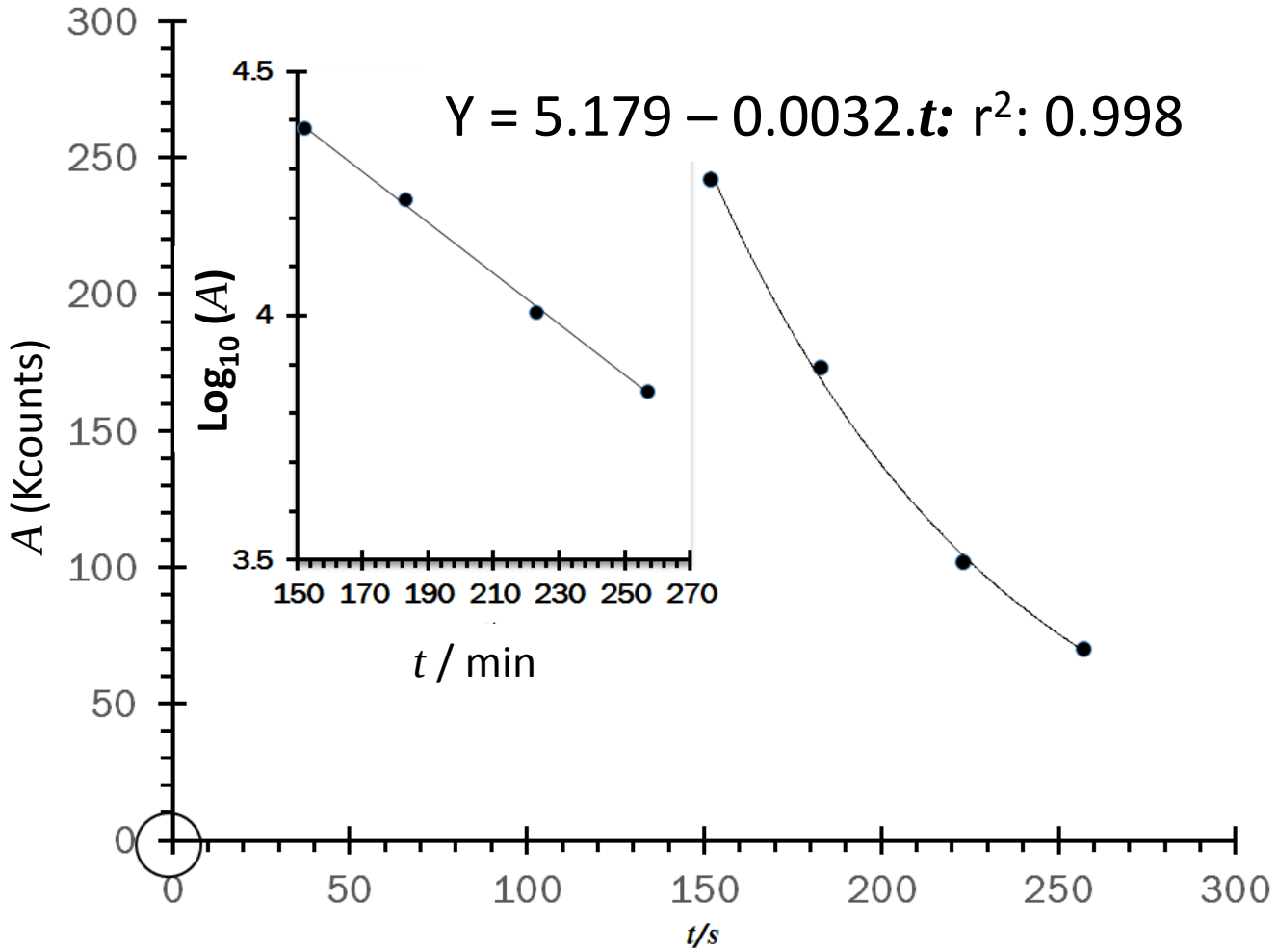


QA: Menthol washout data at 6 hr.



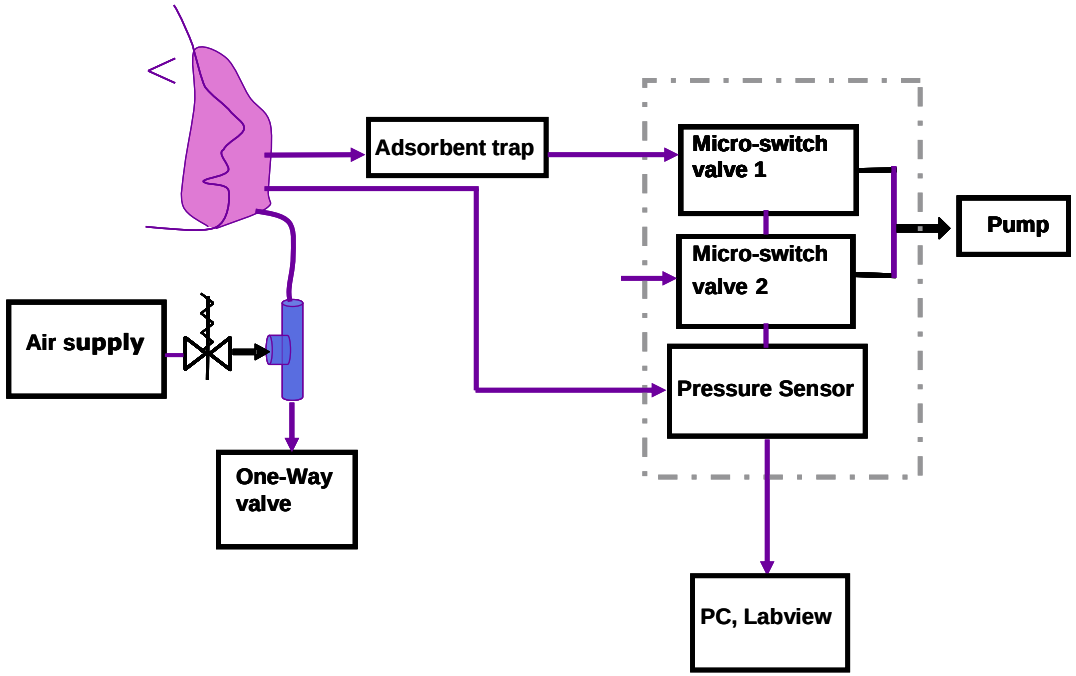
- 20 participants.
- Phenotype variability reflected in data.
  - Able to track across different sampling techniques
  - Able to track across different researchers
  - Able to compare and benchmark different instruments

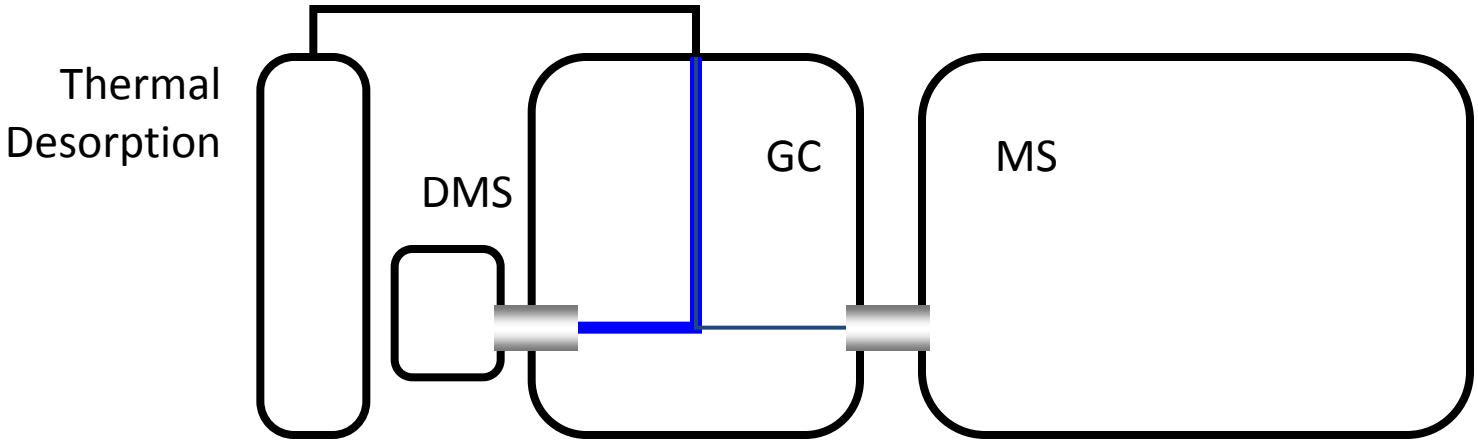
QA: Menthol Washout ¦ Proficiency testing



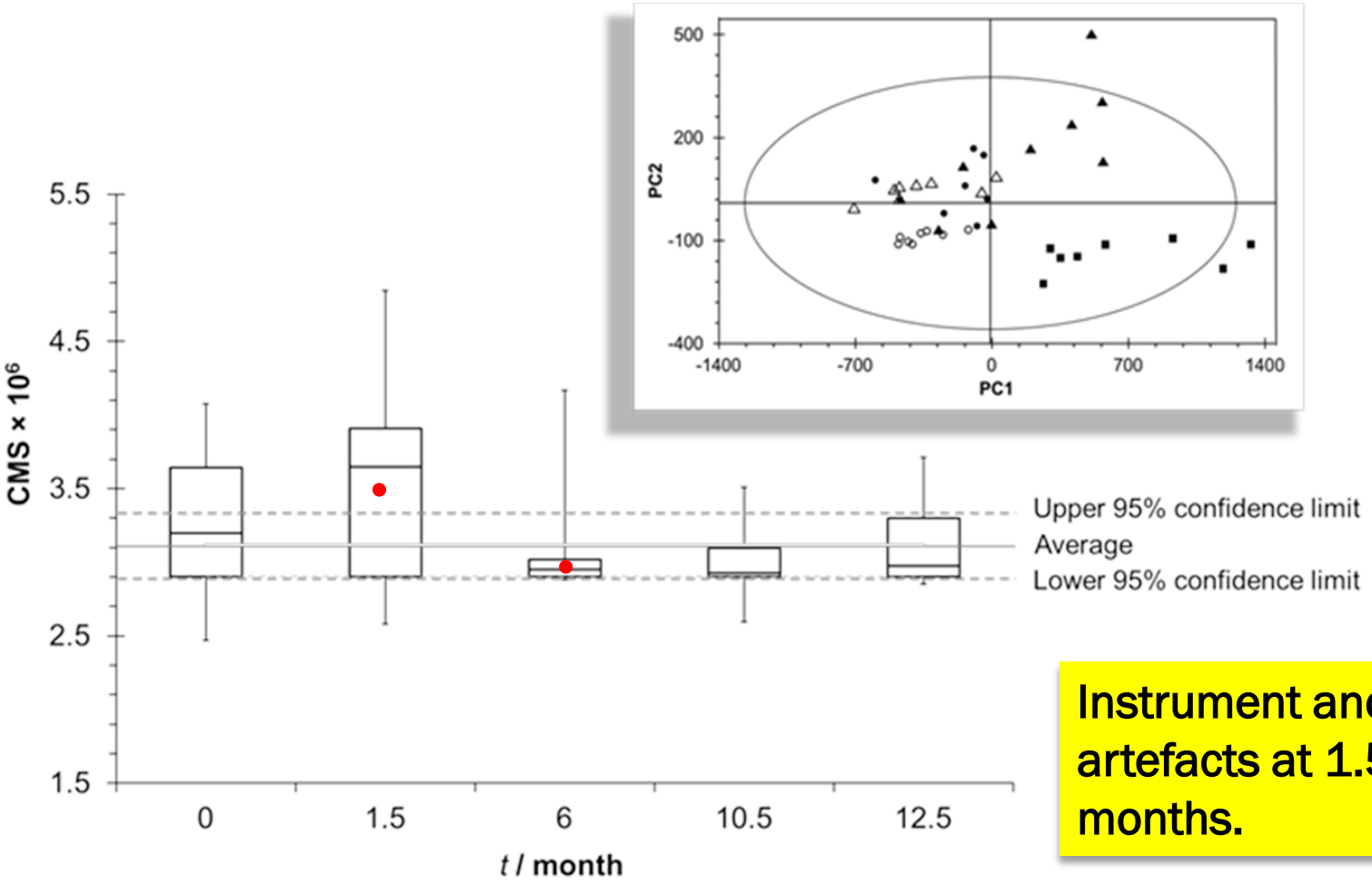
Storage: Conditions and duration

- 20 samples taken from the same participant over the course of an afternoon.
- Care was taken to maintain physiological state.
- Adaptive breath sampling with ventilated mask
- 2.5 dm^3 end-tidal samples trapped on Tenax/Carbograph adsorbent traps
- Randomised and stored at -80^oC and analysed after different storage times.
  - 0, 1.5 month, 6 month, 10.5 month and 12.5 month.
- Field and medair blanks analysed at time zero.





Storage standardisation, PCA on deuterated internal standard mixture



Instrument and /or QA artefacts at 1.5 and 6 months.

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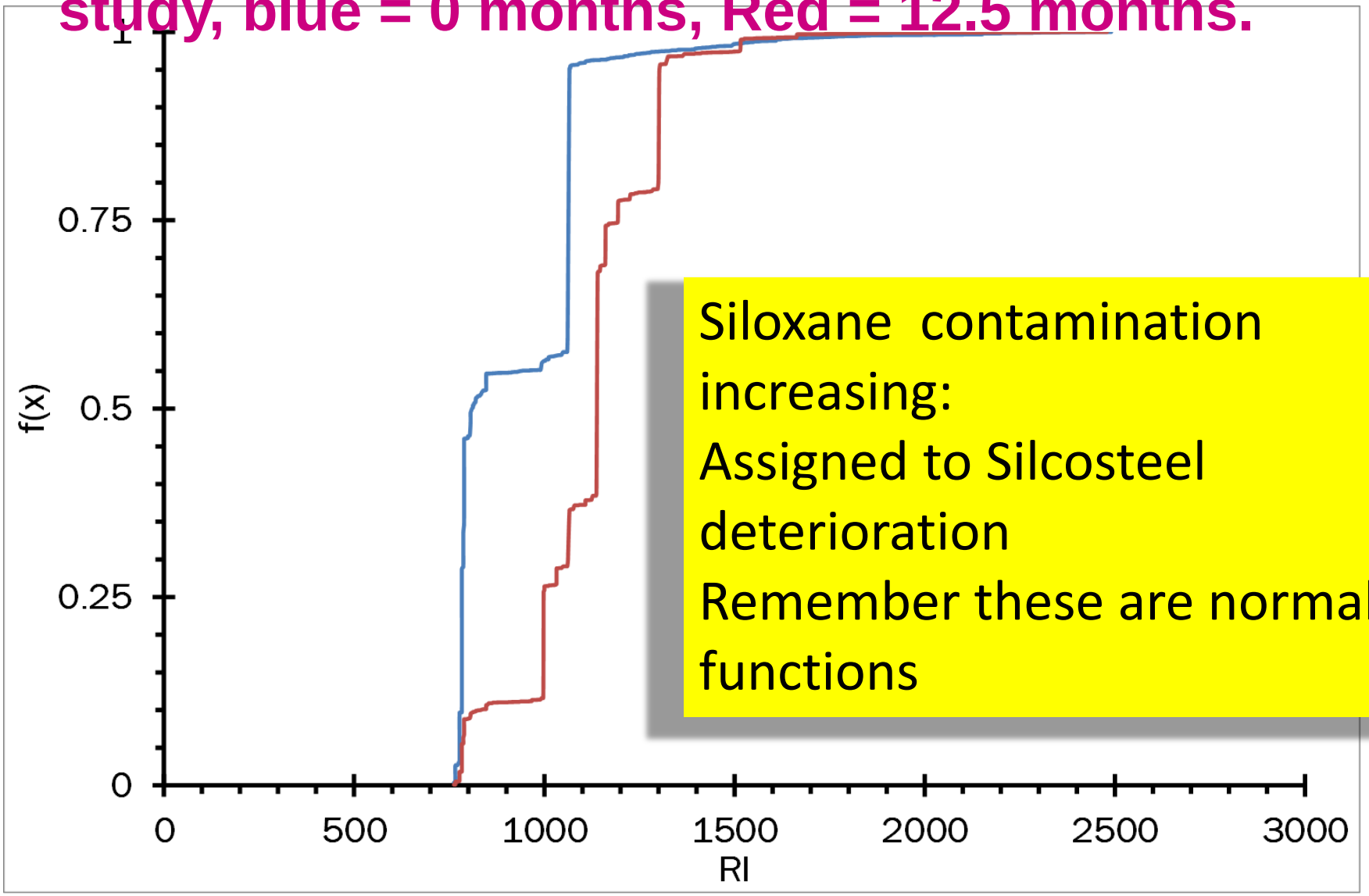
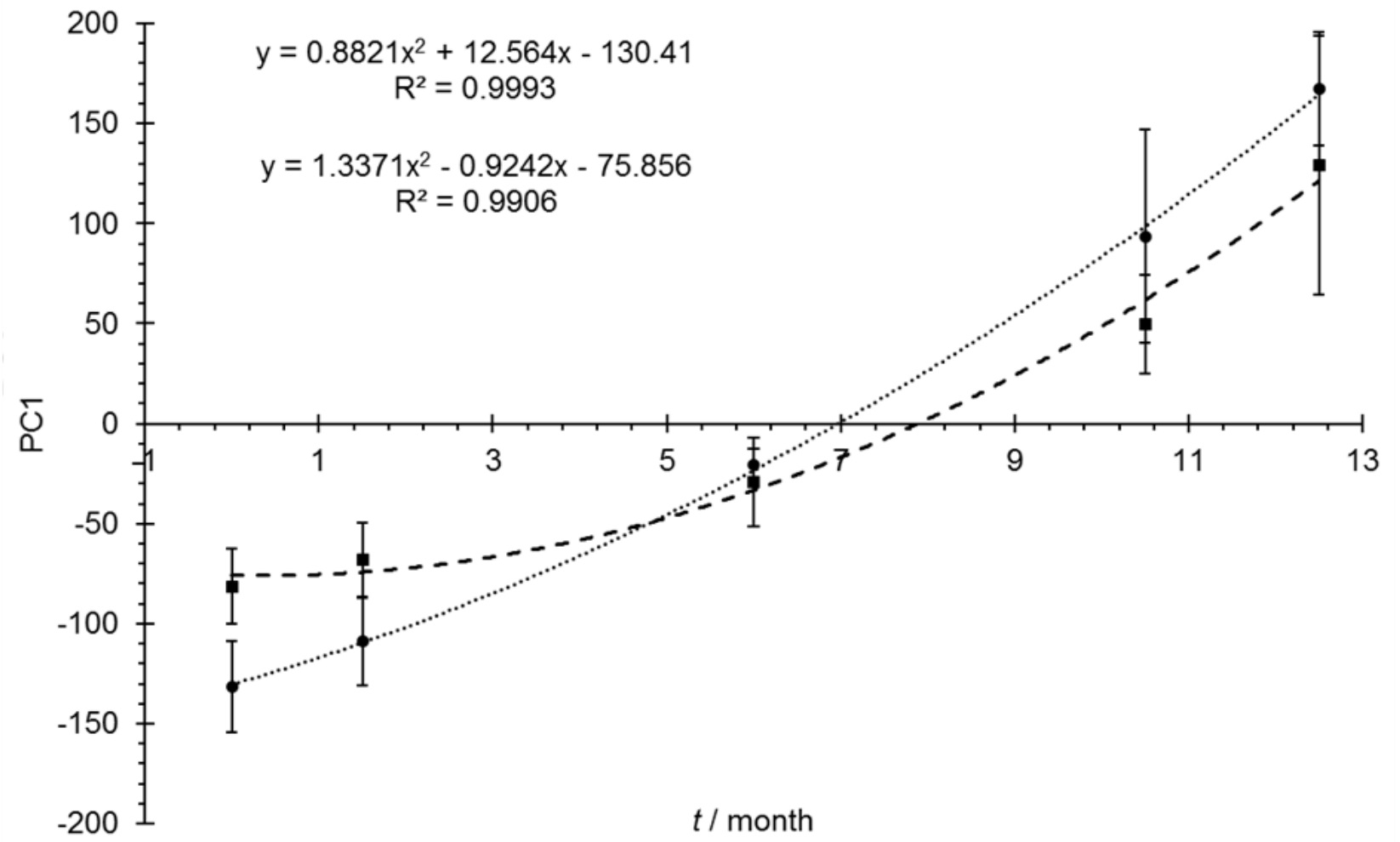
Storage: Tracking instrument operation over the study, blue = 0 months, Red = 12.5 months.



Siloxane contamination increasing:
Assigned to Silcosteel deterioration
Remember these are normalised functions

Storage standardisation, PCA on endogenous features.



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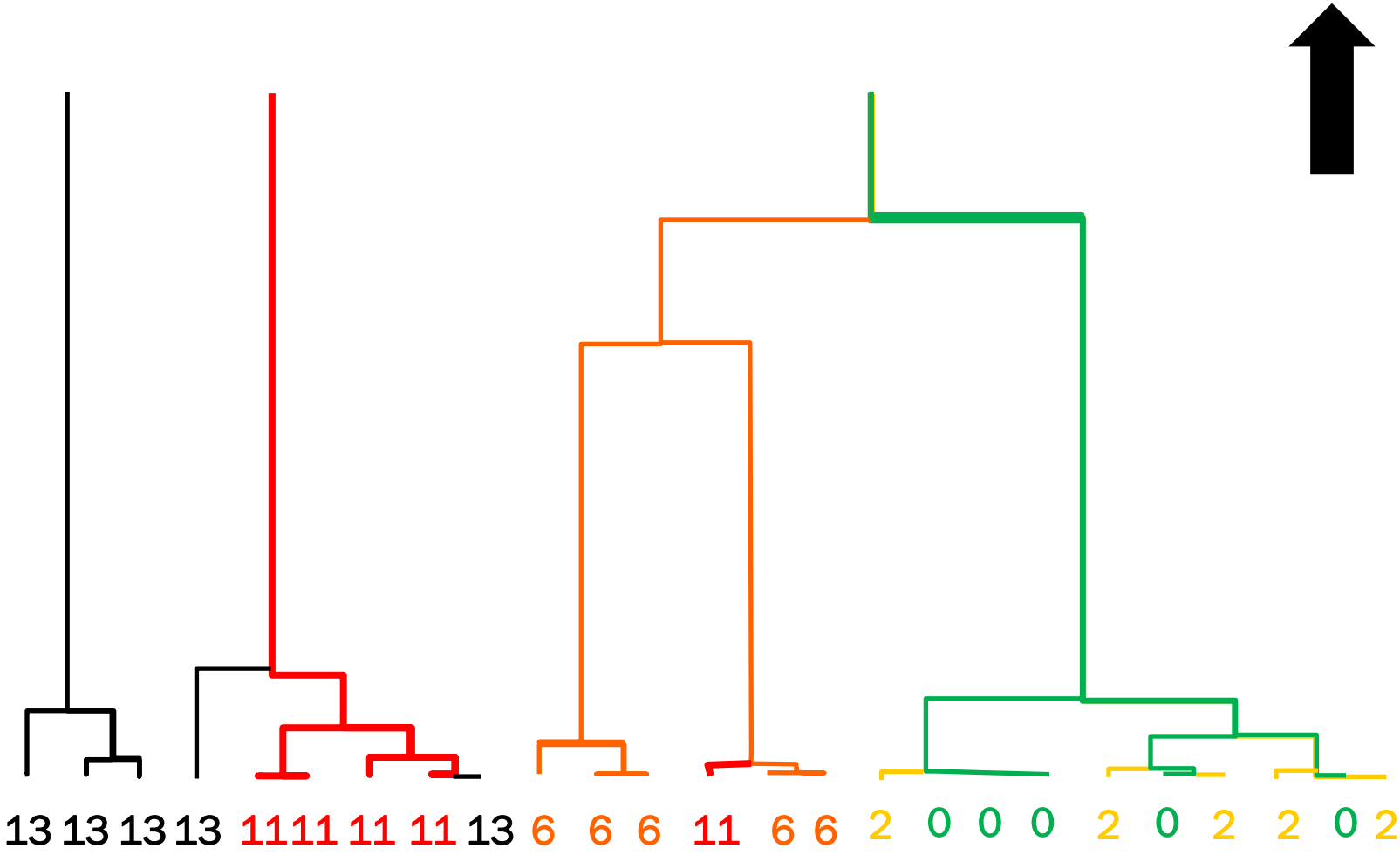
Cluster analysis by time of storage



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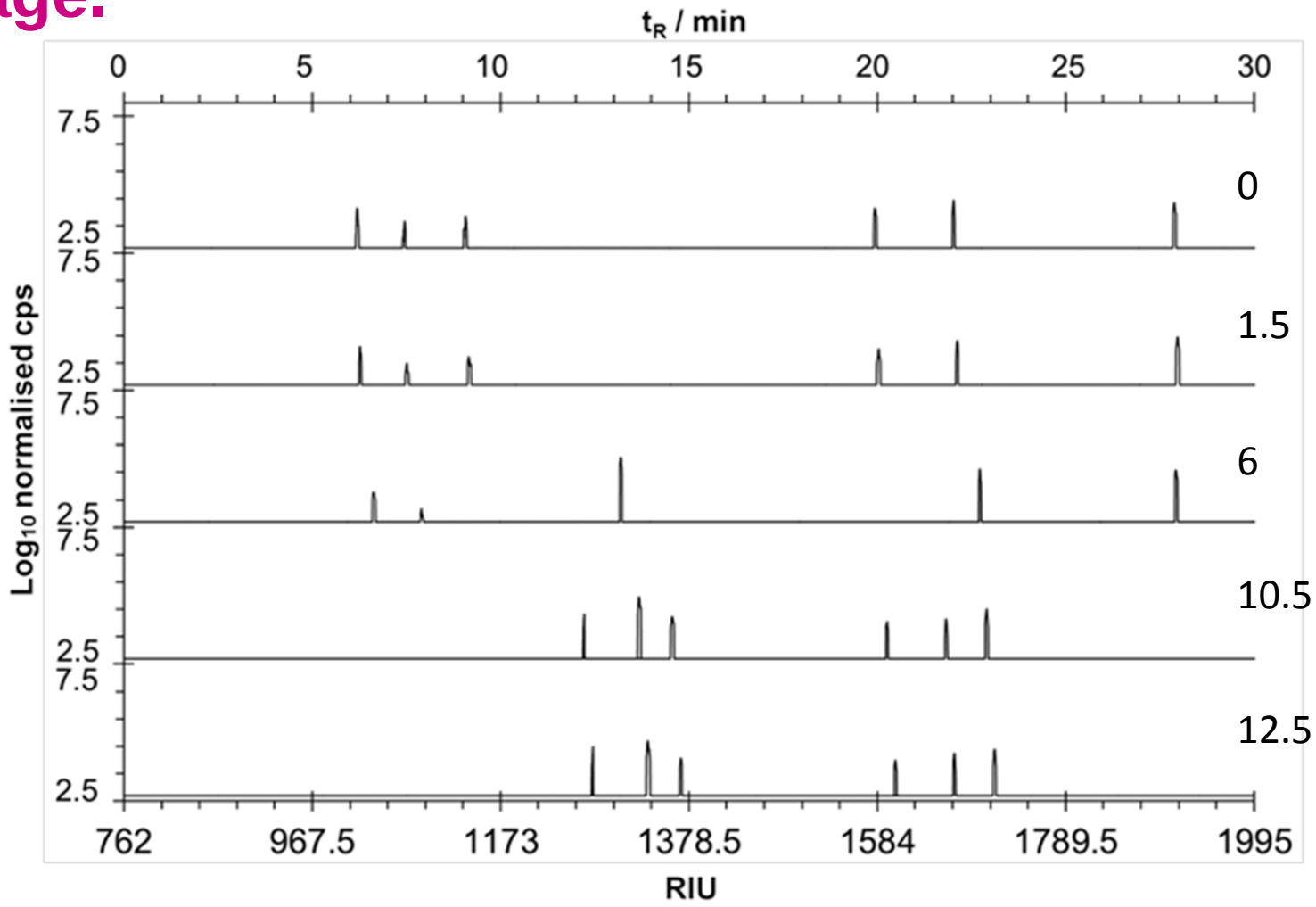
Selected ion chromatography of some egregious changes in endogenous components during storage.



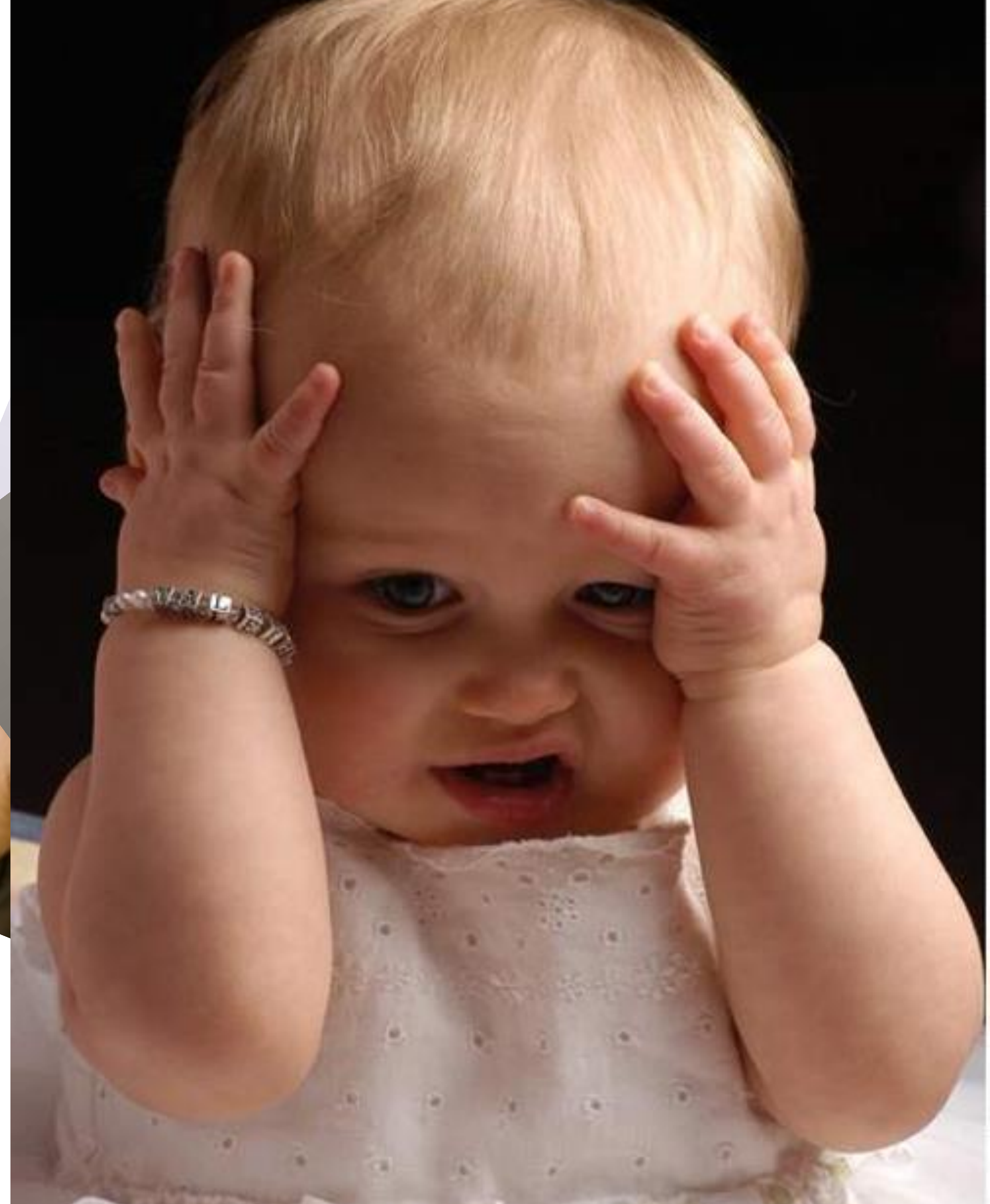
High Level Standardisation Framework

Statistical Process Control (SPC)

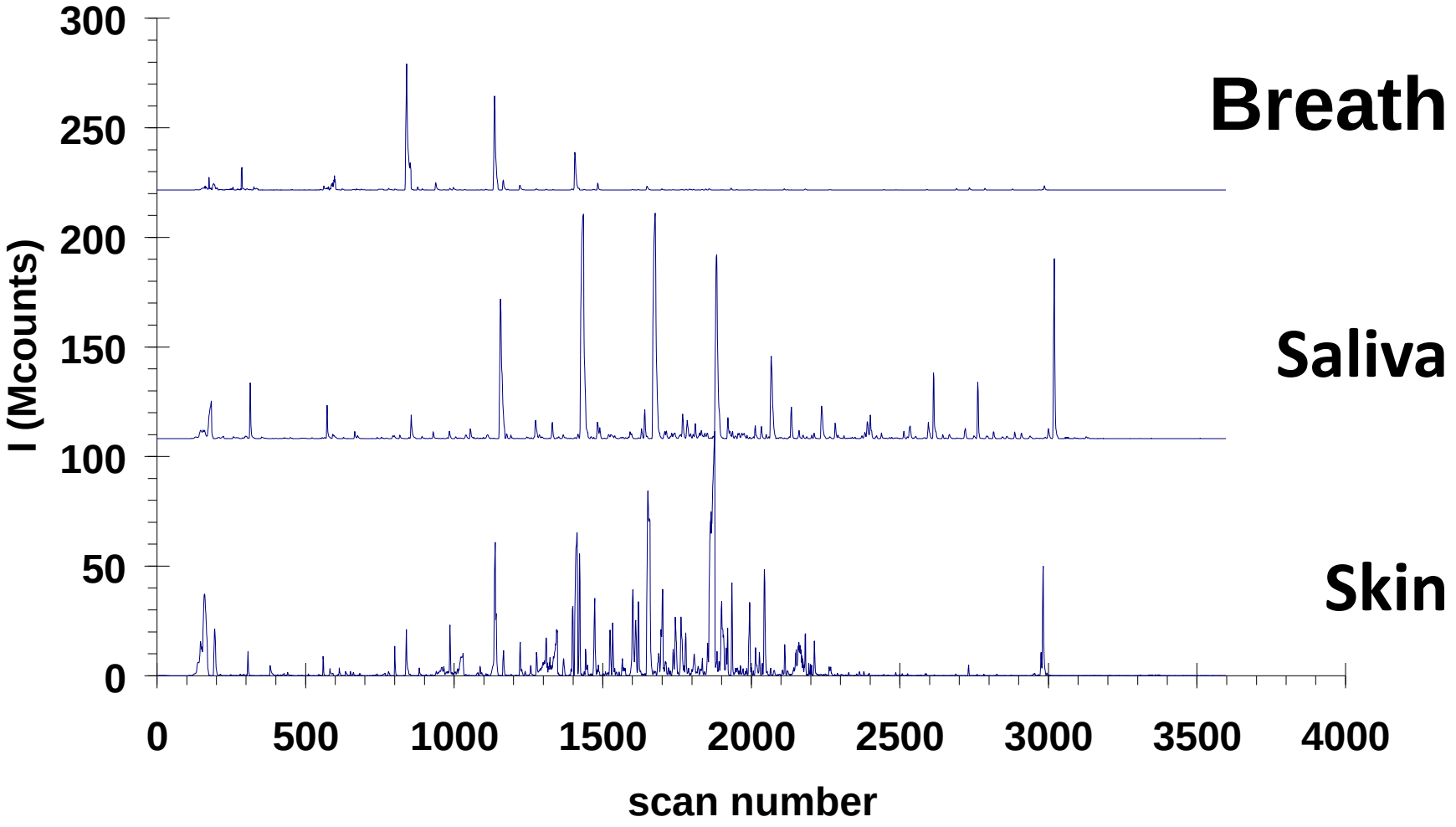
- Defined participant preparation. (SPC)
- Controls: accompanying partners; age, gender and environmental matching.
- Stable and controlled sample station and environment (SPC)
- Quantitative proficiency testing (SPC).
- Internal standards for every sample (SPC).
- SPC for every breath, abnormal patterns identified.
- All samples immediately frozen to <-80^{o}C.
- All samples analysed within 30 days.
- All data open access.
- Libraries generated by public funds made available to all.

The list of factors awaiting standardisation is likely to include:

- Bags/canisters/gas-tight syringes/glass bulbs
- Derivatisation/SPME
- Temperature/
- Residence times / dead-volumes / construction materials / and surface treatments.
- Control: pressure /flow / CO2
- Volume of fixed-volume sample-loops
- Sample flow for continuous inlet capillary aspiration



The human volatalome is just too rich to ignore.



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Markers and detection

- A team of 9 Academics
  - Marker discovery
  - Labels and assays
- Health and Bioanalytics
  - Breath, skin and saliva
  - Diagnosis, screening and assessment.
- Security and Resilience
  - Triage systems
  - Emergency medicine
  - Detection

- **Faster,**
- **more accurate,**
- **lower-cost,**
- **enhanced resolution**

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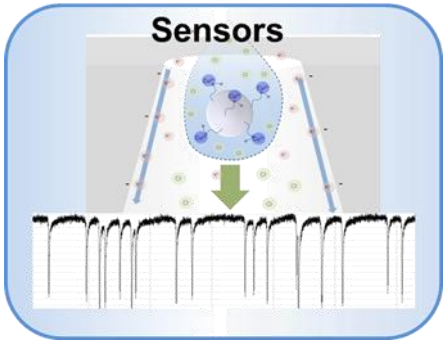
Markers and detection



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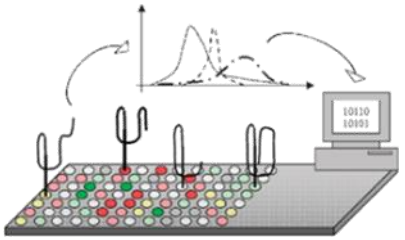
  - Mass spectrometry
  - Separation science
  - Ion mobility spectrometry
  - Sampling
  - Aptamers and sensing
  - Labels and ligands



- Projects, €18M
  - Emergency Medicine
  - IED detection
  - Aptamer discovery
  - Next-generation forensics.
  - Instrument development