HBM4EU project

science and policy
for a healthy future

Training on the Collection of Exhaled Breath Condensate

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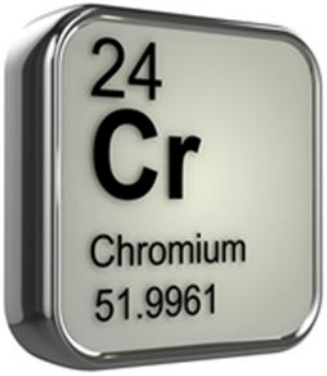
1st HBM4EU Training School 2018



HBM4EU project

1. What is EBC
2. Chromium
3. Chromium in EBC at HSL – The story so far
4. The Collection Process
5. Issues & Limitations
6. Sampling Questions
7. Analysis Questions

What is Exhaled Breath Condensate?

EBC

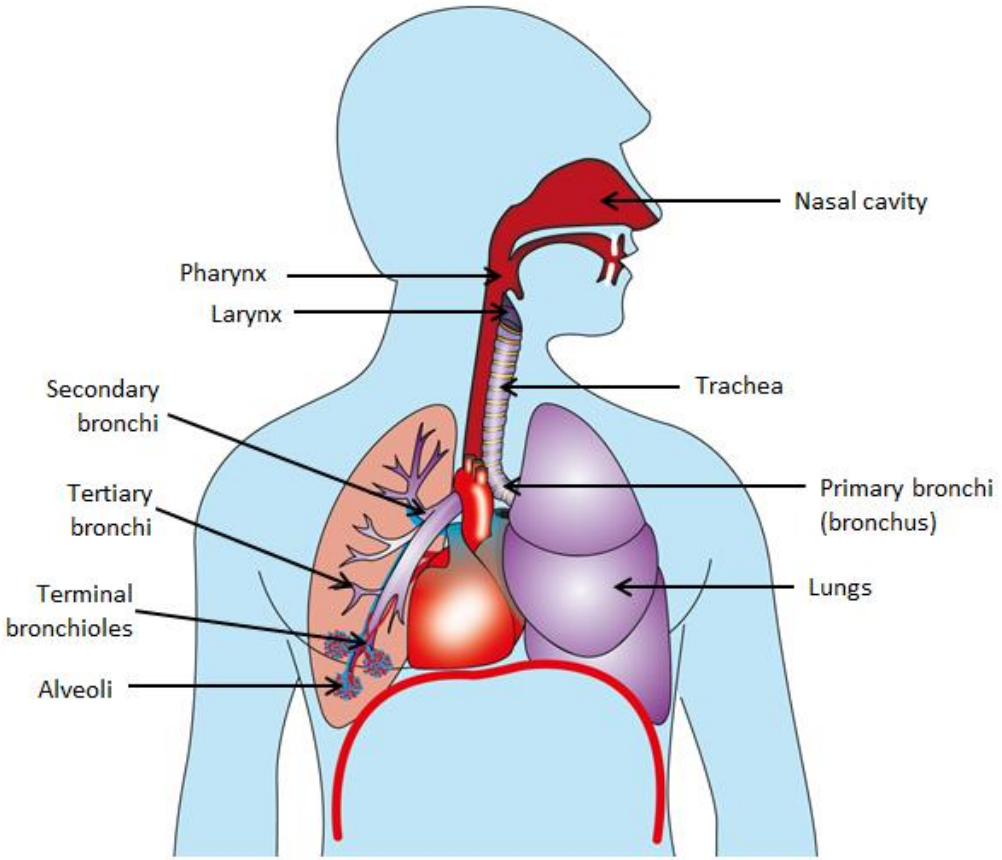
EBC is

- Biological fluid
- Cooled exhaled air
- Condensate solution

Respiratory Tract

EBC Consists:

- Mainly water vapour (>99%)
- Droplets of airway lining fluid from within
  - Bronchial system
  - Alveoli regions
  - Entire respiratory tract



Airway Lining Fluid

Within the droplets of airway lining fluid is an unknown fraction of:

- Volatile substances – water soluble compounds
  - Ammonia, hydrogen peroxide, ethanol, nitric oxides
- Non-volatile substances
  - Cytokines, lipids, proteins, salts
  - Environmental contaminants
  - Occupational contaminants

Formation of EBC

Mechanism is not fully understood

- Turbulent airflow
  - Opening of bronchioles and alveoli creates a turbulent airflow in the respiratory tract aerosolising the fluid

- Bronchiole fluid film burst (BFFB)
  - Bronchioles contract expelling fluid during exhalation. Inhalation creates a bubble which bursts into droplet aerosols which are held in the alveoli until exhalation.

EBC volume

EBC volume will vary from one individual to the next.

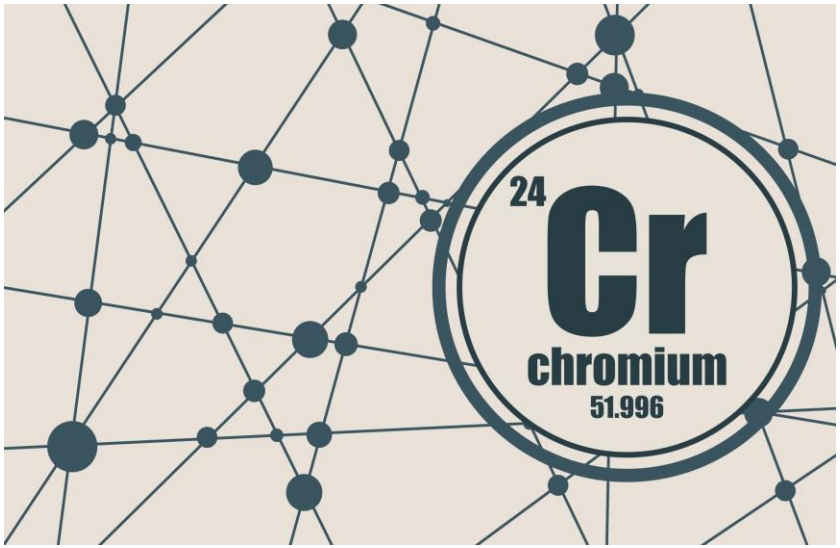
- Age, gender, fitness, smoking status, lung status or disease status are not contributing factors.

EBC volume is directly correlated to:

- Tidal Volume – volume of air displaced in the lungs between normal inhalation & exhalation.
- Minute or Ventilation Volume – amount of gas inhaled or exhaled from the lungs in one minute.

People with higher minute/ventilation volume and/or higher tidal volume will produce more EBC

Chromium



Chromium Species

- Trivalent chromium – CrIII
  - Non toxic
    - Although reactive it cannot permeate cell membranes
  - Essential element
    - Glucose, fat and protein metabolism

- Hexavalent chromium – CrVI
  - Known as chromates, dichromates and chromic acid
  - Carcinogen, respiratory & skin sensitising agent
    - Although unreactive it can permeate cell membranes.
  - Rarely found naturally in the environment with the exception of crocoite

Occupational Cr Exposure








Blood Cr Biomonitoring

No matter the route of exposure CrVI is eventually reduced to CrIII

- Blood
  - Indicative of CrVI exposure only (measured as CrIII)
  - Reduction of CrVI in the plasma prior to cell permeation
  - Levels will be elevated for the life of the red blood cell
  - Invasive & the need for trained medical staff

Urine Cr Biomonitoring

No matter the route of exposure CrVI is eventually reduced to CrIII

- Urine
  - Total chromium measurement (measured as CrIII)
  - Determines all chromium exposure (dietary & occupational)
  - Reflects past & recent exposure with daily accumulation
  - Established background unexposed reference ranges
  - Established Biological Monitoring Guidance Values

EBC Potential Biomonitoring

As the primary route of occupational exposure to CrVI is by inhalation, with EBC we may have the potential:

- EBC
  - To measure both CrVI & CrIII
  - To help understand inhalation exposures and how chromium resides in the lungs
  - To Provide a comprehensive picture of inhalation exposures

Chromium in EBC at HSL

Method Development

Chromium Challenges

At HSL the aim was to detect & measure both CrVI & CrIII

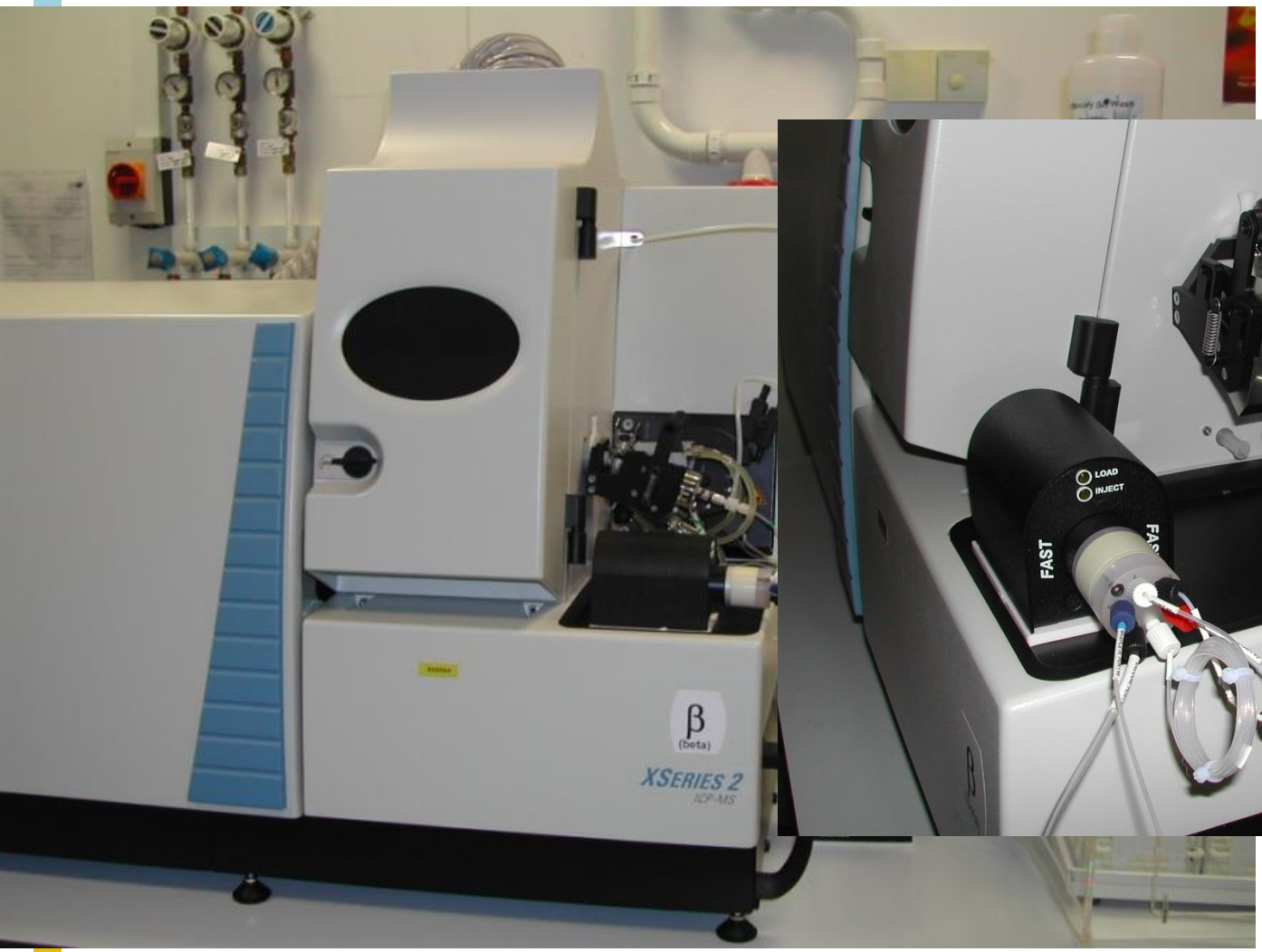
Interconversion between the two oxidation states makes the stability and integrity of the species a challenge during analysis and sample storage.

- CrIII
  - Stable in acidic conditions
  - Cationic in solution
- CrVI
  - Stable in alkaline conditions
  - Anionic in solution

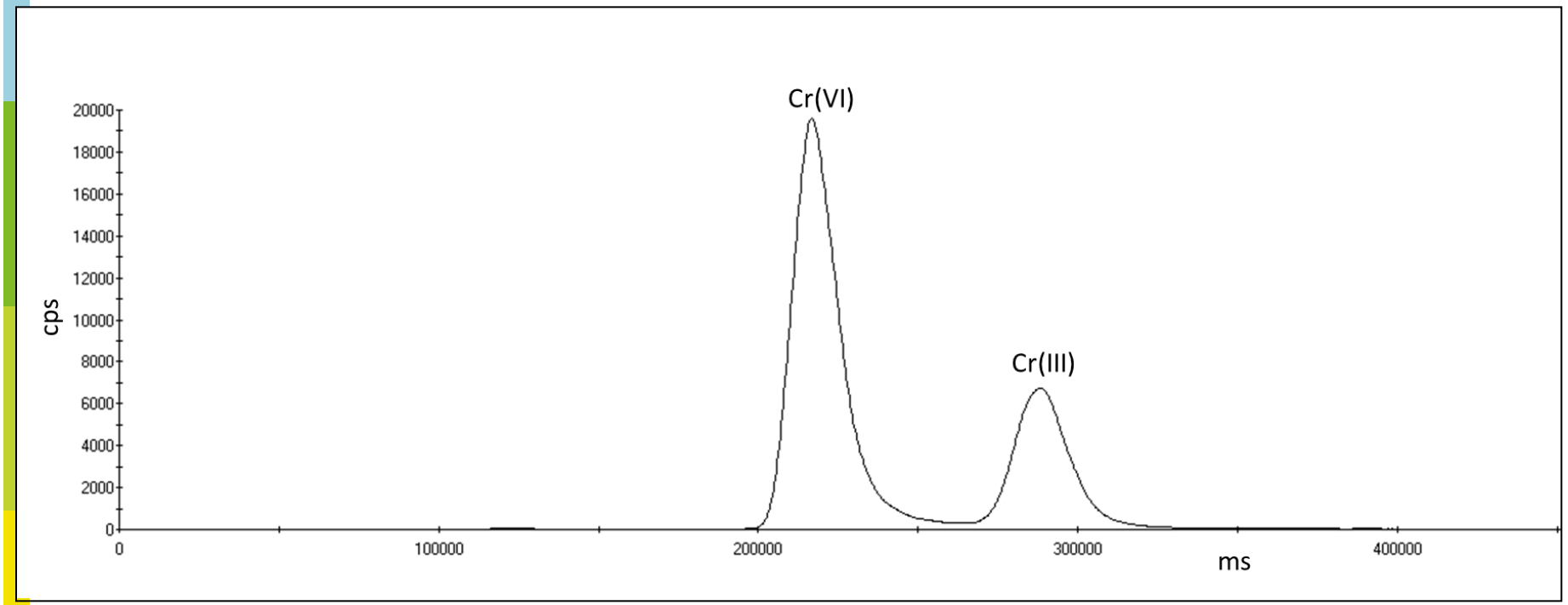
Speciation Set-Up

- Thermo XSERIES2 ICP-MS monitoring ^{52}Cr coupled to an ESI OneFAST
  - CCT mode (7% H2 in He) ~ 3.5 L/min
  - PFA-ST nebuliser
- Dionex IonPAC AG7 speciality column
  - 4 x 50 mm ID, 10 µm
  - Anion exchange column with cationic properties
- Isocratic mobile phase
  - 4 % v/v nitric acid & 3.2 % v/v ammonia – pH 1.8 - 2
  - Flow rate 1 mL/min
  - Total acquisition time 230 s
  - Sample injection 30 s

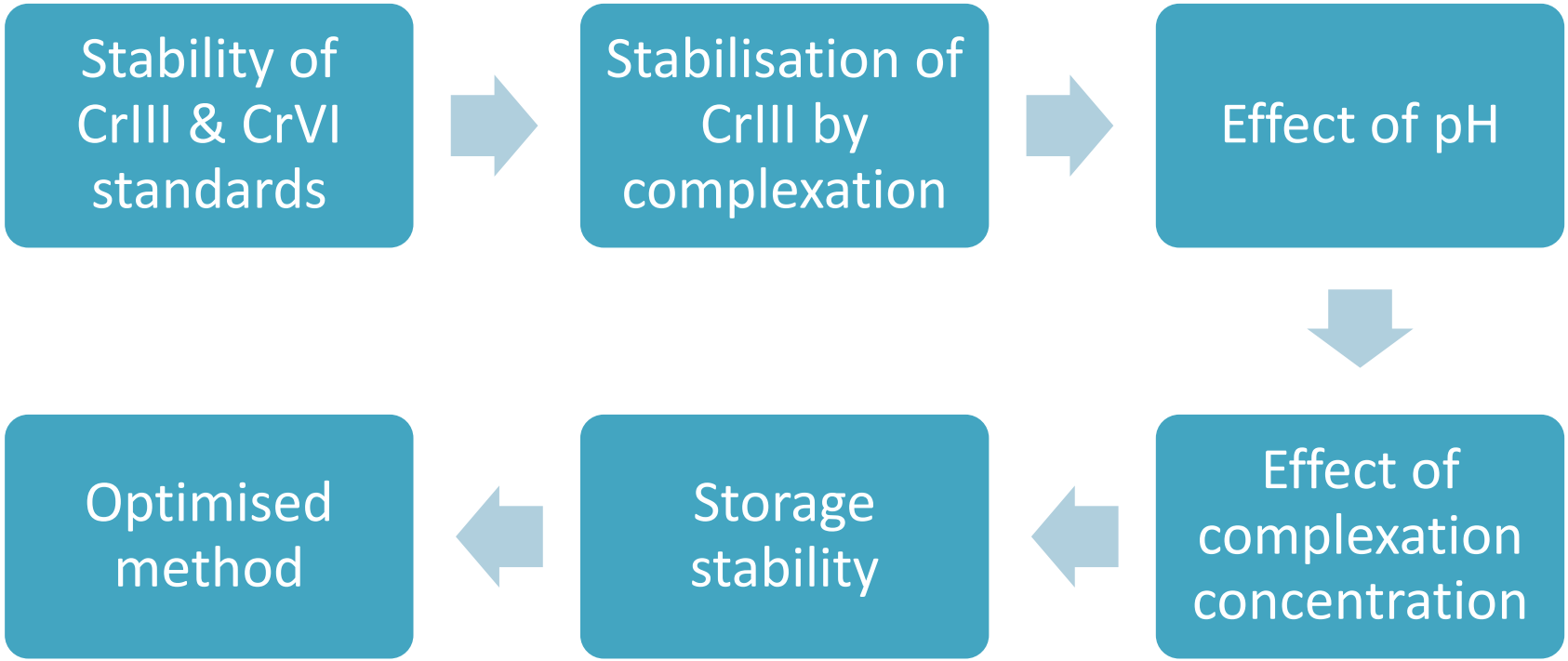
Instrumentation at HSL



If only life was so simple

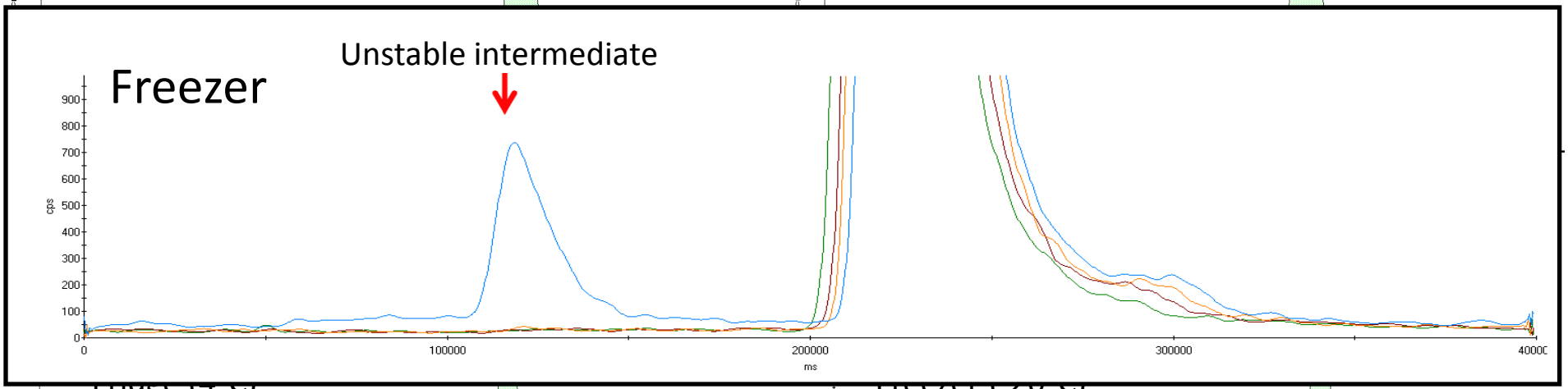


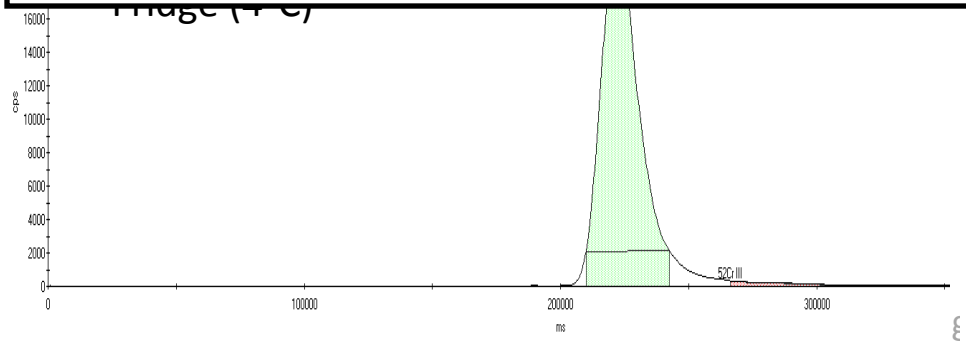
Six Stages of Development

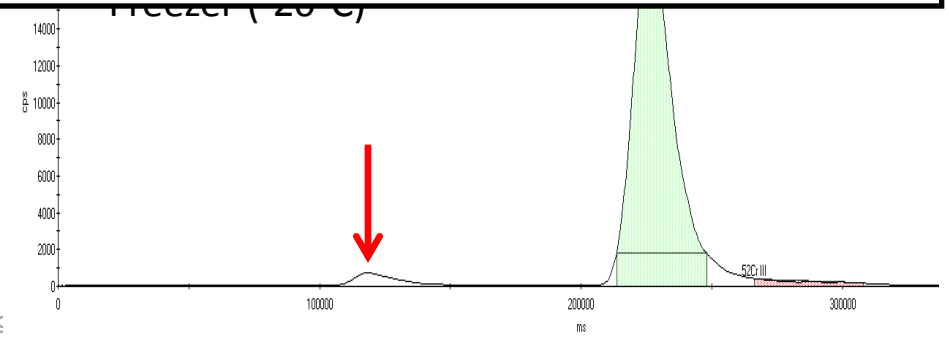


CrVI stability – 24 hours

5µg/L standard - 24 hours storage

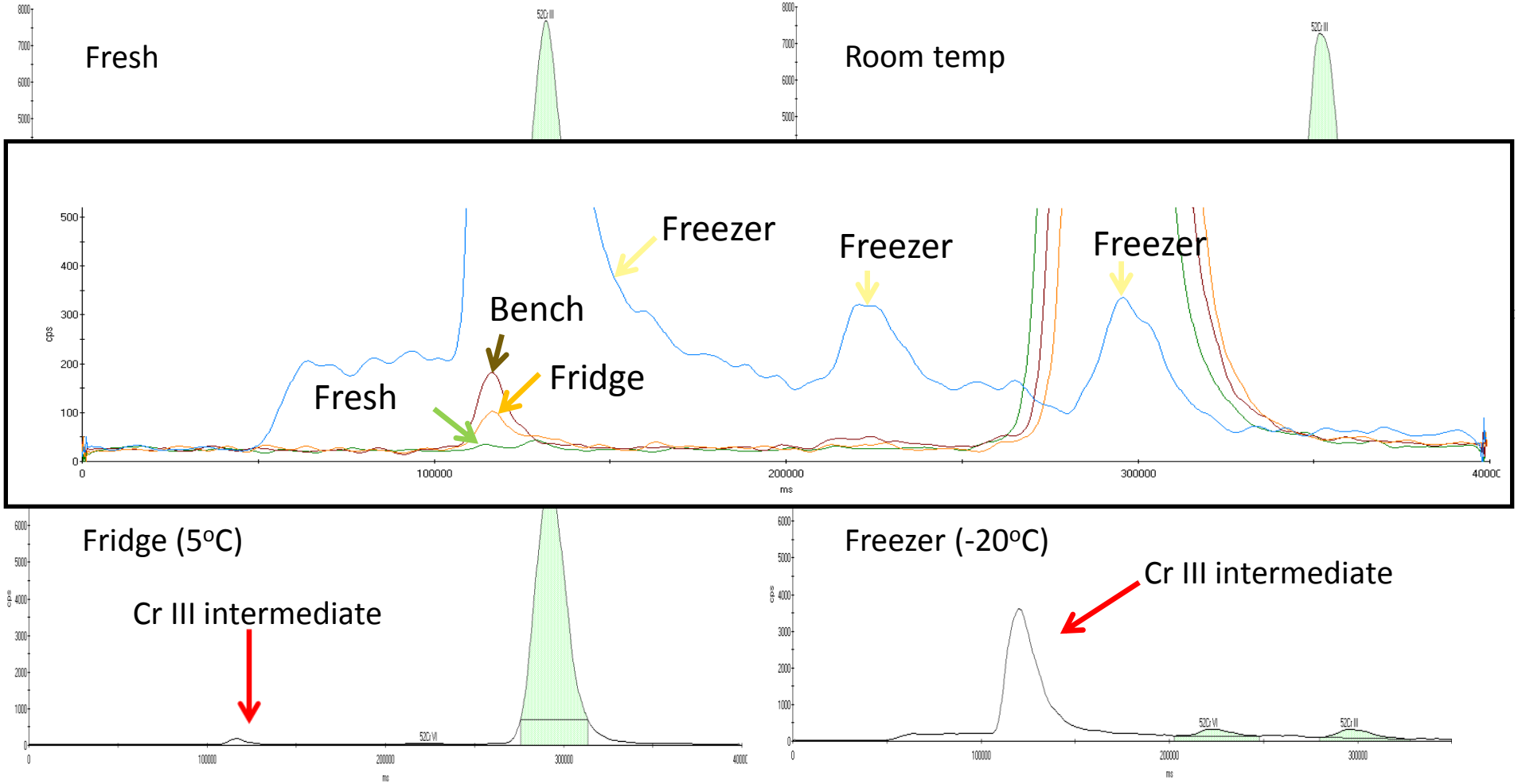






CrIII stability – 24 hours

5µg/L standard - 24 hours storage



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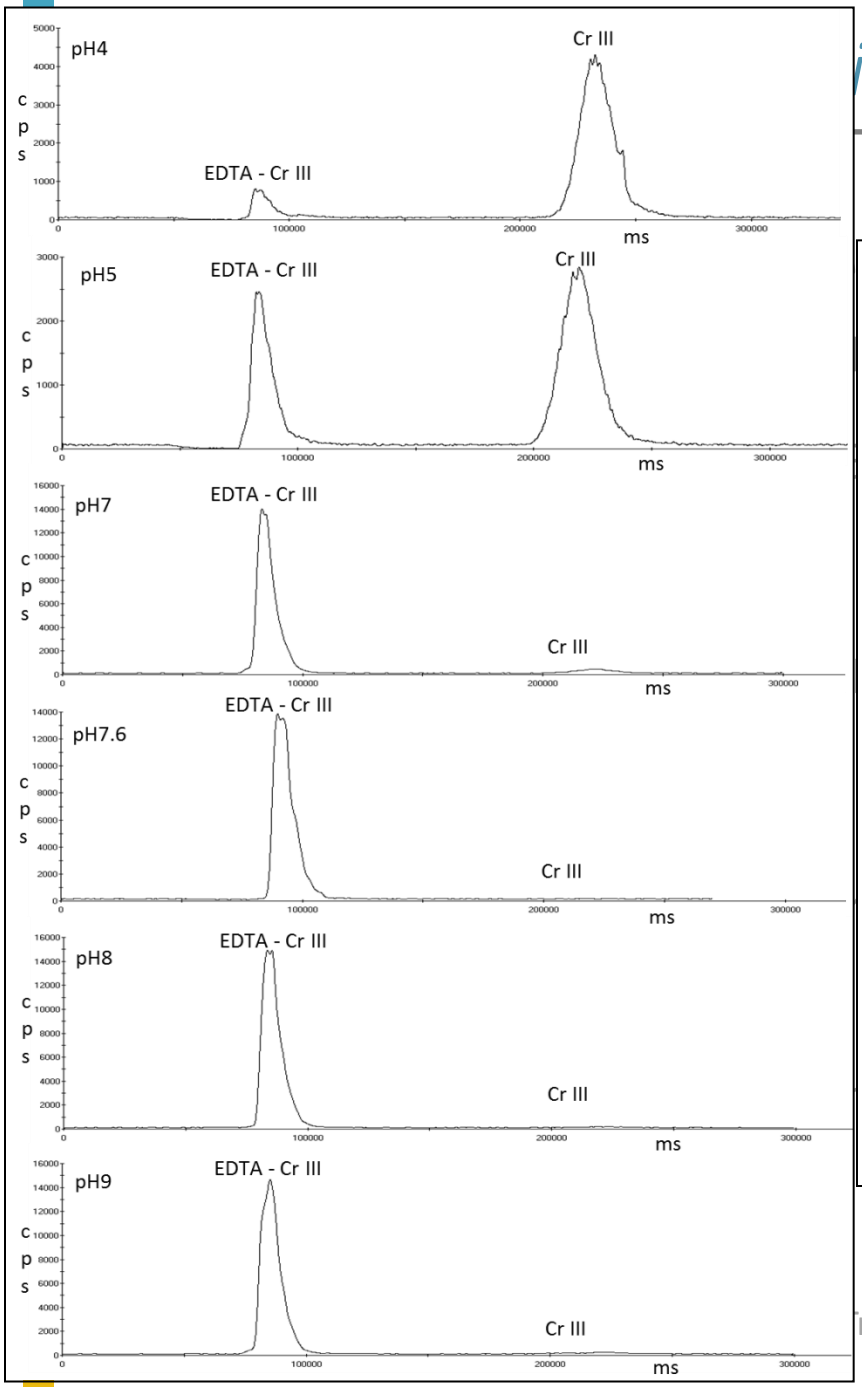
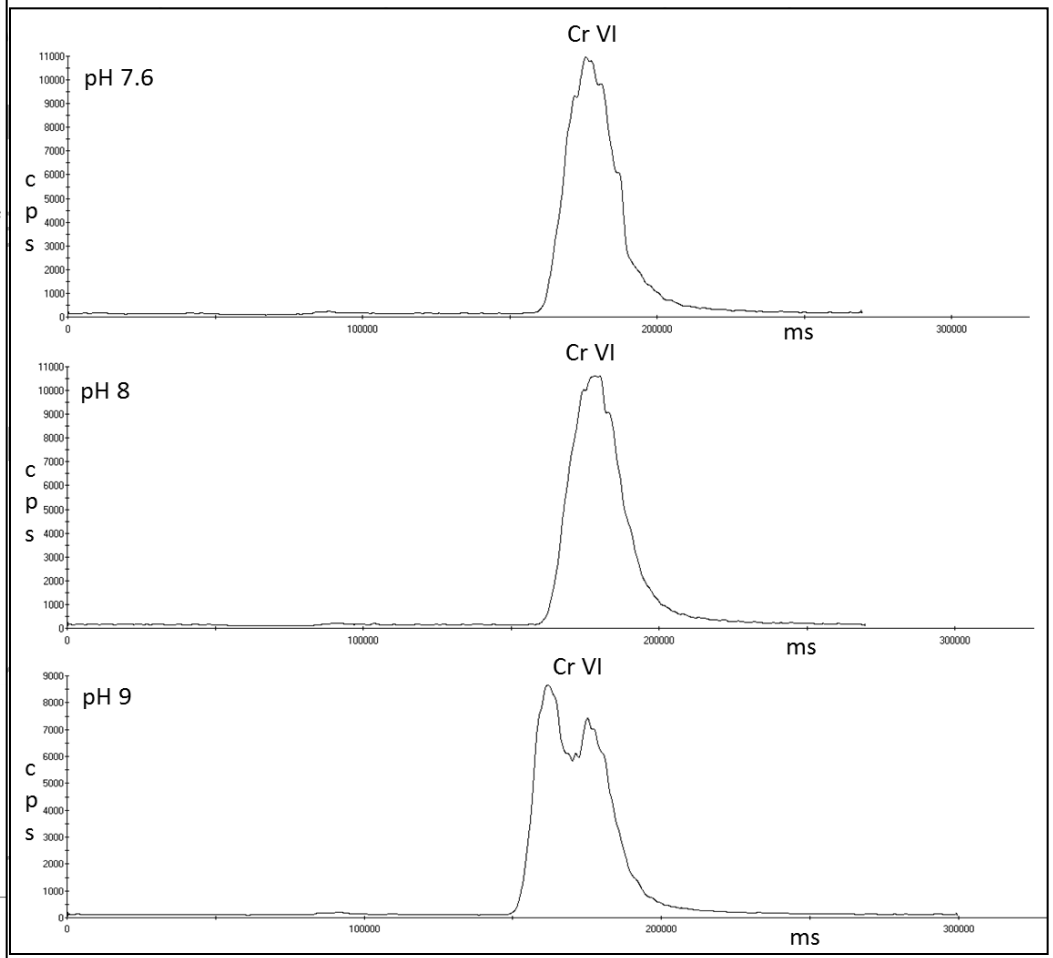

pH4
Cr III
EDTA - Cr III
cps
ms
pH5
EDTA - Cr III
Cr III
pH7
EDTA - Cr III
Cr III
pH7.6
EDTA - Cr III
Cr III
pH8
EDTA - Cr III
Cr III
pH9
EDTA - Cr III
Cr III


Cr VI
pH 7.6
cps
ms
Cr VI
pH 8
Cr VI
pH 9

Storage Stability

- 6 week stability study at pH 7.6 & pH 8
  - Blank EBC spiked & diluted 10 fold with 0.5 mM EDTA solution
    - 5 µg/L CrIII
    - 5 µg/L CrVI
    - 5 µg/L CrIII CrVI mix
  - Stored fridge ~ 4^{o}C

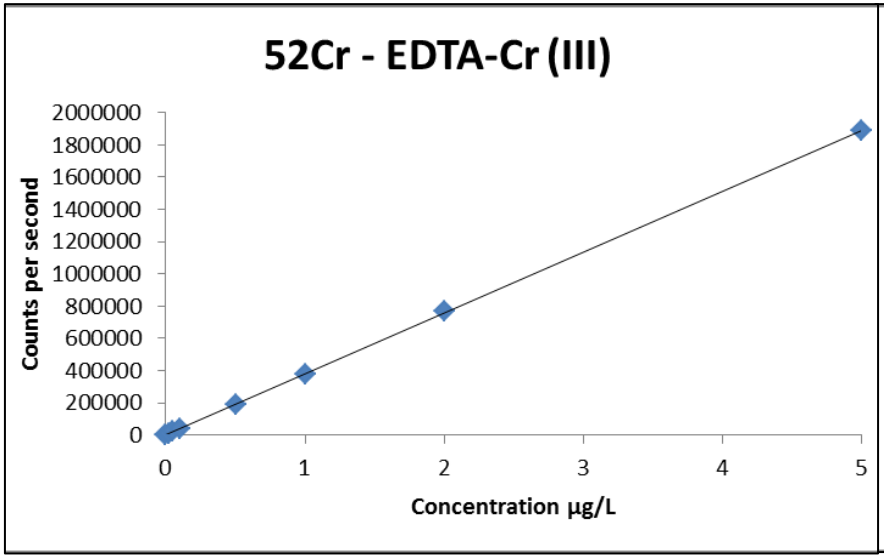
- pH 7.6 showed instability of CrVI after 1 week
- pH 8 showed stability of CrVI for 6 weeks
- CrIII was stable for 6 weeks at both pH levels.

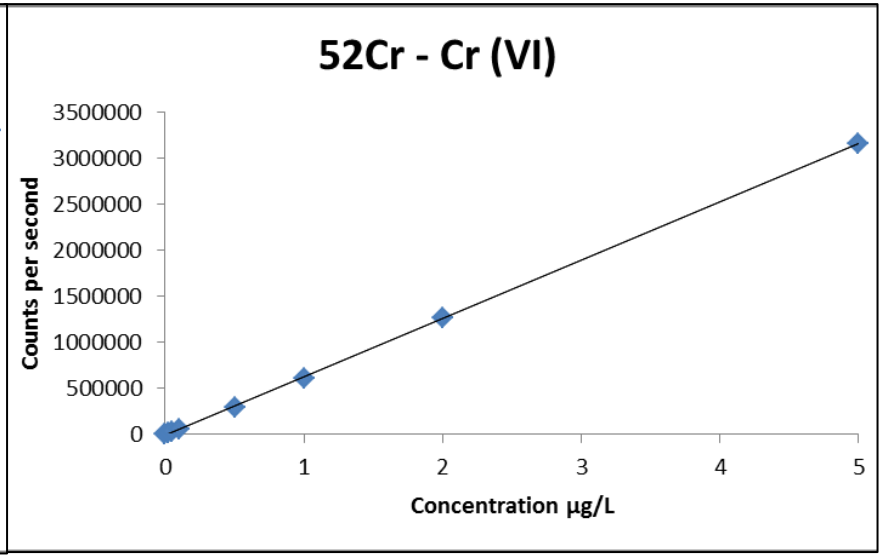
Optimised Method

Calibration range of 0 – 5 µg/L

Standards made in 0.5 mM EDTA adjusted to pH8

- Left for 1 hour to ensure full complexation





Optimised Method

Samples / QCs diluted 10 fold with the 0.5 mM EDTA solution adjusted to pH 8

- QCs left for 1 hour to ensure full complexation

Samples must NOT be FROZEN

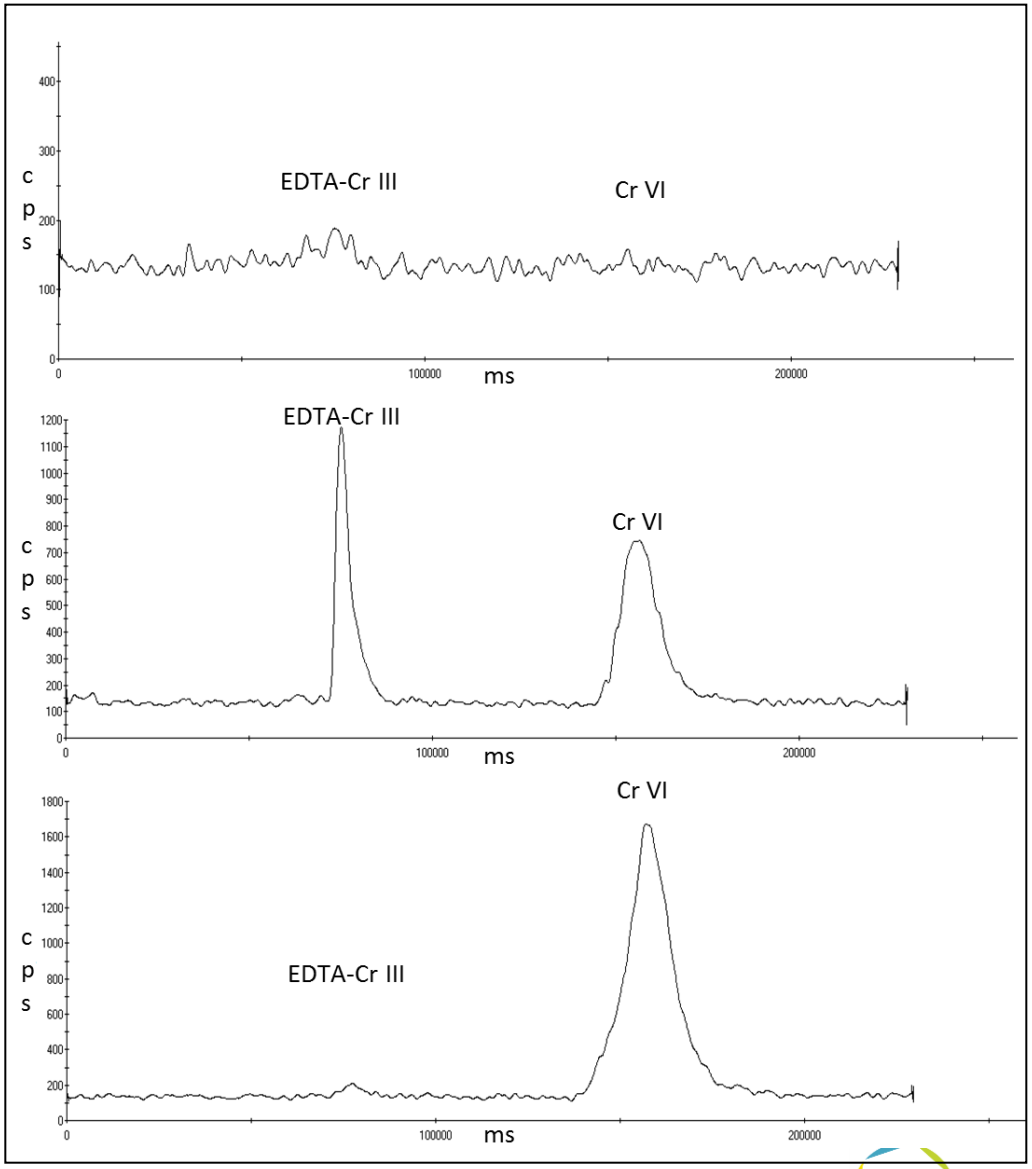
Store refrigerated ~ 4^{o}C

Optimised Method

Blank EBC

2 µg/L spiked blank EBC

Sigma PT scheme CrVI in drinking water sample



Chromium in EBC at HSL

Feasibility Study

Feasibility Study

Aim of the feasibility study at HSL was to determine

- Can CrVI and CrIII be detected and measured in a ‘real’ EBC samples?

If yes

- Is there a difference between unexposed and exposed people

Site Visits

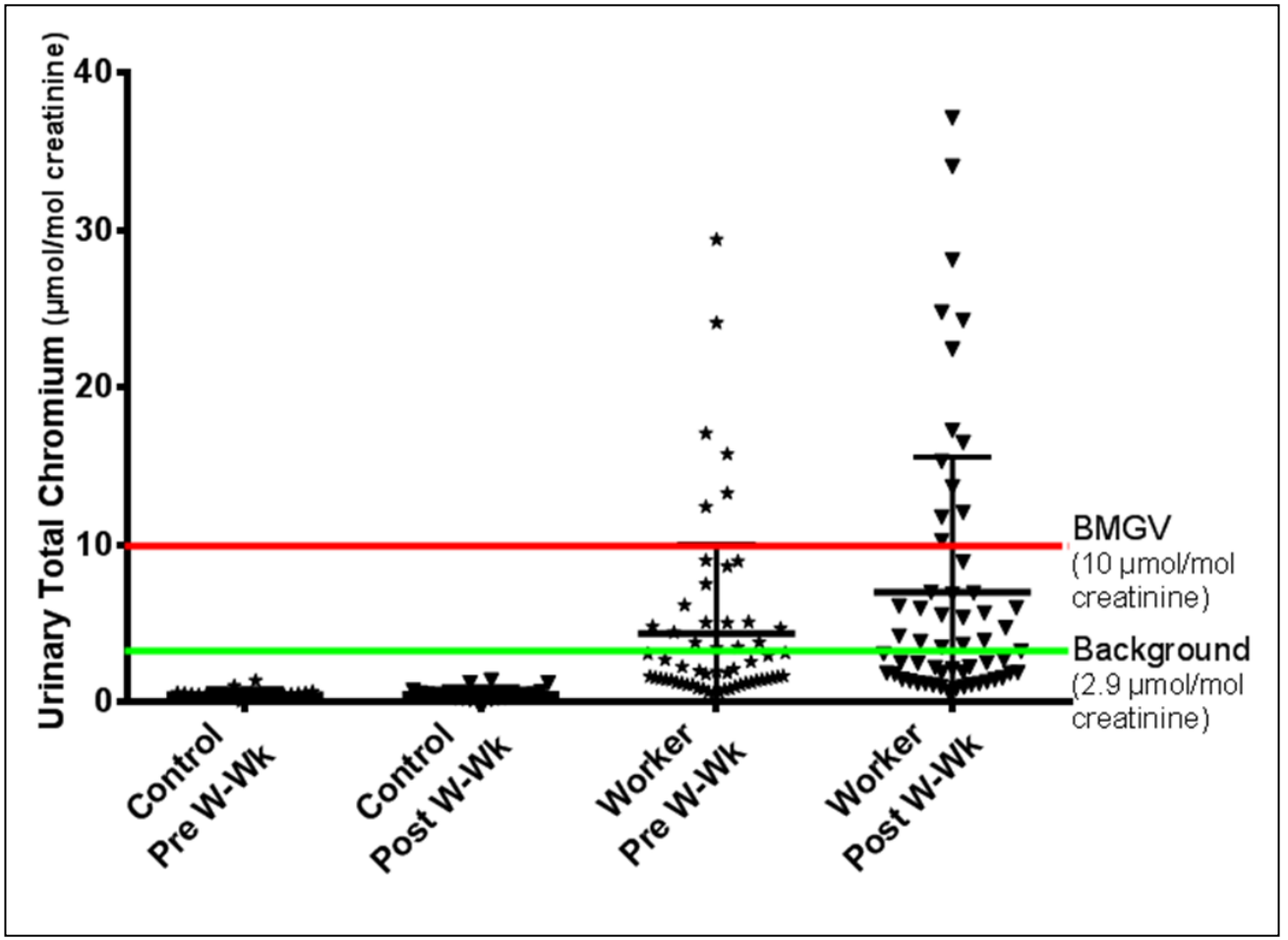
- Any worker potentially occupationally exposed to CrVI by inhalation
- Urine & EBC samples
- Pre shift sampling
  - Monday morning –
- Post shift sampling
  - Thursday afternoon
- Control Group – HSL staff

- n =58 workers
- n = 22 control group

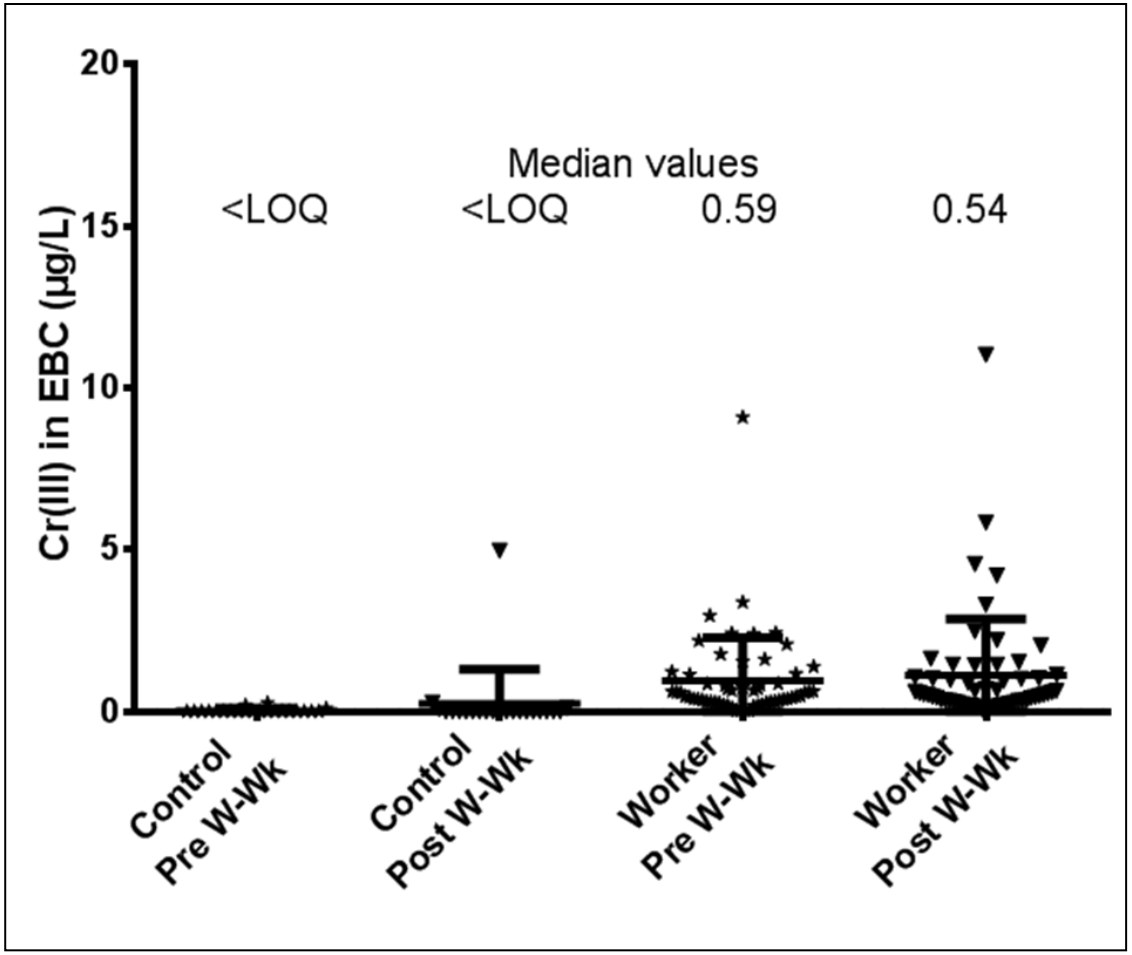
Occupationally Exposed

- 8 companies volunteered
  - Blenders
  - Plasma cutters
  - Electroplaters
    - Anodisers
    - Jiggers & polishers
    - Grinders
    - Welding/ maintenance / repairs
    - Non chrome platers
    - Inspectors & office / managerial staff

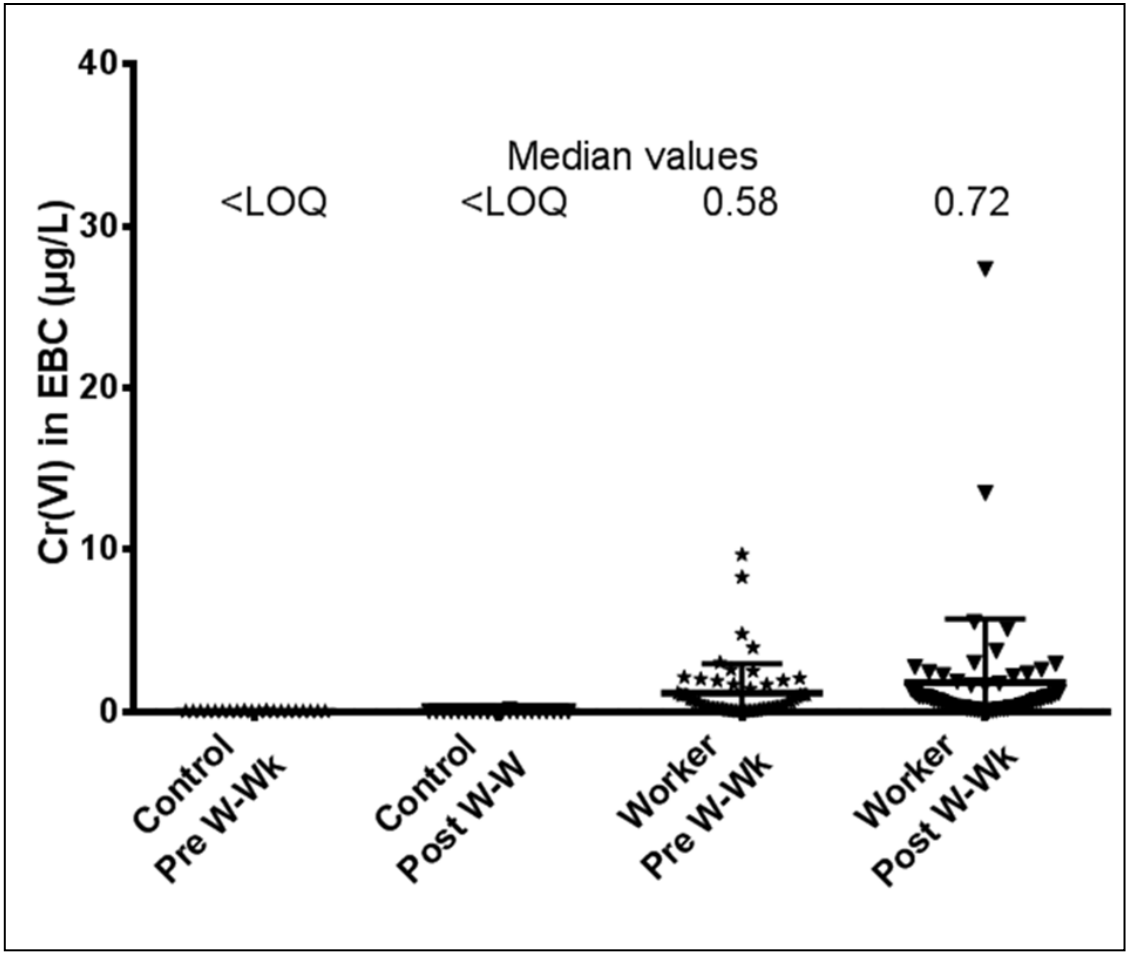
Results – Urinary Chromium



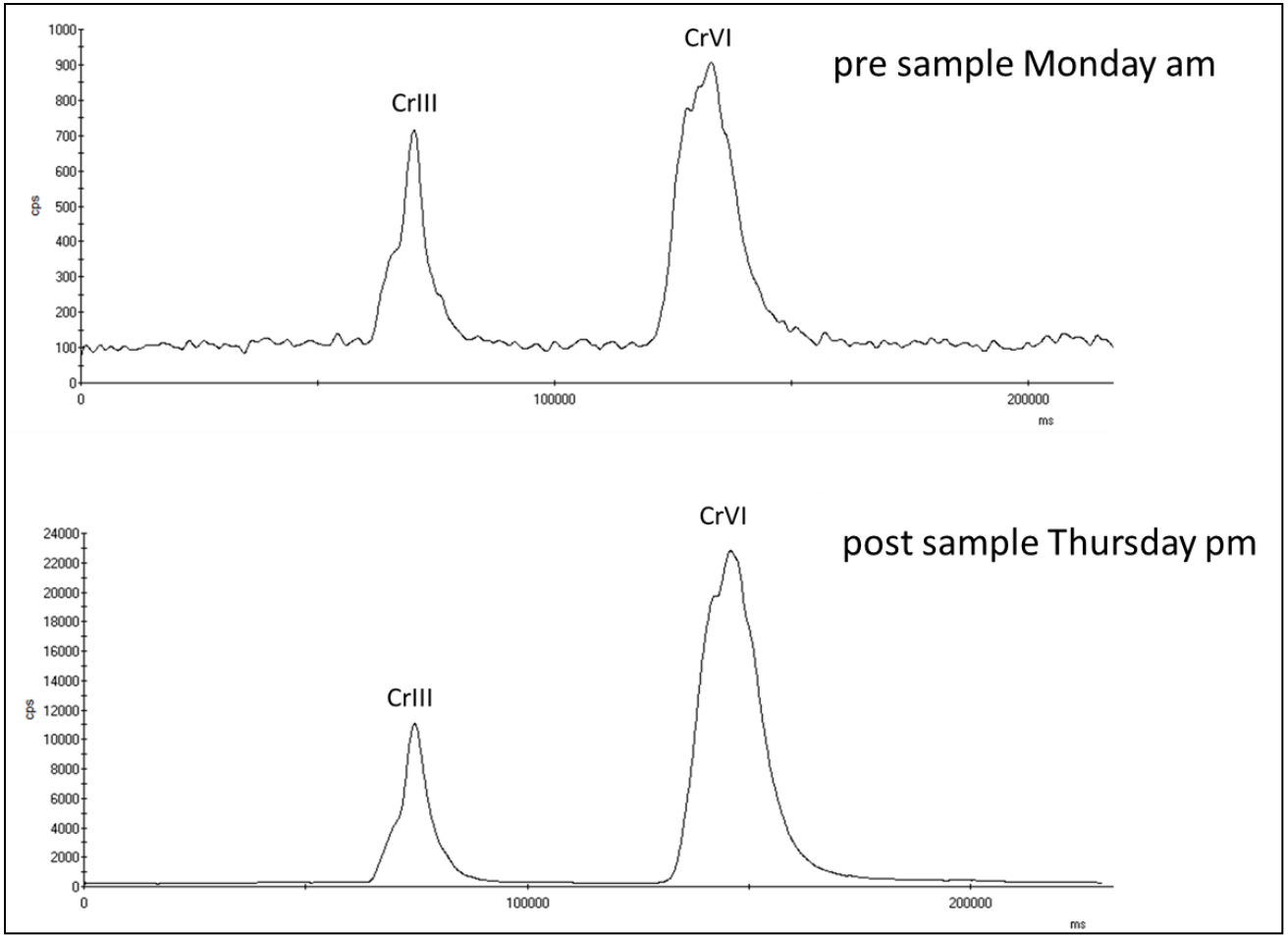
Results – CrIII in EBC



Results – CrVI in EBC



Electroplater Pre v Post EBC



Study Facts

- LOQ
  - CrVI 0.002 µg/L
  - CrIII 0.007 µg/L

- Concentration range of CrVI in EBC samples:
  - 0.01 – 27.3 µg/L
- Concentration range of CrIII in EBC samples:
  - 0.14 – 11.03 µg/L
- Concentration range of urinary total chromium:
  - 0.5 – 37.1 µg/L

Statistics & Observations

Is there a difference between unexposed and exposed people?

- Strong statistical significance between workers and controls
  - Urinary total chromium (p = <0.0001)
  - Cr(III) in EBC (p = <0.0001)
  - Cr(VI) in EBC (p = <0.0001)

- No correlation between urine & EBC

Statistics & Observations

Is there a difference between pre and post working week samples?

- Statistical significance
  - Urinary total Cr from pre and post w/week (p=0.03)

- No statistical significance
  - Cr(III) in EBC from pre to post w/week (p = 0.63)
  - Cr(VI) in EBC from pre to post w/week (p = 0.13)

Limitations

- No environmental monitoring
  - Air monitoring
  - Surface wipes
  - Hand wipes

- Exposure information
  - Length of time since last exposure
  - Levels of exposure
  - Length of time exposed

EBC Collection

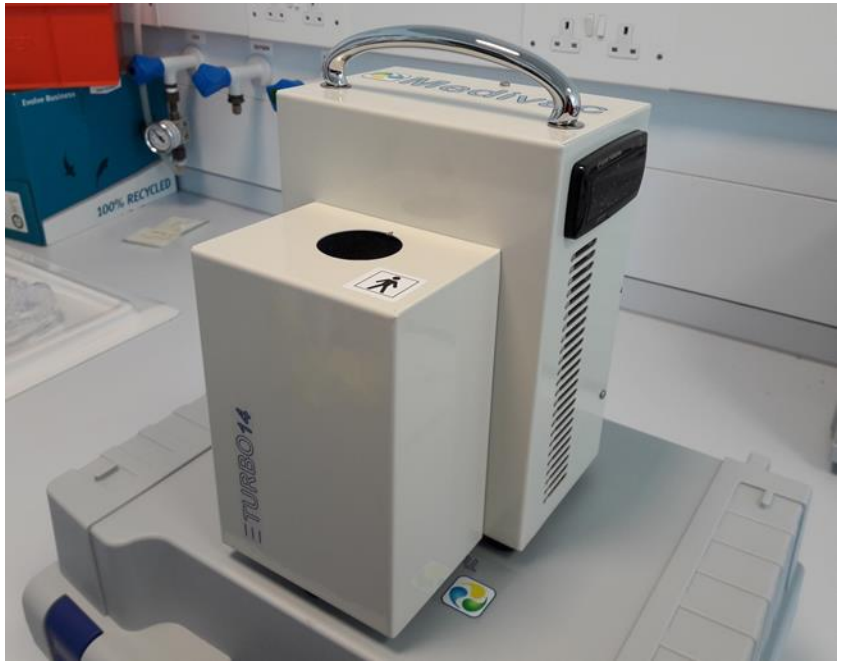
Collection Device

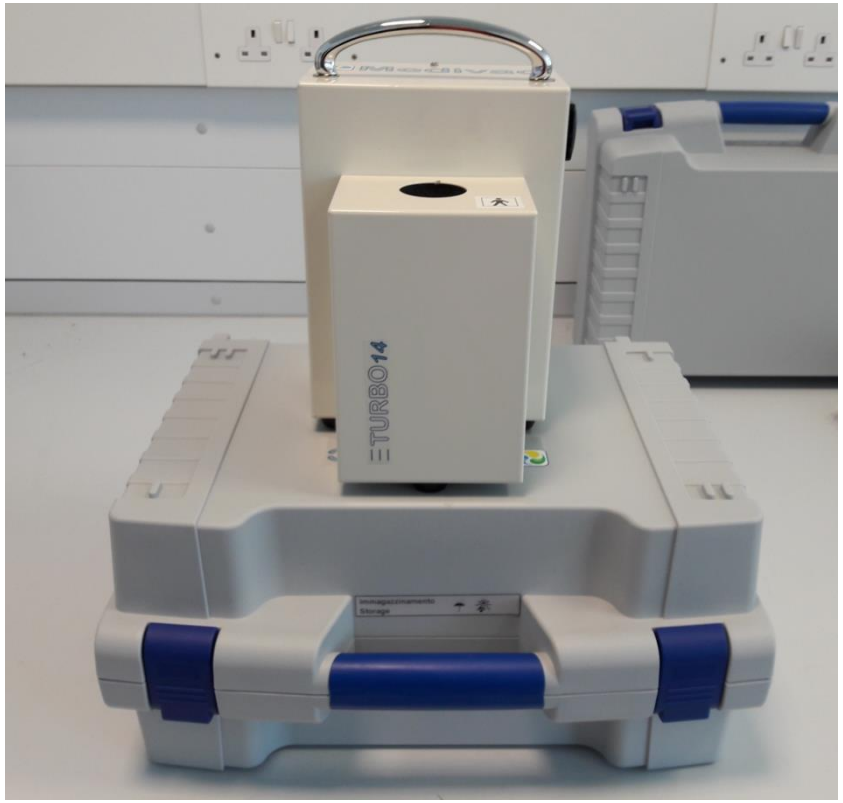
- TURBO - Transportable Unit for the Research on Biomarkers Obtained
- DECCS - Disposable Exhaled Condensate Collections Systems

Aluminium Peltier-cooling system

Disposable polyethylene respiratory system

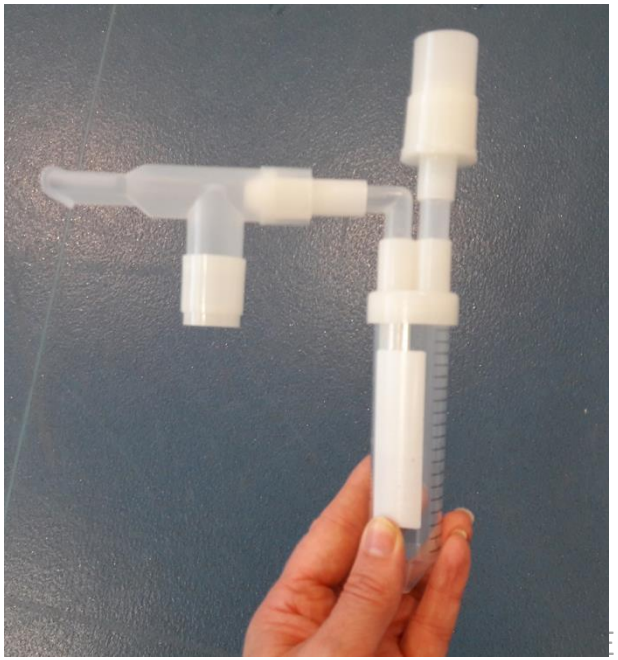
Turbo





DECCS

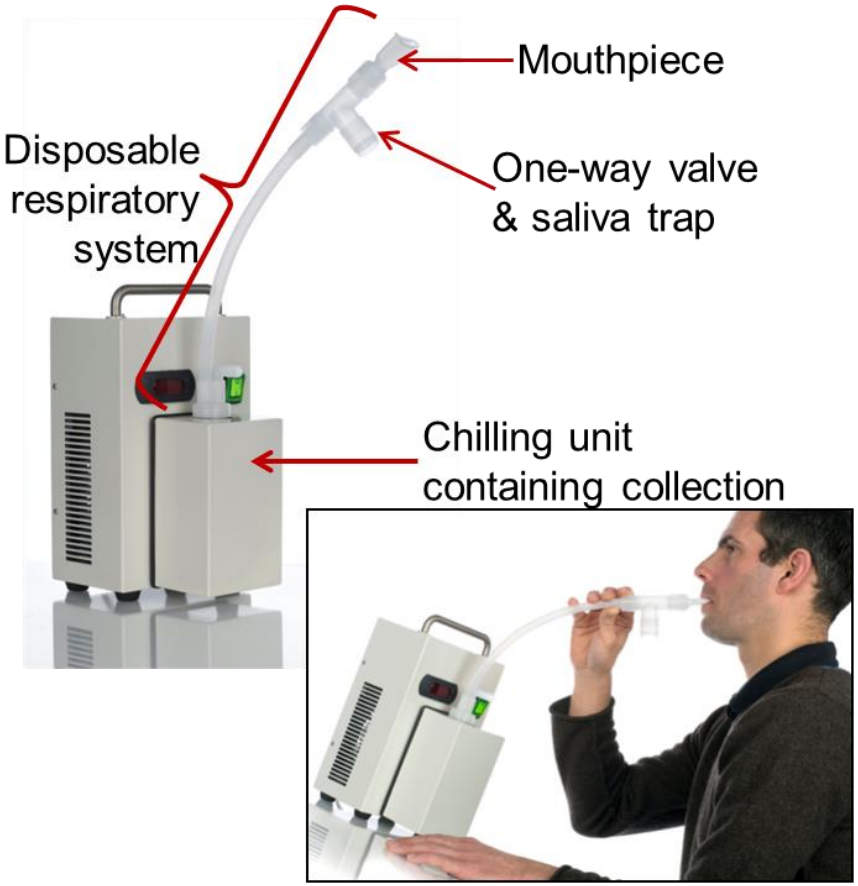

Medivac SRL
LOTTO
DATA
DECCS 14 ST
- Circuito per la raccolta dell'EBC
- EBC's Collection Circuit
KIT MONOUSO / DISPOSABLE KIT
REV. 02
NON RIUTILIZZARE
USARE SOLO PER LA RICERCA
FOR INVESTIGATIONAL USE ONLY
DO NOT REUSE
C3
E
G
C4
C2
C3
C1



Collection of EBC

Regular tidal breathing

- Through the disposable mouth piece.
- Not a forced breath sample
- 15 minutes will collect approximately 1 mL
- Non-invasive
- Does not cause an inflammatory response itself



Starting the Turbo

- Plug the Turbo in to the electrical source & turn on
  - Default temperature setting will be -5^{o}C
  - Can take up to 20 minutes to cool
- Place on top of its box on a suitable table with a chair for the volunteer to comfortably sit at whilst providing the sample
- Unwrap and assemble a DECCS sampling kit (NOT the mouth piece) and place the collection tube into the cooling chamber

DECCs sampling kit

- Each pack is wrapped and sealed to provide a sterile kit.
- For hygiene, open and assemble prior to each volunteer.
  - Leave the mouth piece until last

If the turbo is cooling and a tube is not in the condensing cooling chamber it will begin to freeze over

If a tube containing EBC is inserted into the condensing cooling chamber, the EBC will begin to freeze.

Providing a Sample

- Ask the volunteer to rinse/wash their mouth out with water
- Begin providing the sample
  - Regular tidal breathing through the mouth
  - 15 minutes
  - A complete seal around the mouthpiece with the mouth and lips must be maintained
  - Periodic removal to swallow accumulated saliva in the mouth will be required

Remind them to not breath through their nose

Remind them to keep breathing tidal

Providing a Sample

After the timed 15 minutes alert the volunteer to stop breathing into the mouthpiece

- Remove the entire sampling kit
  - Cap the tube and label
  - Dispose of the remaining sampling kit

Samples are to be kept refrigerated during the site visit and whilst being transported to the laboratory.

- Place another disposable DECCS sampling kit into the Turbo and ensure it has chance to cool for at least 5-10 minutes prior to next volunteer.

EBC EDTA Complexation

At HSL a single batch of EDTA solution adjusted to pH 8 is prepared.

For example 2 L is prepped.

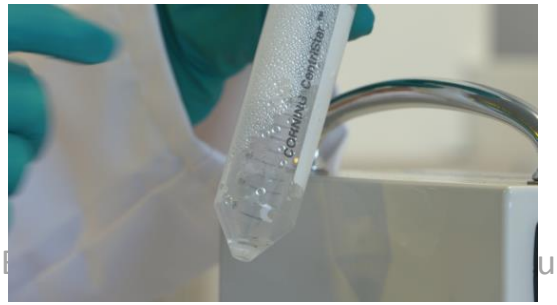
- 1 L is taken to site to complex the EBC samples
- 1 L remains in the laboratory and used for standard and QC preparation when analysing the samples.

- EDTA was made fresh for each set of visit/analysis samples
- I am unaware of stability of the EDTA

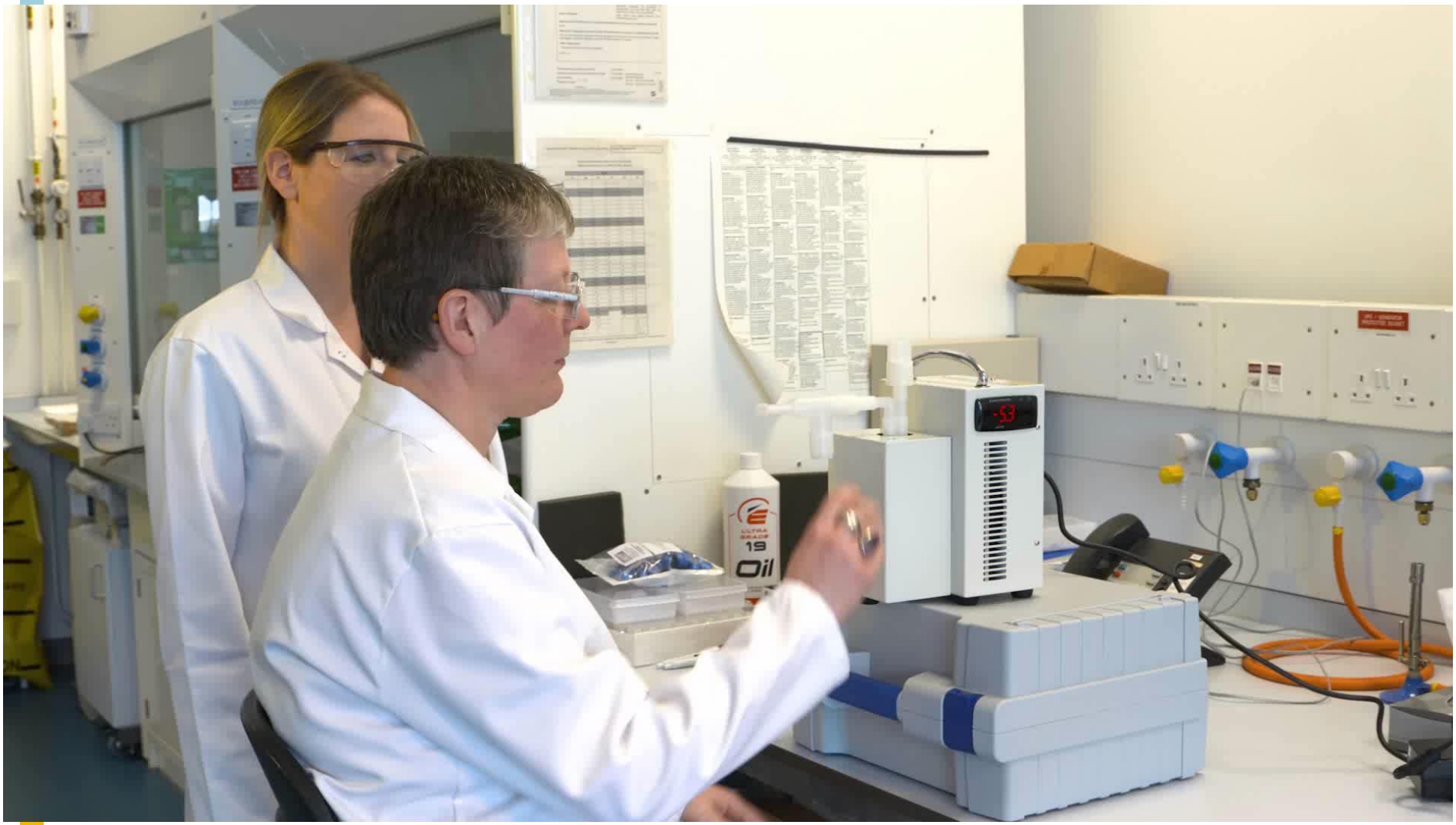
EBC samples not being analysed by the same lab, send a tube of EDTA solution – Blank check/correction

EBC EDTA Complexation

- Label a secondary tube
- Give the collection tube a shake/swirl
- Aliquot a small amount of the EBC sample into this secondary tube and dilute 10 fold with the EDTA solution.
  - Aim to aliquot 1 ml of EBC
- Label/note the amount of EBC aliquoted on the tube or in a workbook.
- Place all samples in a portable refrigeration unit/insulated box with ice pack. DO NOT FREEZE.



HSL Film Stars



Issues & Limitations

Standardisation

- EBC is a relatively novel biological matrix
  - Especially for trace elemental analysis
- Numerous unknowns about EBC
  - Mechanisms
  - What it represents
  - Ratio of exhaled concentration to inhaled exposure
  - Environmental factors
  - Dilution
  - Stability
- Set of specific guidelines to limit as much variation as possible of sample collection which might effect the data between countries / teams

Standardisation – Collection Device

- TurboDECCS will be the only collection device used for this study.
  - Collection temperature
    - Stability of temperature
    - Freezing of sample
    - Efficiency at collecting EBC/water vapour/non-volatile fraction
  - Respiratory device differences
  - Material of cooling chamber
  - Material of collection vessel

Standardisation – Environmental Conditions

Conditions of the location for volunteer to provide EBC sample

- Does the difference in humidity and temperature of inspired air affect the analyte of interest in EBC?
- A study has shown EBC volume is reduced in drier and cooler conditions

- A suitable room away from the primary site of exposure
- Ideally an office / meeting room / first aid room
- General office conditions:
  - 20 – 25^{o}C
  - Air conditioned.

Standardisation – EBC Volume

EBC consists:

Mainly water vapour (>99%)

Unknown fraction of droplets of airway lining fluid

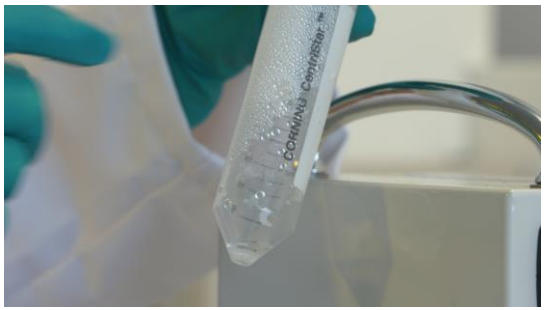
- This dilution will vary from individual to individual.
- Reduction in water vapour does not equal reduction in airway lining fluid.
- Dilution correction ????????????
- Report chromium in EBC results as

  µg/L per volume of EBC collected

Standardisation – EBC Volume

Weigh EBC sample to determine volume

- 1g of EBC = 1 mL of EBC
- Remember to include the aliquot taken for complexation



- Centrifuge the tube
- Weigh an empty 30 mL medicine beaker
- Transfer the EBC sample into the medicine beaker, weigh (g) & record
- Transfer the EBC back to tube

Stability

- Stability study at HSL undertaken for only 6 weeks
- Stability was based on low concentrations of chromium.
- Complexation reaction also based on low concentrations of chromium.
- Room for improvement in the speciation method

Questions about EBC Sampling/Collection

Questions about Speciation Analysis

Contacts

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HEALTH & SAFETY LABORATORY

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Speaker's information

Liz Leese PhD is a senior scientist at the Health & Safety Laboratory, United Kingdom. In HBM4EU she is the lead in chromates in EBC and is part of the UK research team (with IOM) collecting and analysing urine and EBC samples for chromium.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.