science and policy
for a healthy future

HBM4EU project

Blood and urine:
sample collection, aliquoting and storage

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1. Urine

Urinary biomarkers

Sample definitions

Density adjustment

Pitfalls and contingencies

2. Blood

Blood biomarkers

Sample definition

Pitfalls and contingencies

Urinary biomarkers

- Water soluble (organic) metabolites (MW ≈< 350)
- Inorganic ions including e.g. metal ions
- Some cell material (so-called exfoliated cells from which DNA may be extracted)
- Also ultrafine inert particles (silicates, soot)

How to define a urine sample

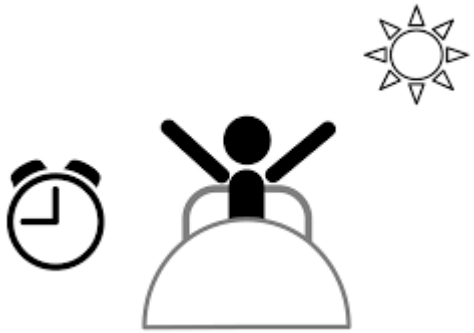
24 h collection sample

'all urine collected during a day and following night'

Spot urine sample

Urine micturition 'between two toilet visits'

How to define a spot urine sample



Spot urine

Random or specified (e.g. in workers as '*pre-shift*' or '*post-shift*'
What was the time of the previous toilet visit?

Morning urine

First urine collected after awakening
What was the time of the previous toilet visit?

Urine

How to define urine as a biological matrix

- Collected in the bladder over time
- Density depends fluid intake/loss
- Usually sterile (except in the case of an infection)
- Homogeneous solutions of salts with peptide/protein
- Excretion of waste products (urea, creatinine)

Sample Integrity

Total void, midstream or collection of a small aliquot?

How the subject can keep the sample (light/dark, cool/freeze)?

How, when and where to do the aliquoting?

When start to freeze?

Dilemmas

Chemical stability/ photosensitivity. Add a nontoxic preservative?

Freezing may cause loss of homogeneity (formation of (in)organic precipitates)

Add a (toxic) preservative

Take a sample for density adjustment when the homogeneity is ensured

How to adjust for urine density?

Creatinine

- Clinical parameter of kidney function
- Artefacts (physical activity; diet/supplements)
- Cost/effective

Density

- Much (manual) work

Osmolality

- Can be done high throughput (more expensive)

Pitfalls	Contingencies
Contamination of material	Select/pre-treat container
Contamination by subject	Wash hands also before
Missing samples	Provide support
Low fluid intake	Provide drinks
Overfill container	Check before freezing
Microbial and chemical integrity	Ask for infection risk and/or add a preservative
Losses	Limit freeze/thaw cycles
Precipitates (e.g morning void)	Dilute with aqua pur

Choice of materials for urine sample collection

- Type of sample container is not much standardized
- Transparent plastic container (visual inspection)
- Wide opening and screw cap
- Add preservative (you may need to label)
- Required sample size should be well thought-through
- Labels attached with non-water soluble glue
- For non-toilet trained children there are solutions like diaper, collection bag, adsorptive pad, free catch

Support to prevent/report missing samples

Support/instructions

e.g. ‘place the container on a closed toilet seat’

Messaging

e.g. send out a reminder at a well selected moment using a text message or App

Reporting

Make sure that the subject does not feel embarrassed over a missed sample

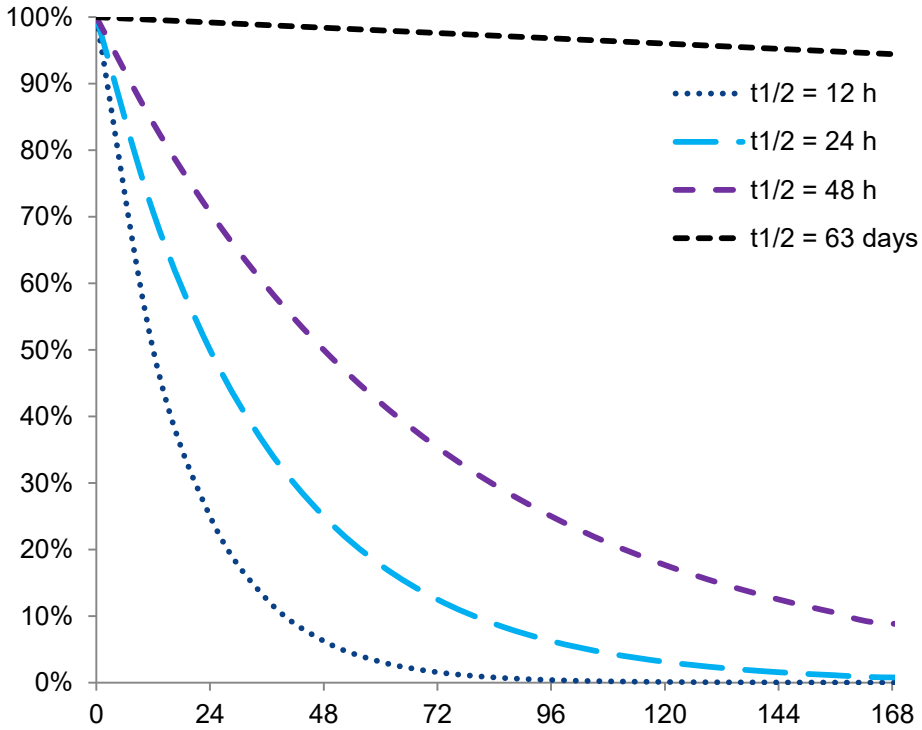
Take home

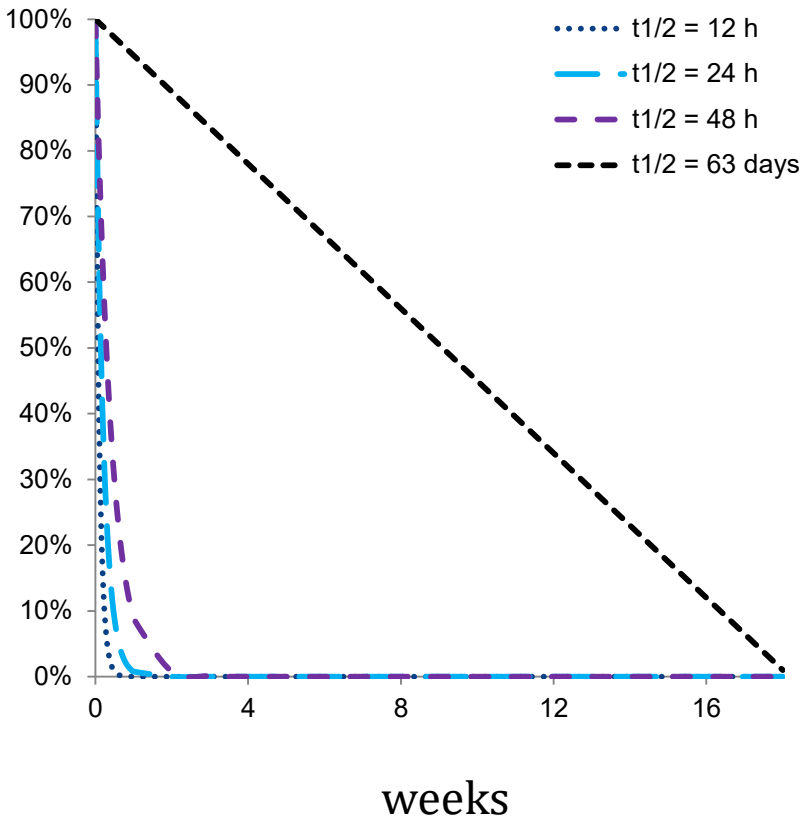
Urine is an attractive medium to study elimination of water-soluble biomarkers but it should not be underestimated for problems with sample collection and interpretation of biomarkers

Blood biomarkers

- Parent substances and metabolites
- Biomarkers are free solution or protein bound
- Partitioning with air (volatile substances) and with (target) organs and adipose tissue
- Some biomarkers may cross blood-brain barrier or blood-placenta barrier
- Some biomarkers enter blood cells and interact with proteins, RNA and/or DNA to form adducts

Free metabolites follow first order kinetics; Intracellular biomarkers follow zero order kinetics





How to define a sample

Venous blood sample

Collected by venipuncture

Capillary blood sample

Collected by finger prick

Blood

How to define blood as a biological matrix

- Well characterised buffered physiological fluid
- Complex matrix rich in proteins and lipids
- Plasma with blood platelets, erythrocytes and white blood cells
- Metabolism will continue in fresh sample
- Blood will coagulate if no anticoagulant is added

Sample Integrity

Slow down metabolism by cooling to 4°C

If blood fractionation is required the best time is within 24 h.

Lysis of cells can be caused after time, mechanical force, addition of aqua pur and freezing

Dilemmas

When should the sample be collected, regarding limited storage time of untreated full blood?

Take blood before or after a meal?

What coagulant to use?

How to ship the sample?

Pitfalls

Contamination from used sample collection materials (e.g. needle and glass in case of metals)

Forget to measure and register full blood volume (for later calculations)

Contingencies

Use special needles and blood tubes for trace metals

Place a mark of the blood level on the collection tube or (more accurate) determine blood volume gravimetrically

Choice of materials for blood sample collection

- Choice of blood tube type with proper coagulant
- Skin prick equipment for capillary blood collection
- Needles with (inner) silicone lining for trace metals
- Plastic tubes with specified low trace metal content
- Use cryo tubes for aliquoting and proper labels for -80 °C storage

Support to prevent/report missing samples

Instructions

e.g. invite the subject to sit or lie down to prevent injury in case of fainting

Take time and special care for young subjects

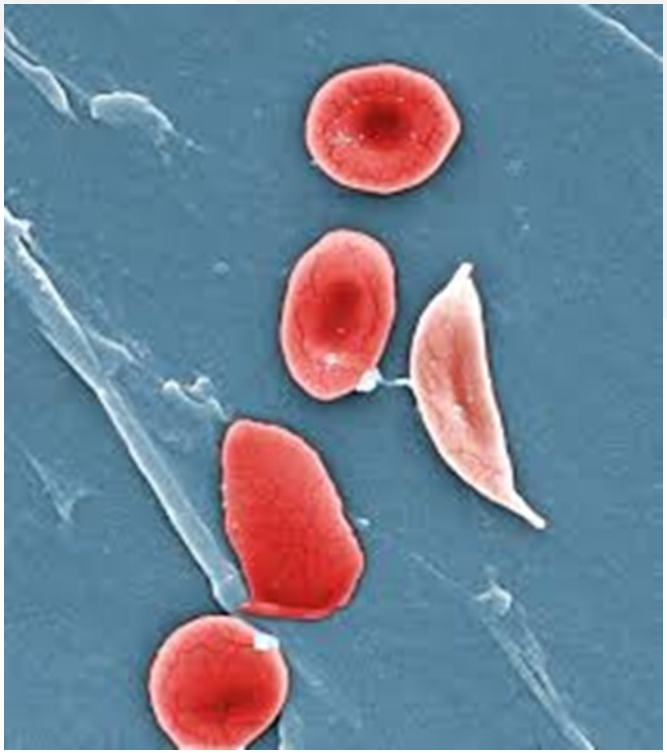
Collection procedure

e.g. involve a well-trained experienced person and follow procedures to prevent infection

Apply pressure for a couple of minutes on the puncture site to prevent hemorrhage.

Take home

Blood is a complex matrix and many precautions need to be taken during collection and pretreatment. Once analysed blood biomarkers can provide very valuable results.



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Speaker's information

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