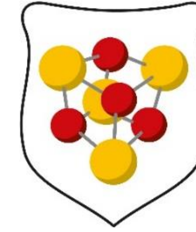


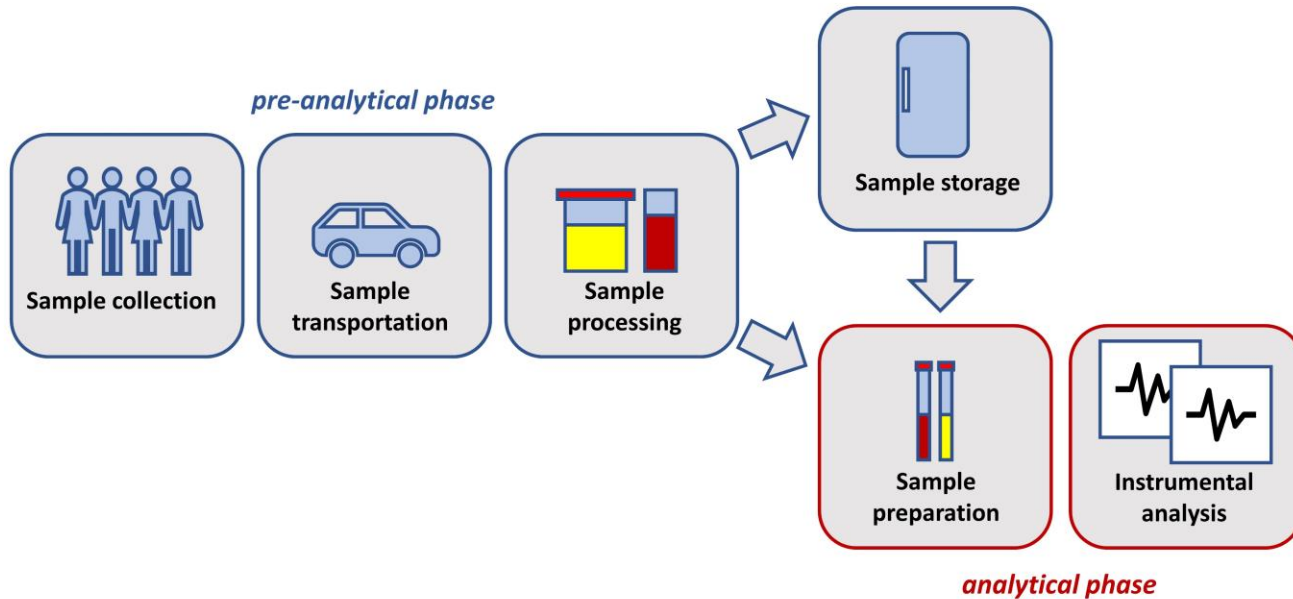

# Roundtable
# Sample collection and preparation: impact on the quality of the data

Veronica Ghini (CERM - UNIFI)

# Why SOPs are so important?

**The preservation of the chemical composition (in terms of both nature and concentration of metabolites) of the "original" metabolome of a sample during the entire workflow that ends with metabolomic analysis represents a key factor in ensuring highly accurate and reproducible results.**

# Preservation of the original metabolome




Both phases comprise several steps that may influence the composition of the sample metabolome.
Degradation occurs along all steps (that inevitably occur prior to the actual laboratory analysis, starting from sample collection, to sample stabilization, transport, and storage

- Contaminations
- Enzymatic reactions
- Redox reactions
- …

Validated and detailed procedures are required to reduce chemical changes in the metabolome as far as possible.

# Evidenced-based SOPs

Within the EU SPIDIA and SPIDIA4P projects, NMR approaches to evaluate the impact of different pre-analytical treatments on the quality of urine and blood serum and plasma samples for metabolomics were developed.

J Biomol NMR (2011) 49:231–243
DOI 10.1007/s10858-011-9489-1


ARTICLE

**Standard operating procedures for pre-analytical handling of blood and urine for metabolomic studies and biobanks**


Patrizia Bernini · Ivano Bertini · Claudio Luchinat · Paola Nincheri · Samuele Staderini ·



Metabolomics (2015) 11:872–894
DOI 10.1007/s11306-014-0746-7

REVIEW ARTICLE

**Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review**


Abdul-Hamid Emwas · Claudio Luchinat · Paola Turano · Leonardo Tenori · Raja Roy · Reza M. Salek · Danielle Ryan · Jasmeen S. Merzaban · Rima Kaddurah-Daouk · Ana Carolina Zeri · G. A. Nagana Gowda · Daniel Raftery · Yulan Wang · Lorraine Brennan · David S. Wishart



New BIOTECHNOLOGY 52 (2019) 25–34

Contents lists available at ScienceDirect
New BIOTECHNOLOGY
journal homepage: www.elsevier.com/locate/nbt

Full length Article

NMR for sample quality assessment in metabolomics


Veronica Ghini[a], Deborah Quaglio[b], Claudio Luchinat[a,c], Paola Turano[a,c,*]

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[c] Department of Chemistry, University of Florence, Sesto Fiorentino FI, Italy

# Evidenced-based SOPs

CEN
**CEN/TS 16945:2016** (MAIN)

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for metabolomics in urine, venous blood serum and plasma

BACK | 18-May-2016 | #11.100.10 | CEN/TC 140 | follow

EUR | 0 | SIGN IN | REGISTER

ABSTRACT | GERMAN | FRENCH | SLOVENIAN

This Technical Specification covers the preanalytical phase and recommends the handling, documentation and processing of urine, venous blood plasma and serum intended for metabolomics analysis. This Technical Specification is applicable to metabolomics examinations and is of importance to biomedical laboratories, customers of laboratories, in vitro diagnostics developers and manufacturers, institutions and companies performing biomedical research, biobanks, and regulatory authorities.
The adoption of the described procedures for the preanalytical phase make it possible to compare and evaluate the results obtained from metabolic profiling analysis.

BUY STANDARD

TECHNICAL SPECIFICATION

-TS CEN/TS 16945:2016 - BARVE na PDF-str 17,18
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ICS › 11 › 11.100 › 11.100.10

# ISO 23118:2021

## Molecular in vitro diagnostic examinations — Specifications for pre-examination processes in metabolomics in urine, venous blood serum and plasma

# Evidenced-based SOPs

## BLOOD SERUM / PLASMA

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of collection tube**

For serum use anticoagulant free tubes.
For plasma use tubes with anticoagulant (preferred EDTA, avoid heparin).
Avoid tubes with other additives.

**Blood collection**

Collect blood after a minimum of 8 hours fasting.
Specify, if collected at different times or under non-fasting conditions.

**Information on the sample**

Time and date of blood collection.

**Transport and storage**

Keep the whole blood sample at RT for a maximum of 30 min.

**Biomedical Lab / Biobank**

**Blood sample processing**

SERUM: allow the blood to clot in an upright position from 30-60 min at RT. Centrifugation at 1500 g for 10 min at RT.
PLASMA: centrifugation at 820 g for 10 min at 4° C.

**Long-term storage requirements**

Aliquot the processed sample into cryo-vials. Storage at < -70° C.

**Transfer**

Keep the aliquot(s) at < -70° C .

## URINE

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of containers**

Avoid containers with additives.

**Urine collection**

Collect the first midstream urine of the morning after a minimum of 8 hours fasting.
Specify, if collected at different times, or for 24 hour collection .

**Information on the sample**

Time and date of urine collection.

**Transport and storage**

Keep the whole urine sample refrigerated at 2° C to 8° C for a maximum of 2 h.

**Biomedical Lab / Biobank**

**Urine sample processing**

Centrifugation (1000 g to 3000 g for 5 min at 2° C to 8° C) followed by filtration (0,20 um cut-off filter).

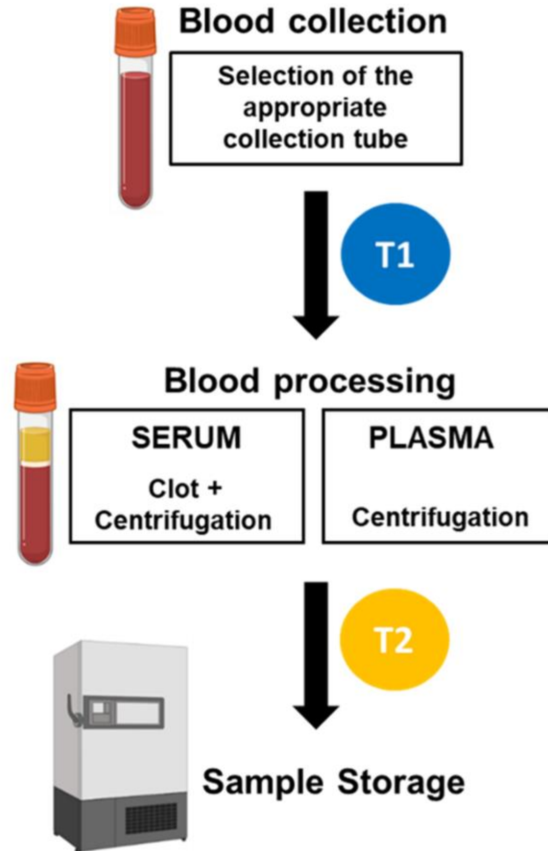
**Long-term storage requirements**

Aliquot the processed sample into cryo-vials. Storage at < -70° C.

**Transfer**

Keep the aliquot(s) at < -70° C .

# Blood- Critical steps along the preanalytical phase



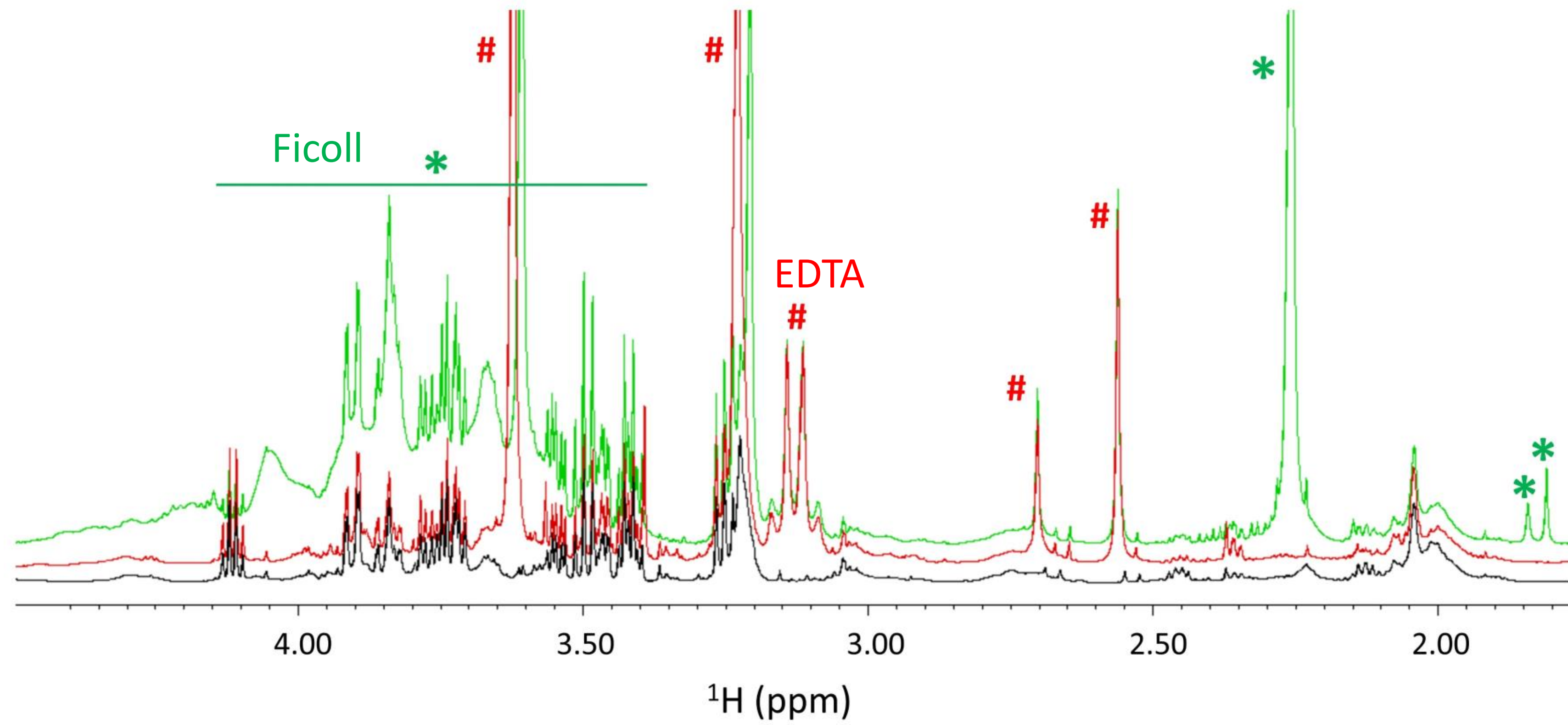

**Factors influencing the stability of blood serum/plasma profiles over time:**

Presence of cells

Oxidation reactions

# Selection of the appropriate collection tube

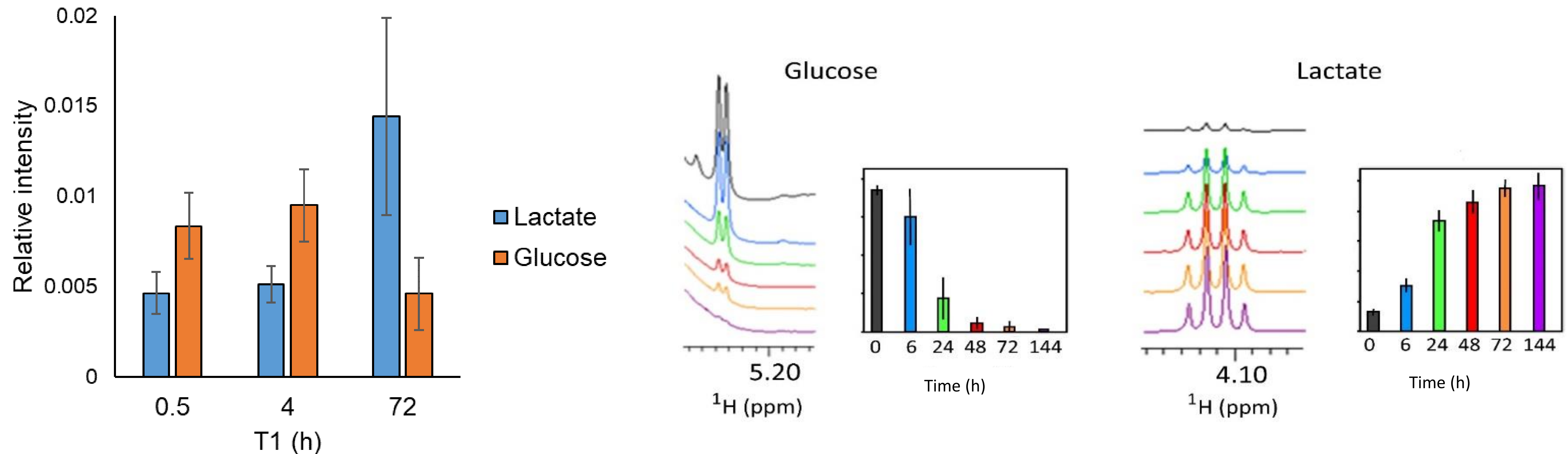
Avoid additives that could make difficult metabolite observation



# Presence of cells. Critical step-T1

**Cellular activity- main source of changes.**

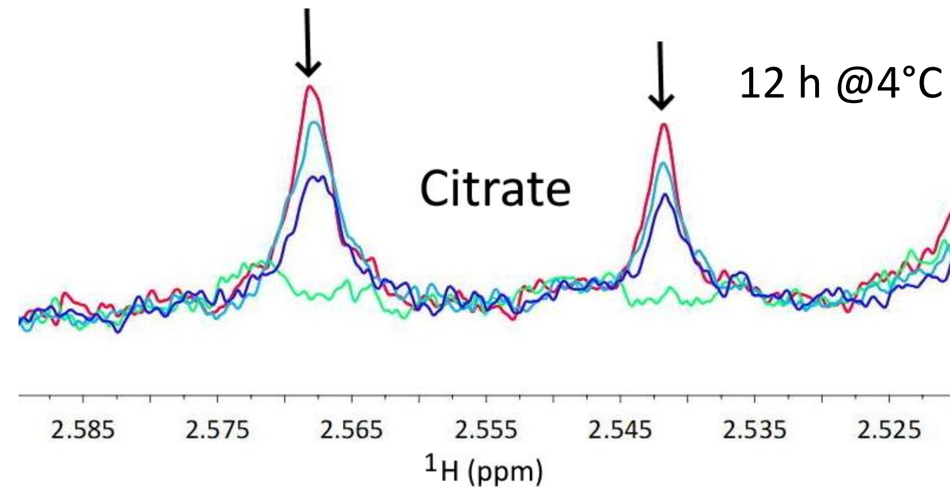
The processing should be initiated within 30 min after the blood collection in order to minimize these alterations.




V. Ghini, et al. N Biotechnol. NMR for sample quality assessment in metabolomics 2019;52:25-34. doi: 10.1016/j.nbt.2019.04.004.

# Oxidation reactions. Critical step-T2

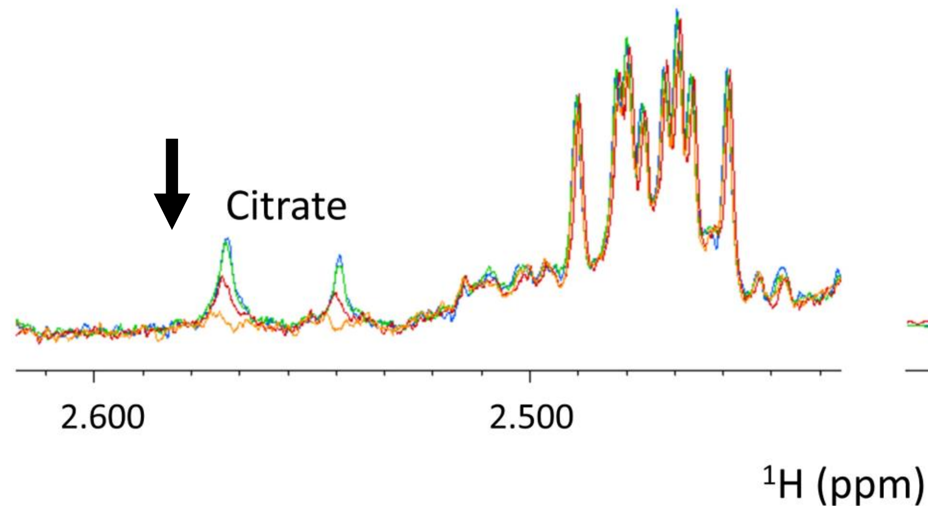
After blood processing, the sources of variation are attributable to **oxidation reactions** occurring under aerobic conditions that cause concentration changes of metabolites such as proline, citrate and lipoproteins

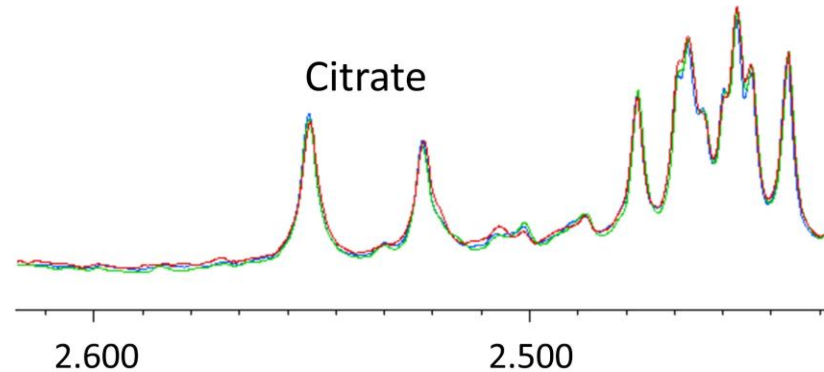



Serum sample maintained at 4°C for 0, 4, 8 and 12 h after processing.
Freezing at -80°C immediately after serum obtainment is sufficient to quench this effect.

V. Ghini, et al. N Biotechnol. NMR for sample quality assessment in metabolomics 2019;52:25-34. doi: 10.1016/j.nbt.2019.04.004.

# Long term storage at -80° C



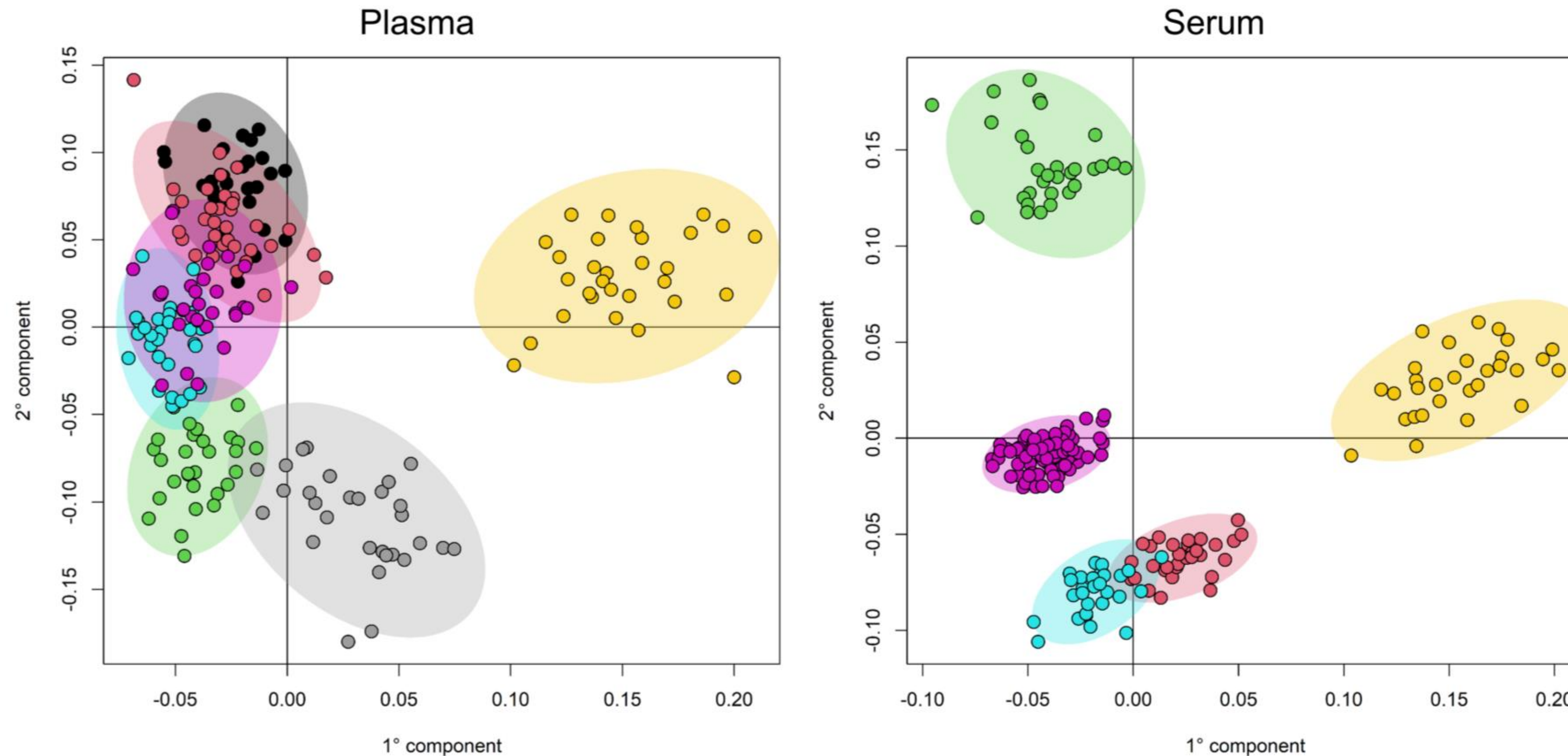




T0; 4h; 8h; 12 h

The metabolic phenotype of urine samples was followed over 5-year storage. T0; 4 year-storage; 5 year-storage

V. Ghini, et al. N Biotechnol. NMR for sample quality assessment in metabolomics 2019;52:25-34. doi: 10.1016/j.nbt.2019.04.004.

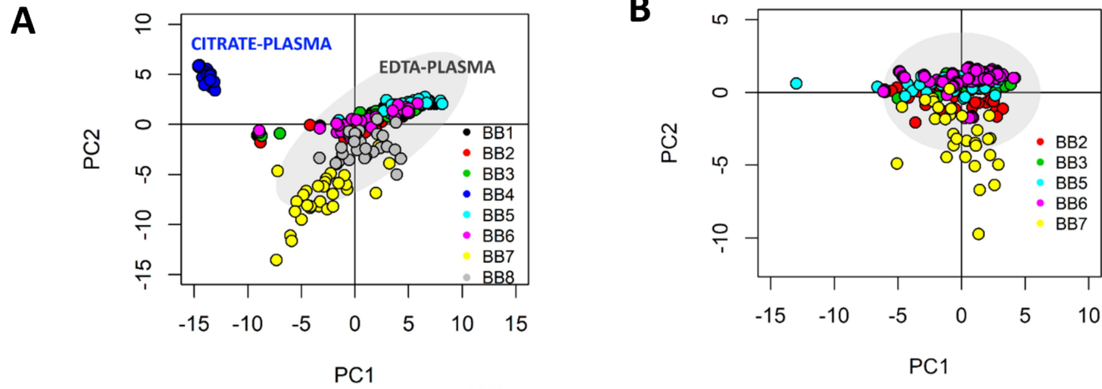
# The problem of multicenter studies: plasma and serum from different biobanks



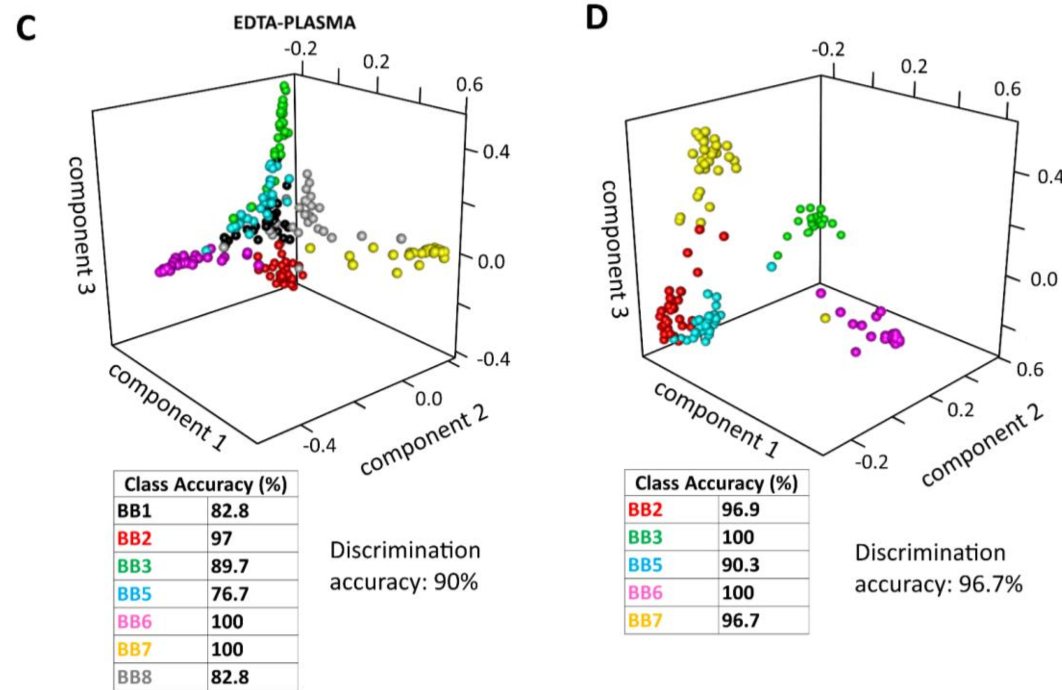

Ghini V., Abuja P.M., Polasek O., Kozera L., Laiho P., Anton G., et al. Impact of the pre-examination phase on multicenter metabolomic studies. 2022, N Biotechnol, 68, 37-47.

Ghini V., Abuja P.M., Polasek O., Kozera L., Laiho P., Anton G., et al. Metabolomic fingerprints in large population cohorts: Impact of pre- analytical heterogeneity. 2021, Clin Chem, 67(8), 1153–1155.

# Multivariate analysis-PCA




# Multivariate analysis-RF



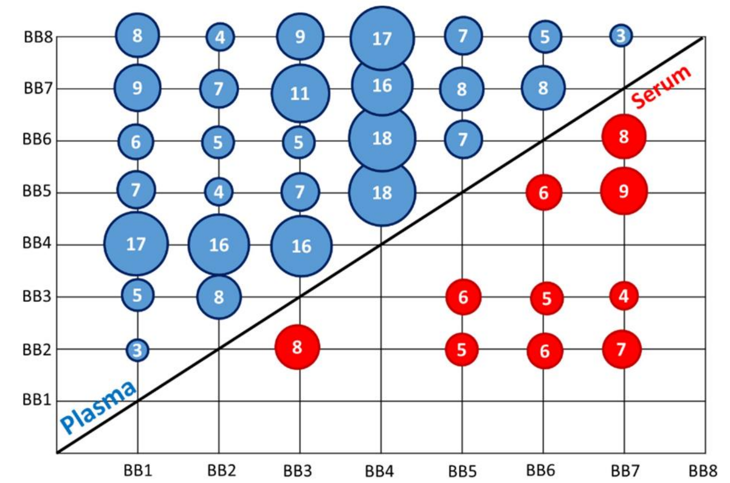

| Class Accuracy (%) | |
|---|---|
| BB1 | 82.8 |
| BB2 | 97 |
| BB3 | 89.7 |
| BB5 | 76.7 |
| BB6 | 100 |
| BB7 | 100 |
| BB8 | 82.8 |

Discrimination accuracy: 90%

| Class Accuracy (%) | |
|---|---|
| BB2 | 96.9 |
| BB3 | 100 |
| BB5 | 90.3 |
| BB6 | 100 |
| BB7 | 96.7 |

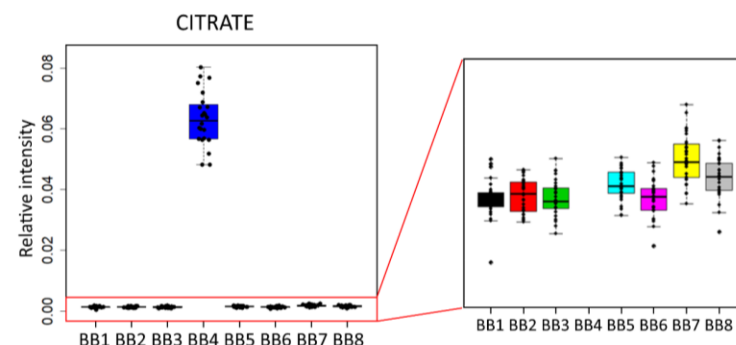
Discrimination accuracy: 96.7%

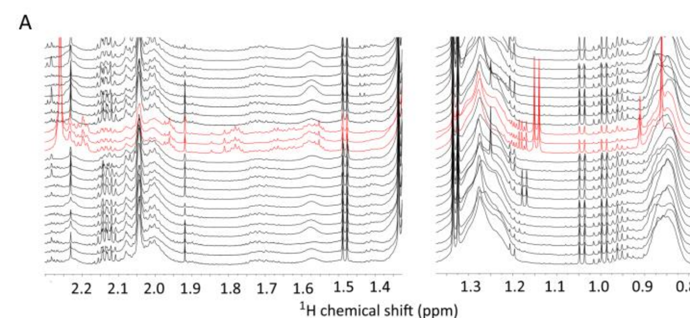
# Univariate analysis

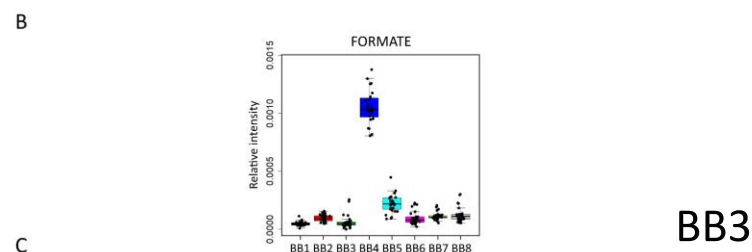


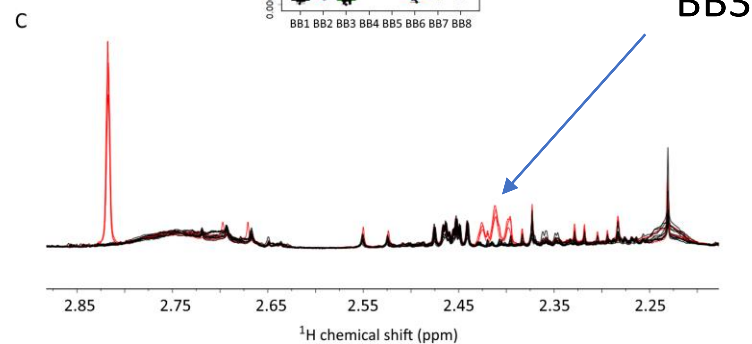
# Collection tubes in the SOPs of different biobanks

| Biobank | Serum | Plasma |
|---|---|---|
| CEN/TS 16945:2016 / ISO 23118:2021 | **Anticoagulant free tubes with no other additives** | **Anticoagulant tubes with no other additives** |
| BB1 | - | EDTA-tubes (brand not specified) |
| BB2 | Tubes with clot activator (brand not specified) | EDTA-tubes (brand not specified) |
| BB3 | Tubes with separation gel and clotting activator (VACUETTE) | EDTA-tubes (VACUETTE) |
| BB4 | - | Cell preparation tube with sodium citrate (BD) |
| BB5 | Tubes with separation gel and clotting activator (SARSTED) | EDTA-tubes (SARSTED) |
| BB6 | Tubes with separation gel and clotting activator (BD) | EDTA-K3 tubes (BD) |
| BB7 | Tubes with separation gel and clotting activator (BD) | EDTA tubes (BD) |
| BB8 | - | K2 EDTA tubes (brand not specified) |



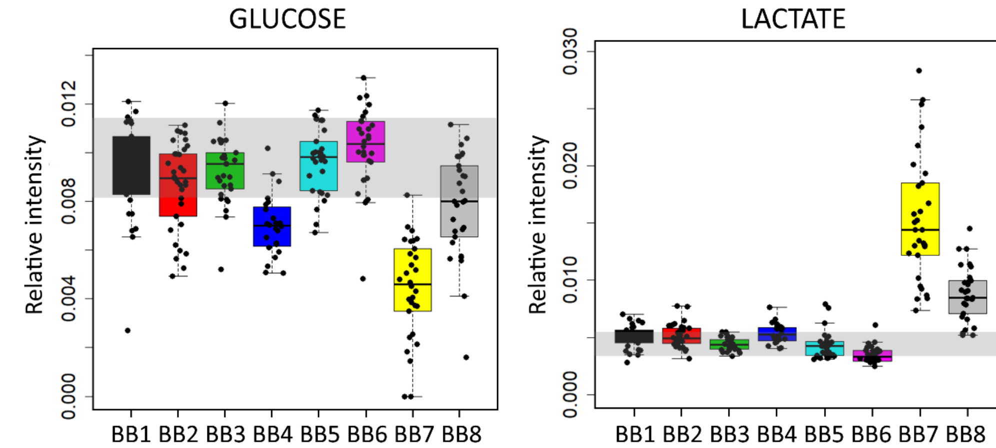



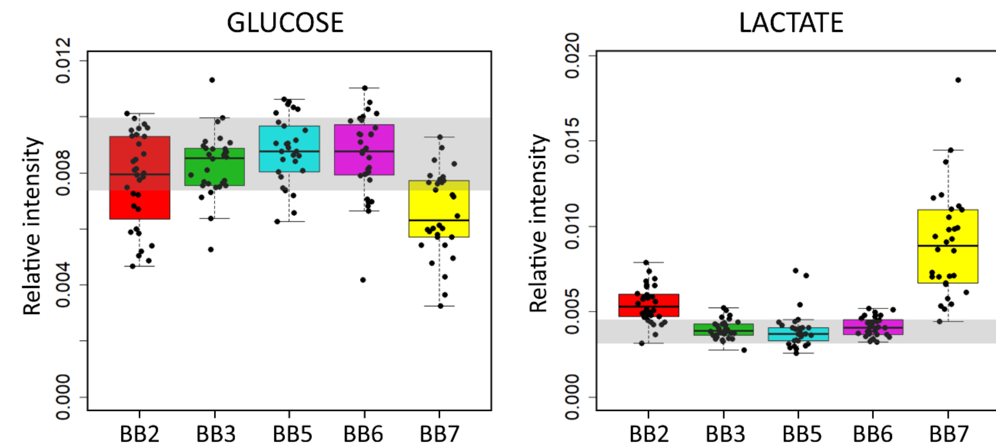
# T1 in the SOPs of different biobanks

| Biobank | T1 |
|---|---|
| CEN/TS 16945:2016 / ISO 23118:2021 | Max. of 30 min at RT |
| BB1 | Max. of 30 min (T not specified) |
| BB2 | Max. of 4 h (T not specified) |
| BB3 | Max. of 60 min at RT |
| BB4 | Max. of 120 min at RT |
| BB5 | Max. of 30 min at RT |
| BB6 | Max. of 2 h (T not specified) |
| BB7 | Max. of **72 h** at 4°C |
| BB8 | Max. of **72 h** at 4°C |

Plasma



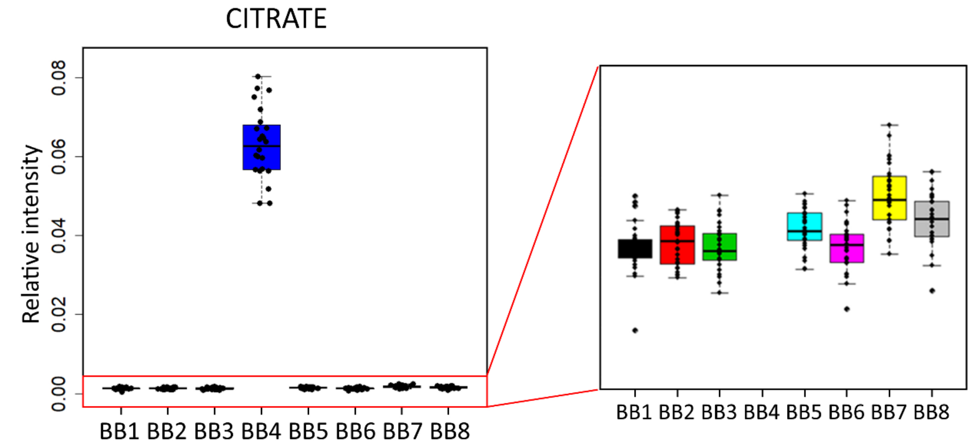

Serum



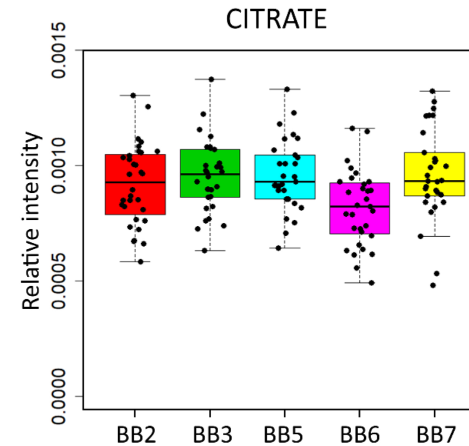
# T2 in the SOPs of different biobanks

| Biobank | T2 |
|---|---|
| CEN/TS 16945:2016 / ISO 23118:2021 | Immediate freezing |
| BB1 | Not specified |
| BB2 | Not specified |
| BB3 | Not specified |
| BB4 | Not specified |
| BB5 | Put the tubes in ice water |
| BB6 | dry ice at -80° C |
| BB7 | Not specified |
| BB8 | storage at +4 – 8° C then transfer to the storage straws within 2h |

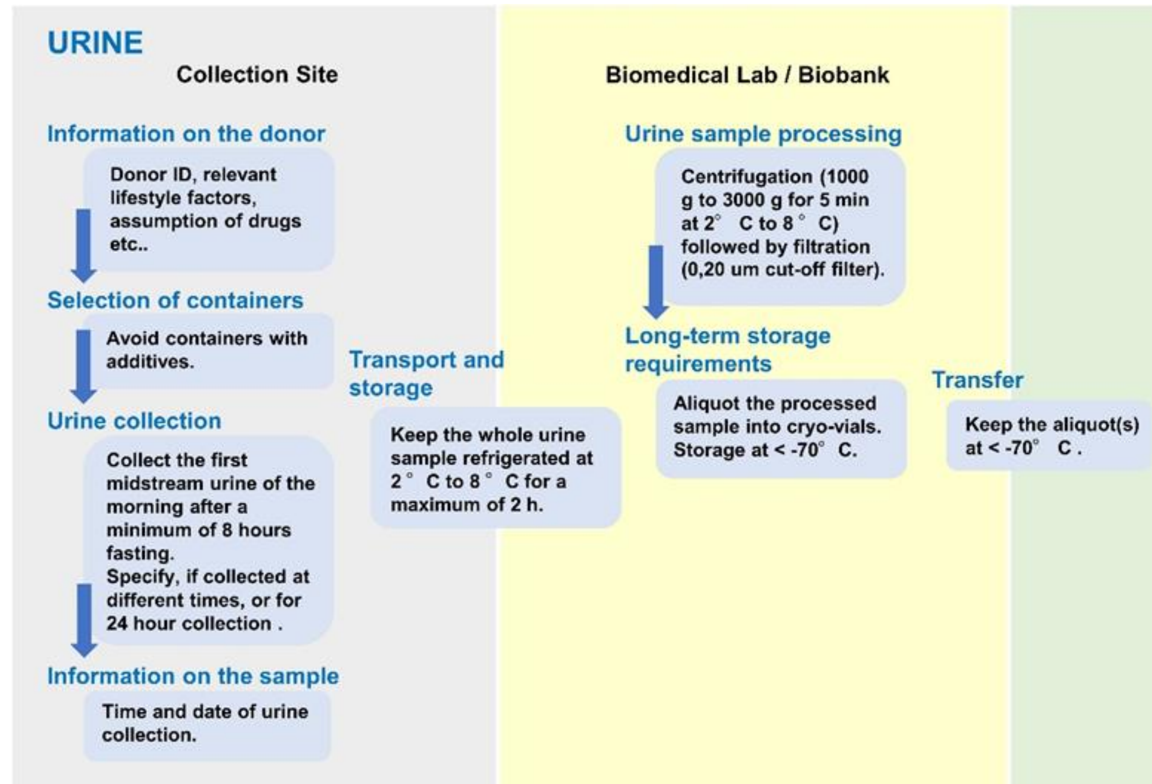
Plasma




Serum



# URINE SAMPLES



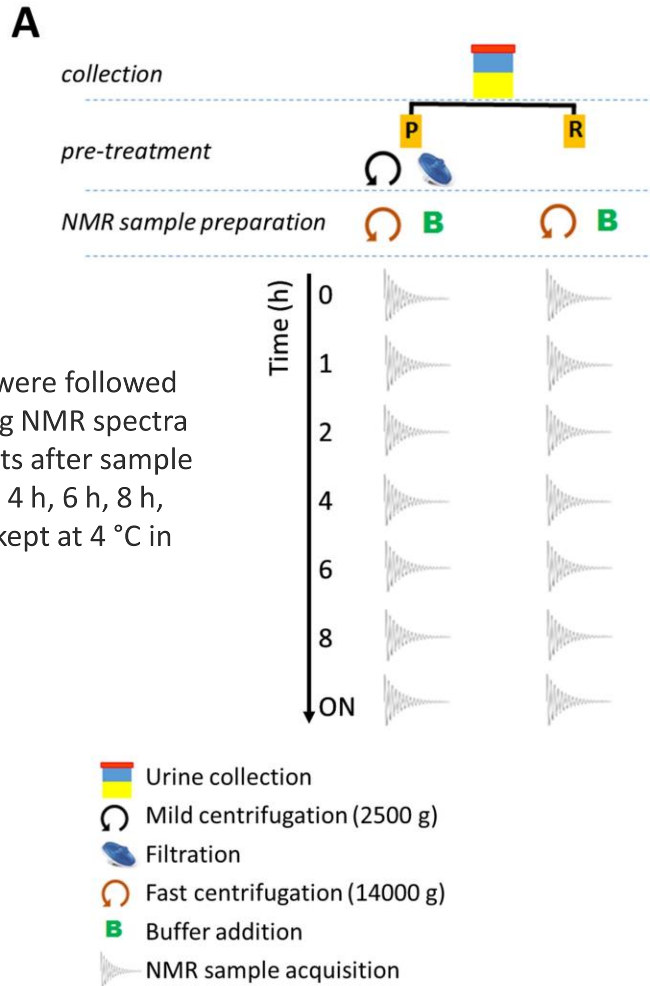

## Factors influencing the stability of urine profiles over time:

Presence of cells:
→ shift of signals over time

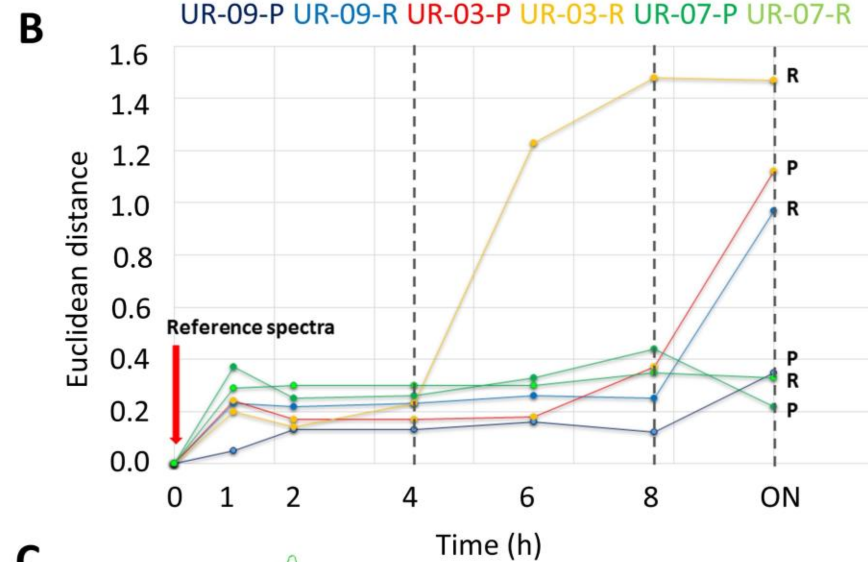
Chemical and enzymatic reactions
→ concentration changes over time

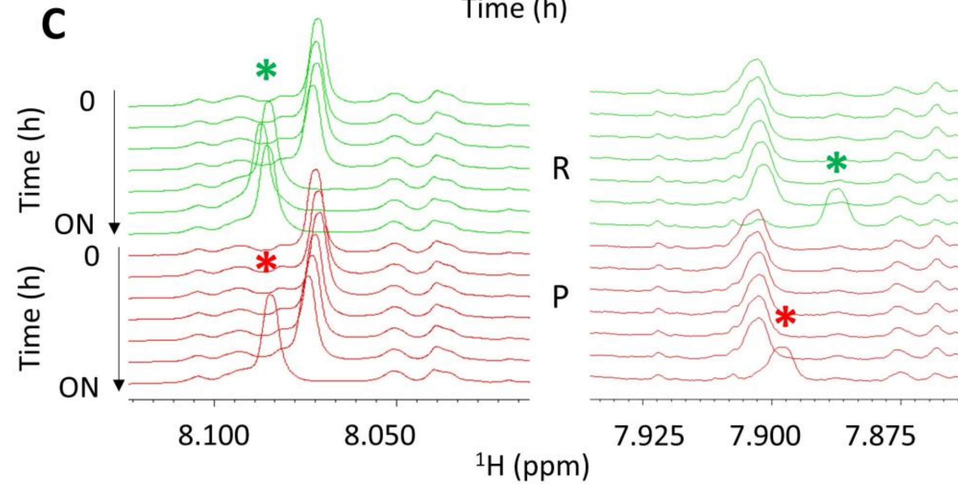
V. Ghini, et al. N Biotechnol. NMR for sample quality assessment in metabolomics 2019;52:25-34. doi: 10.1016/j.nbt.2019.04.004.

# The presence of cells




The profiles of urine were followed over time by acquiring NMR spectra at different time points after sample collection (0, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h). Samples were kept at 4 °C in between spectra.



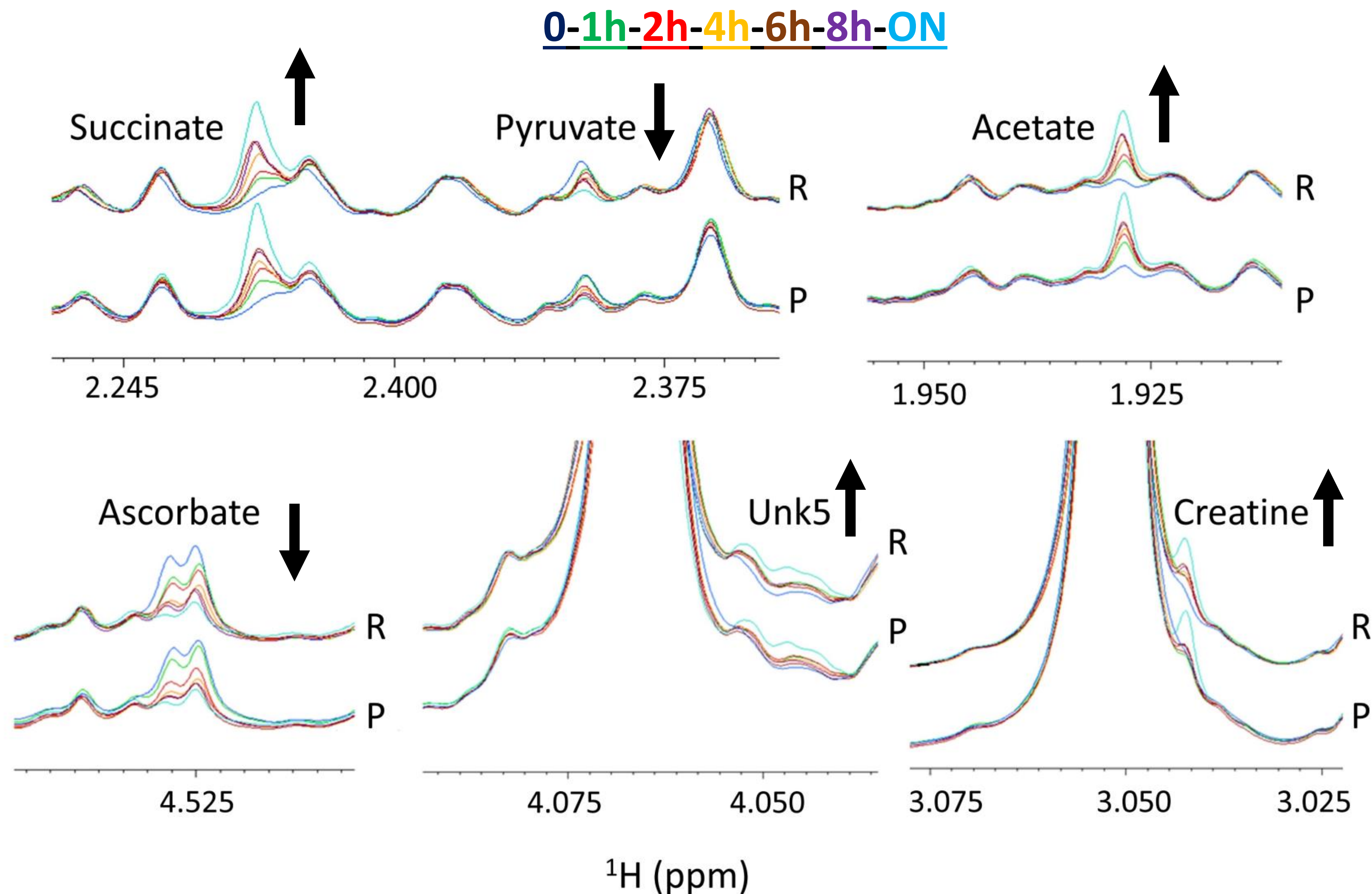

**UR-03**: very high cellular contents
**UR-09**: modest
**UR-07**: low

**Mild centrifugation and/or filtration are strongly suggested to remove cells from urine**

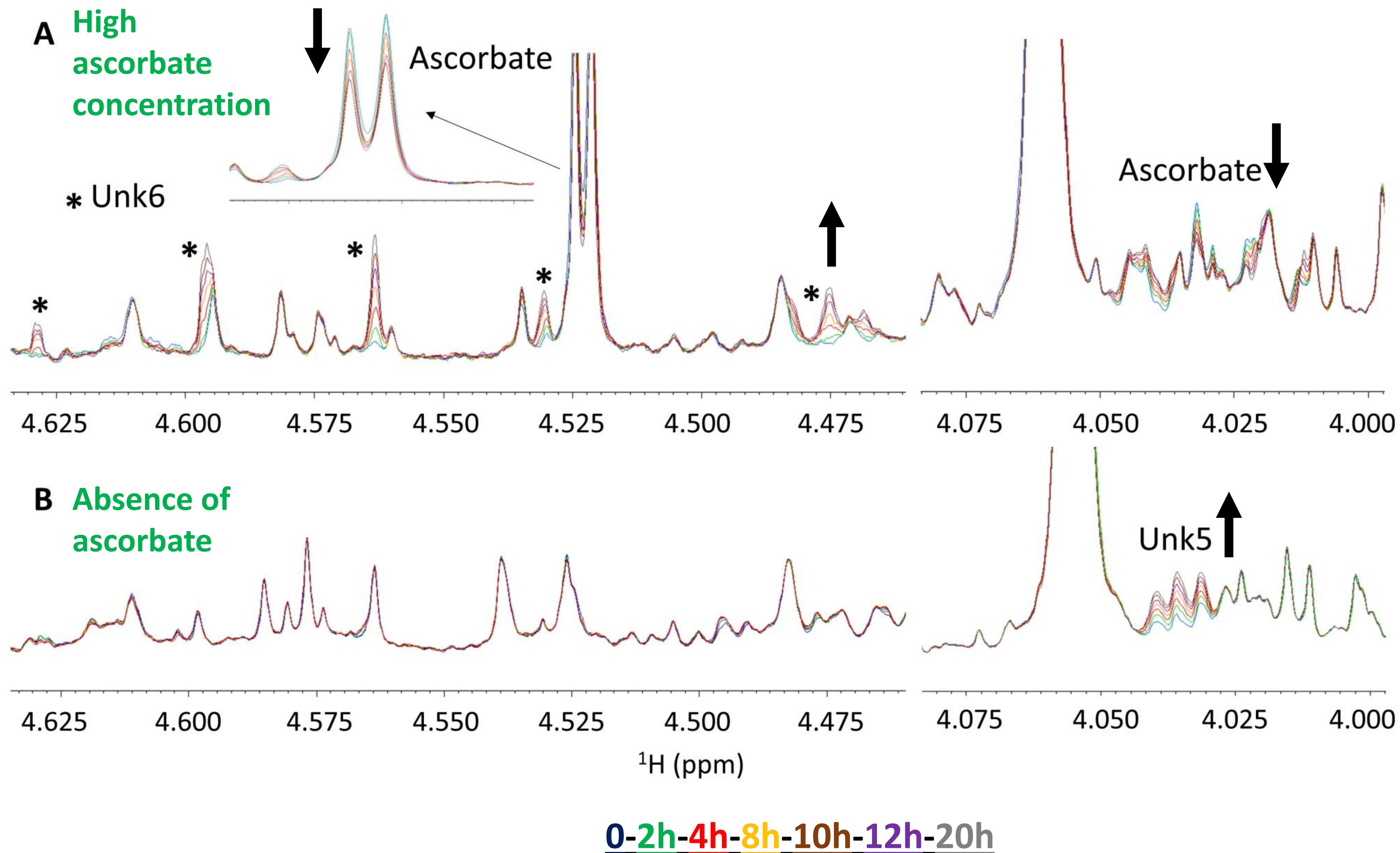
# Chemical and enzymatic reactions

*The metabolic profiles of 6 urine samples were followed for 24 h, acquiring an NMR spectrum every 2h; samples were kept at 4° C in between spectra*

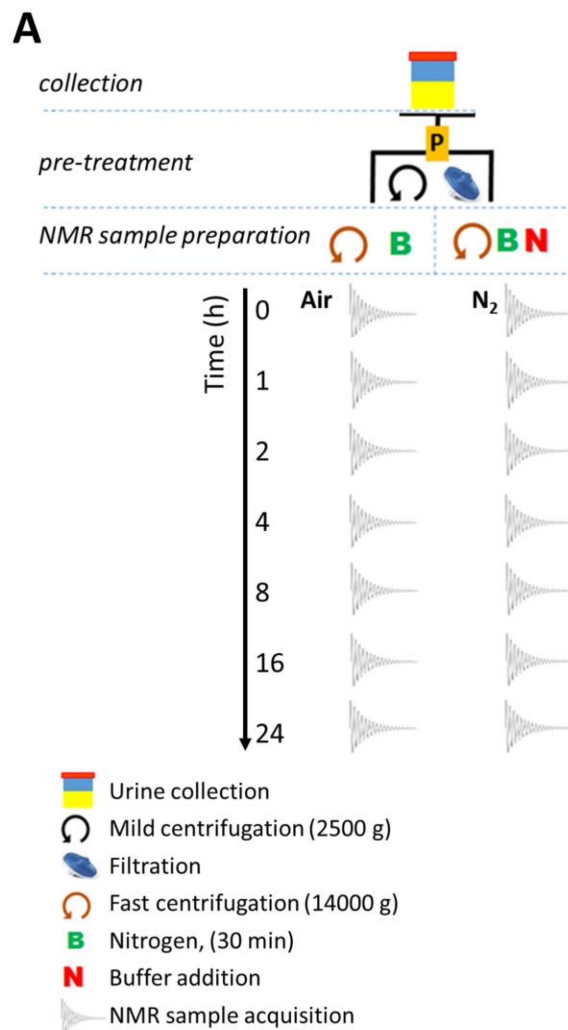
| Metabolite | HMDB | Occurrence (%) presence/absence in the spectra | Chemical shift (ppm) | Changes |
|---|---|---|---|---|
| **Acetate** | HMDB0000042 | 100 | 1.92 (s) | ↑ |
| **Unk2** | - | 70 | 2.17 (s) | ↑ |
| **Unk3** | - | 70 | 2.18 (s) | ↓ |
| **Pyruvate** | HMDB0000243 | 100 | 2.38 (s) | ↓ |
| **Succinate** | HMDB0000254 | 100 | 2.40 (s) | ↑ |
| **2-Oxoglutarate** | - | 100 | 2.44 (t)<br>3.00 (t) | ↓ |
| **Creatine** | HMDB0000064 | 100 | 3.04 (s)<br>3.93 (s) | ↑ |
| **Creatinine** | HMDB0000562 | 100 | 3.05 (s)<br>4.06 (s) | ↓ |
| **Unk4** | | -* | 3.35 (m) | ↑ |
| **Ascorbate** | HMDB0000044 | 14 | 3.74 (m)<br>4.01 (m)<br>4.51 (d) | ↓ |
| **Unk5** | | -* | 4.05 (t) | ↑ |
| **Unk6** ***ascorbic acid oxidation by-products*** | - | -* | 4.48 (t)<br>4.53 (s)<br>4.56 (s)<br>4.59 (s)<br>4.63 (s) | ↑ |
| **Unk7** | - | 100 | 5.61 (d) | ↓ |
| **Urea** | HMDB0000294 | 100 | 5.80 (s) | ↓ |



*The patterns of changes correlated with the presence or absence of or absence of certain urinary metabolites. In particular, the presence/absence of ascorbate strongly influenced the changes overtime*



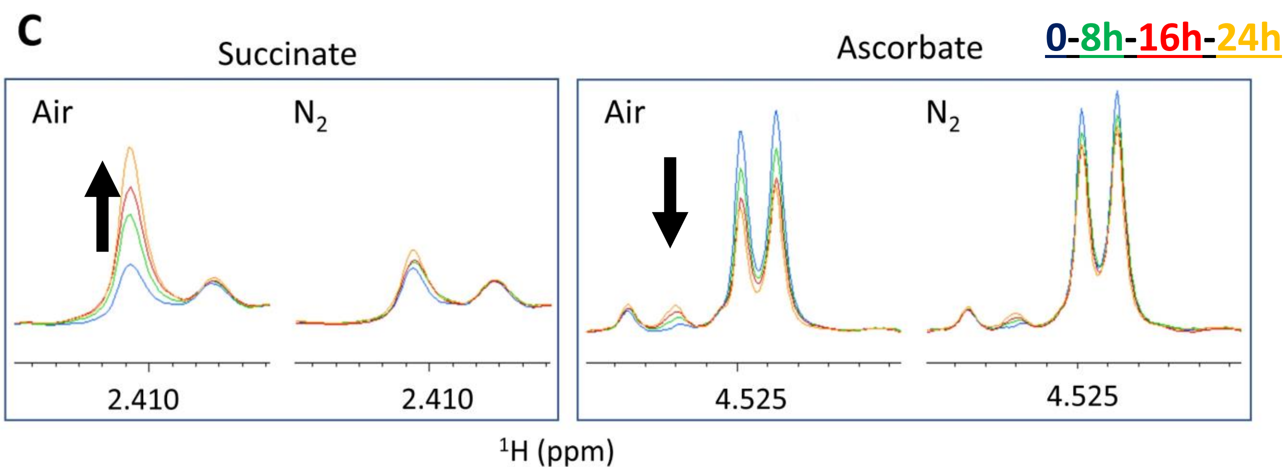
*To investigate the nature of these changes, variations in the urine metabolome of 4 subjects were followed over time both under aerobic atmosphere and under an **inert atmosphere ($N_2$)**. During the intervals between experiments, the samples were kept at 4 °C and NMR spectra for each sample were acquired every 2 h for a total of 24 h.*




**B**

| Metabolites | | Air (mean) | $N_2$(mean) |
|---|---|---|---|
| Acetate | ↑ | 19.15% | 7.9%* |
| Unk2 | ↑ | 7.6% | 7.5% |
| Unk3 | ↓ | 5.9% | 6.4% |
| Pyruvate | ↓ | 13.6% | 2.8%** |
| Succinate | ↑ | 64.6% | 15.9%* |
| 2-oxoglutarate | ↓ | 7.5% | 1.5%* |
| Creatine | ↑ | 20.5% | 17.2% |
| Creatinine | ↓ | 7.0% | 7.0% |
| Unk4 | ↑ | 13.4% | 8.3%* |
| Acorabate | ↓ | 11.47% | 4.3%* |
| Urea | ↓ | 5.0% | 0.5%* |
| Unk5 | ↑ | 33.3% | 29.7% |
| Unk6 | ↑ | 49.0% | 33.3%* |
| Unk7 | ↓ | 17.3% | 16.7% |

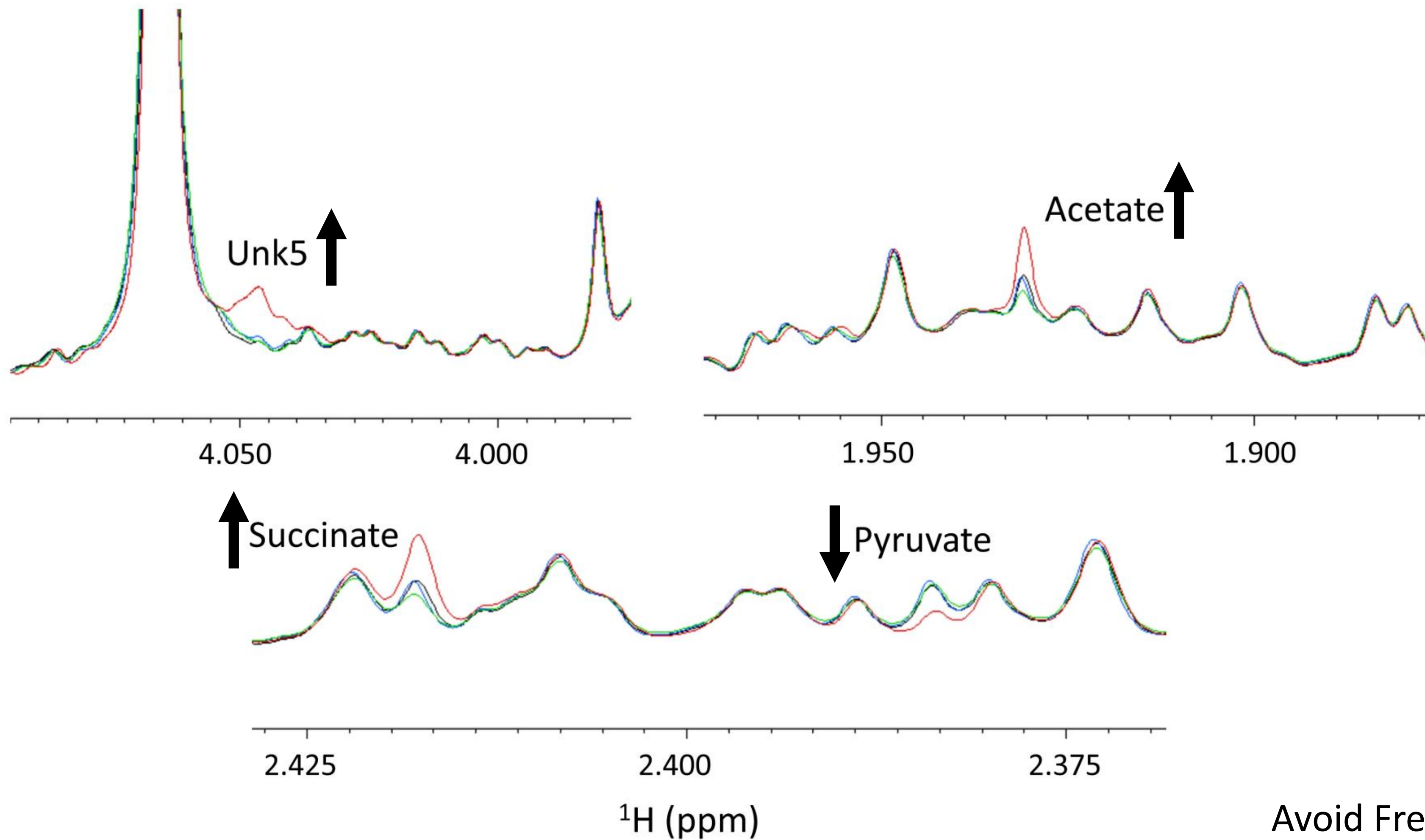
<u>Under $N_2$, the urine metabolic profiles were more stable over time.</u>




**Chemical and enzymatic redox reactions** are the main factors inducing changes in concentration levels of urinary metabolites. These effects cannot be avoided but can be reduced by <u>maintaining the samples at low temperatures</u> throughout the analytical and pre-analytical processes and reducing exposure to air.

| Reaction | Midpoint Reduction Potential at pH 7 ($E_{m,7}$) |
|---|---|
| Acetate + $CO_2$ → Pyruvate | -0.7 V |
| Succinate + $CO_2$ + $2H^+$ → 2-Oxoglutarate + $H_2O$ | -0.6 V |
| Dehydroascorbic acid → Ascorbic acid | +0.06 V |

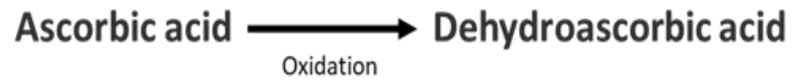
# Long-term storage at -80

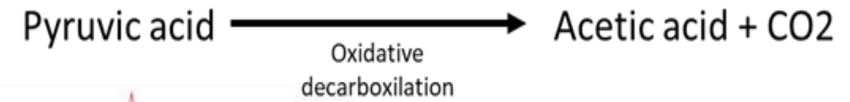


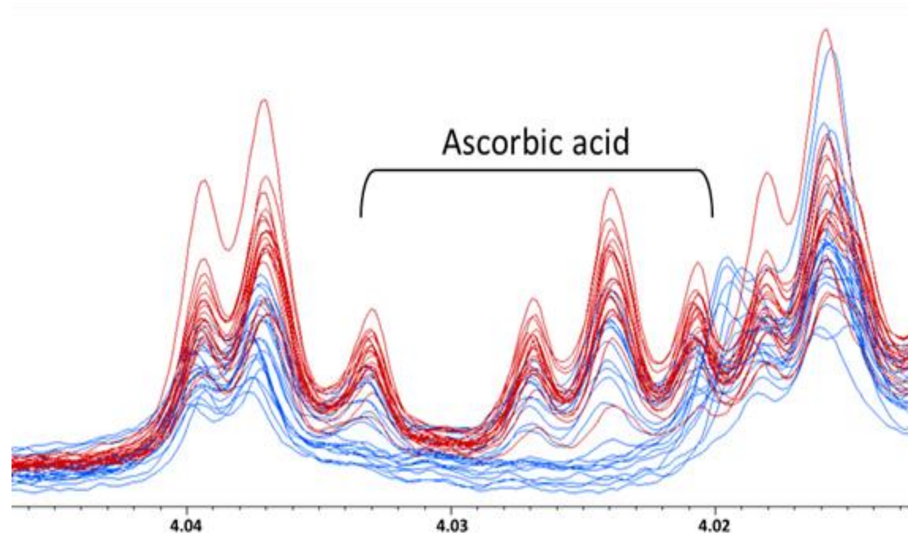

Avoid Free/thaw cycles

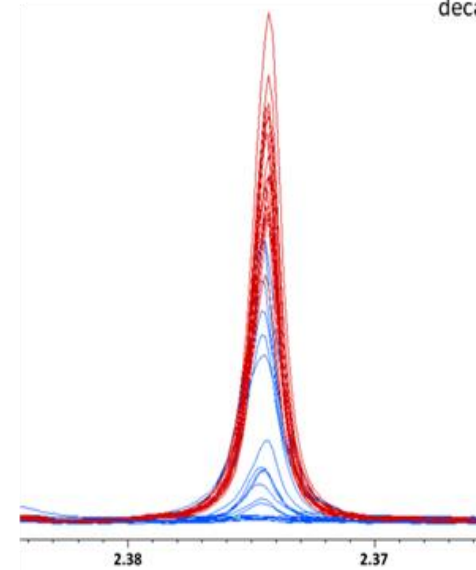
The metabolic phenotype of urine samples was followed over 5-year storage. **T0**; **4 year-storage**; **5 year-storage**; **5 year-storage + 48 h at 4–6 °C**

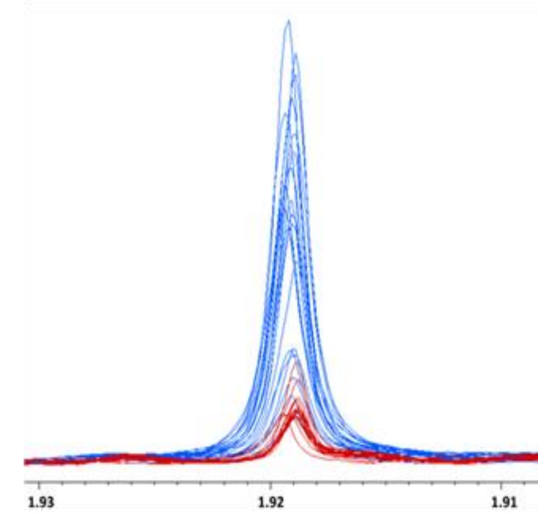
# CSF - same reporters as in urine




$^{1}$H ppm

Freezing > 1 h from collection
Freezing within 1 h from collection

Vignoli et al., J Proteome Res 19:4, 1696–1705 (2020)

# Information on the donor

## BLOOD SERUM / PLASMA

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of collection tube**

For serum use anticoagulant free tubes.
For plasma use tubes with anticoagulant (preferred EDTA, avoid heparin).
Avoid tubes with other additives.

**Blood collection**

Collect blood after a minimum of 8 hours fasting.
Specify, if collected at different times or under non-fasting conditions.

**Information on the sample**

Time and date of blood collection.

**Transport and storage**

Keep the whole blood sample at RT for a maximum of 30 min.

**Biomedical Lab / Biobank**

**Blood sample processing**

SERUM: allow the blood to clot in an upright position from 30-60 min at RT. Centrifugation at 1500 g for 10 min at RT.
PLASMA: centrifugation at 820 g for 10 min at 4° C.

**Long-term storage requirements**

Aliquot the processed sample into cryo-vials. Storage at < -70° C.

**Transfer**

Keep the aliquot(s) at < -70° C .

## URINE

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of containers**

Avoid containers with additives.

**Urine collection**

Collect the first midstream urine of the morning after a minimum of 8 hours fasting.
Specify, if collected at different times, or for 24 hour collection .

**Information on the sample**

Time and date of urine collection.

**Transport and storage**

Keep the whole urine sample refrigerated at 2° C to 8° C for a maximum of 2 h.

**Biomedical Lab / Biobank**

**Urine sample processing**

Centrifugation (1000 g to 3000 g for 5 min at 2° C to 8° C) followed by filtration (0,20 um cut-off filter).

**Long-term storage requirements**

Aliquot the processed sample into cryo-vials. Storage at < -70° C.

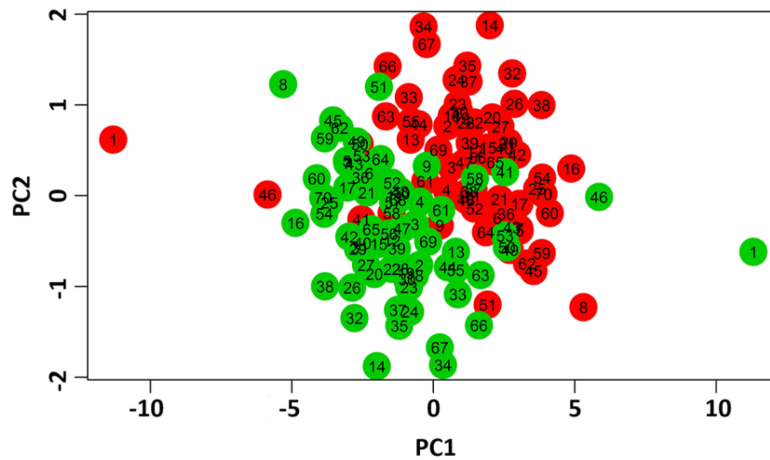
**Transfer**

Keep the aliquot(s) at < -70° C .

# The effect of anesthesia

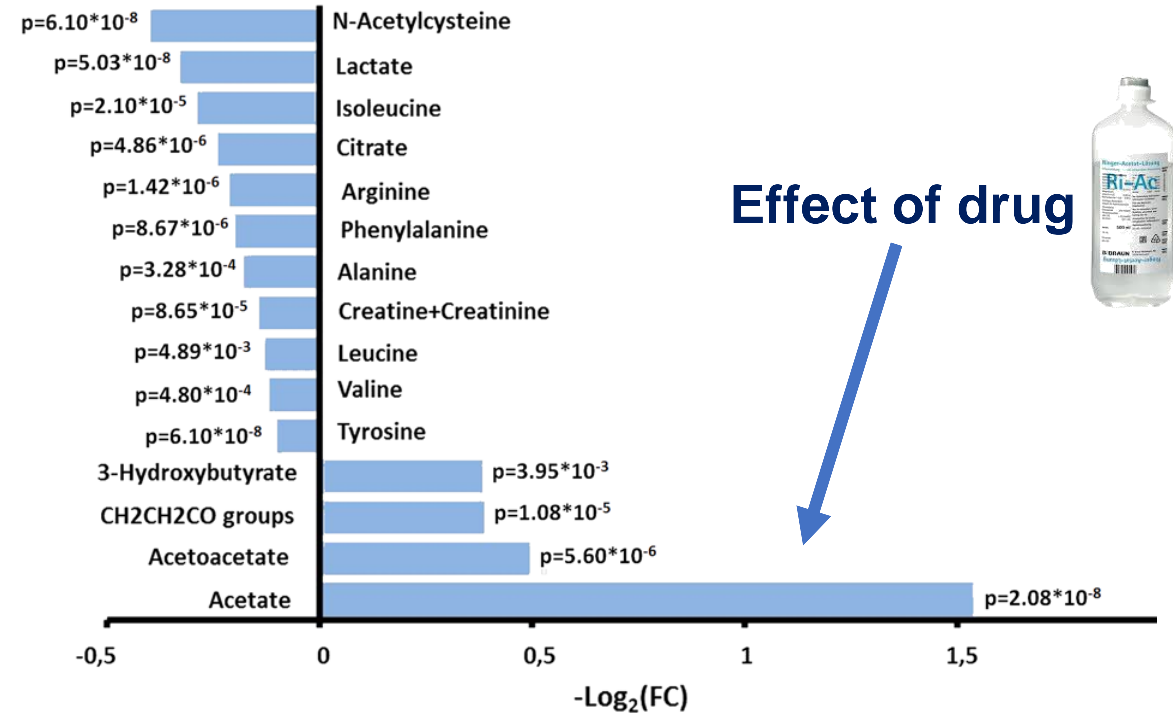
- Avoid anesthesia
- Annotation of drugs administered to the patient

**Pre- & post- anesthesia blood**




**Discrimination accuracy 92.60%**

| Confusion matrix | Pre | Post |
|---|---|---|
| Pre | *92.6 | 7.4 |
| Post | 7.4 | **92.6 |



Ghini V, Unger FT, Tenori L, Turano P, Juhl H, David KA. Metabolomics. 2015;11(6):1769-1778.

# Information on the sample

## BLOOD SERUM / PLASMA

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of collection tube**

For serum use anticoagulant free tubes.
For plasma use tubes with anticoagulant (preferred EDTA, avoid heparin).
Avoid tubes with other additives.

**Blood collection**

Collect blood after a minimum of 8 hours fasting.
Specify, if collected at different times or under non-fasting conditions.

**Information on the sample**

Time and date of blood collection.

**Transport and storage**

Keep the whole blood sample at RT for a maximum of 30 min.

**Biomedical Lab / Biobank**

**Blood sample processing**

SERUM: allow the blood to clot in an upright position from 30-60 min at RT. Centrifugation at 1500 g for 10 min at RT.
PLASMA: centrifugation at 820 g for 10 min at 4° C.

**Long-term storage requirements**

Aliquot the processed sample into cryo-vials. Storage at < -70° C.

**Transfer**

Keep the aliquot(s) at < -70° C .

## URINE

**Collection Site**

**Information on the donor**

Donor ID, relevant lifestyle factors, assumption of drugs etc..

**Selection of containers**

Avoid containers with additives.

**Urine collection**

Collect the first midstream urine of the morning after a minimum of 8 hours fasting.
Specify, if collected at different times, or for 24 hour collection .

**Information on the sample**

Time and date of urine collection.

**Transport and storage**

Keep the whole urine sample refrigerated at 2° C to 8° C for a maximum of 2 h.

**Biomedical Lab / Biobank**

**Urine sample processing**

Centrifugation (1000 g to 3000 g for 5 min at 2° C to 8° C) followed by filtration (0,20 um cut-off filter).

**Long-term storage requirements**

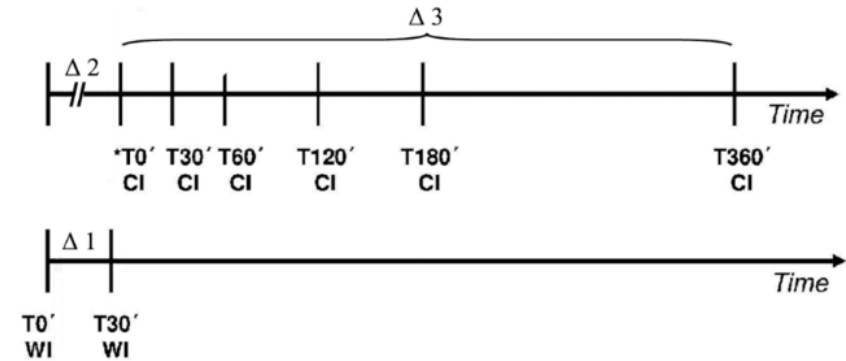
Aliquot the processed sample into cryo-vials. Storage at < -70° C.

**Transfer**

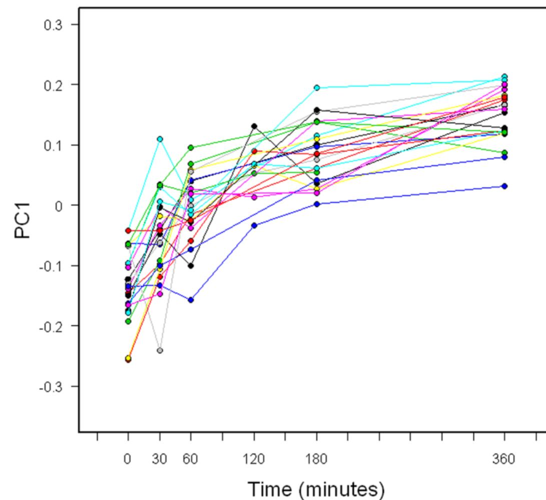
Keep the aliquot(s) at < -70° C .

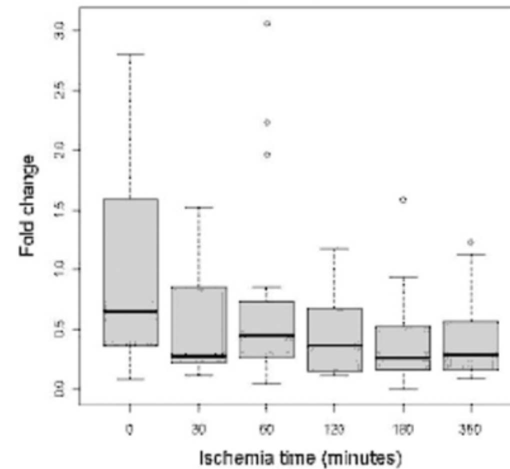
# Human Liver: Cold and Warm Ischemia

STUDY DESIGN




Individual NMR profile changes during CI




Mean intensity changes of metabolites during CI

- Annotation of the ischimia time

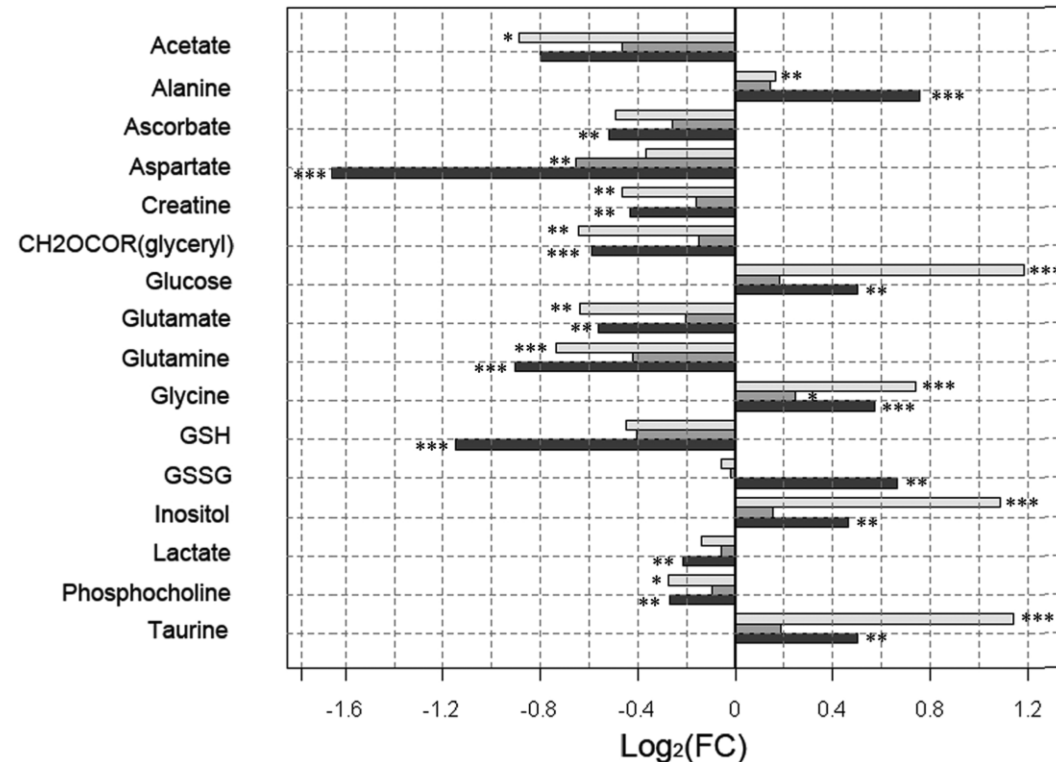
Cacciatore S, Hu X, Viertler C, Kap M, Bernhardt GA, Mischinger HJ, Riegman P, Zatloukal K, Luchinat C, Turano P. J Proteome Res. 2013;12:5723-9.

# GSH/Gln consumption

- Oxidative stress associated with ischemia was the likely cause for the consumption of the antioxidant GSH (and the concomitant production of GSSG).

- Consumption of glutamine, a GSH precursor, was also observed.

- GSH consumption was much more prominent at prolonged CI times compared to T30'WI, suggesting that in CI non-enzymatic reactions, such as those in which GSH acts as a reactive-oxygen-species scavenger, may be dominant over enzymatic reactions.



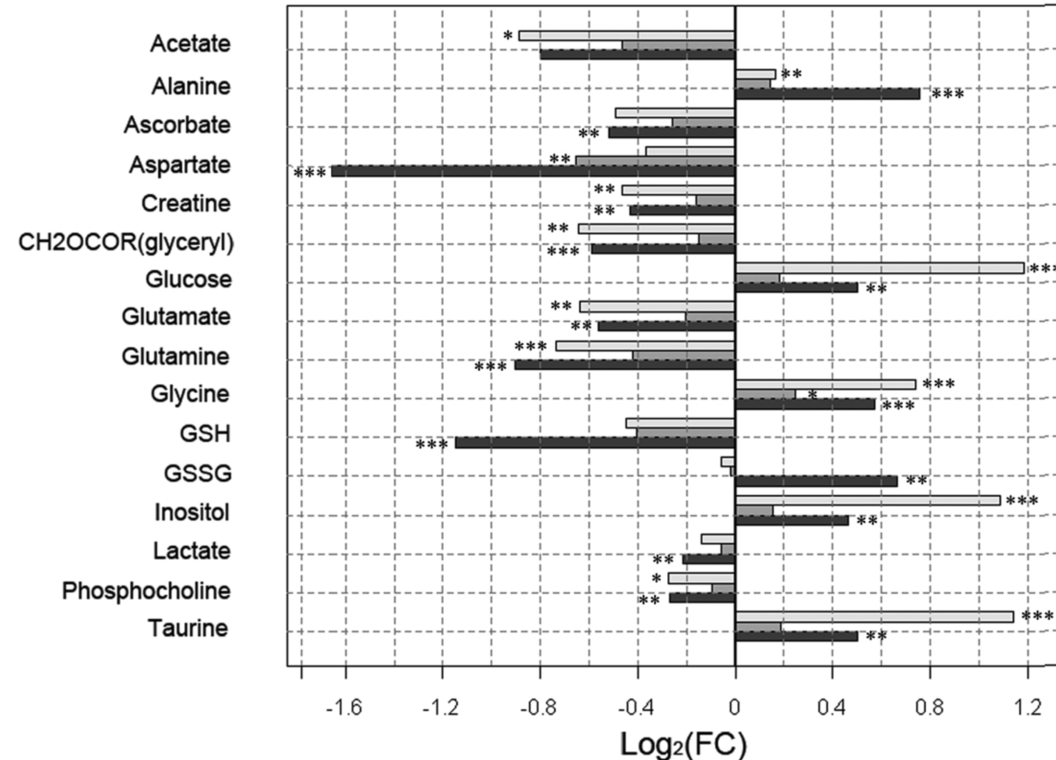

$\log_2$(T360' CI/T0' CI) in black
$\log_2$(T30' WI/T0' WI) in light grey
$\log_2$(T30' CI/T0' CI) in dark grey

Cacciatore S, Hu X, Viertler C, Kap M, Bernhardt GA, Mischinger HJ, Riegman P, Zatloukal K, Luchinat C, Turano P. J Proteome Res. 2013;12:5723-9.

# Glucose, taurine inositol

- Glucose increase during ischemia possibly reflected glucose uptake by the cells and changes in glycogenolysis or gluconeogenesis.

- Taurine, a robust apoptotic biomarker, increased mainly in WI.
- Inositol, which regulates many cellular processes in eukaryotes, including stress responses and apoptosis also increased
- Changes in taurine and inositol were strongly correlated.




$\log_2$(T360' CI/T0' CI) in black
$\log_2$(T30' WI/T0' WI) in light grey
$\log_2$(T30' CI/T0' CI) in dark grey

Cacciatore S, Hu X, Viertler C, Kap M, Bernhardt GA, Mischinger HJ, Riegman P, Zatloukal K, Luchinat C, Turano P. J Proteome Res. 2013;12:5723-9.

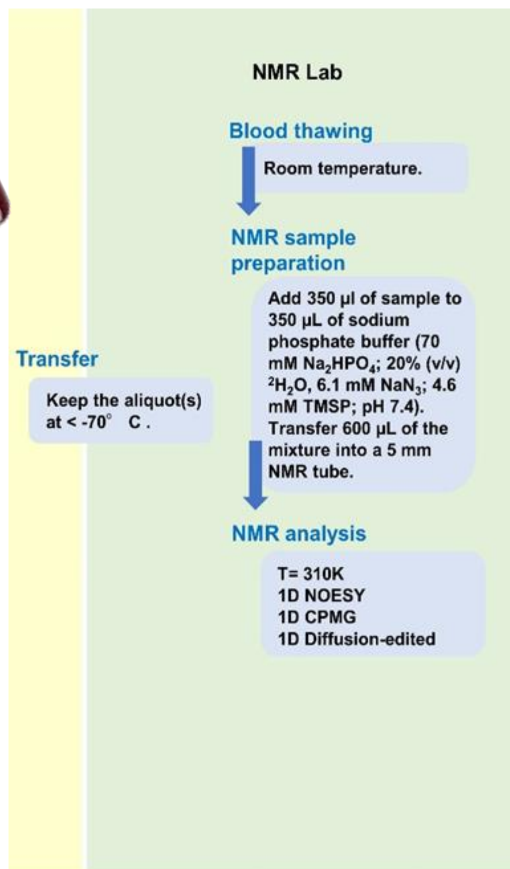
# General considerations

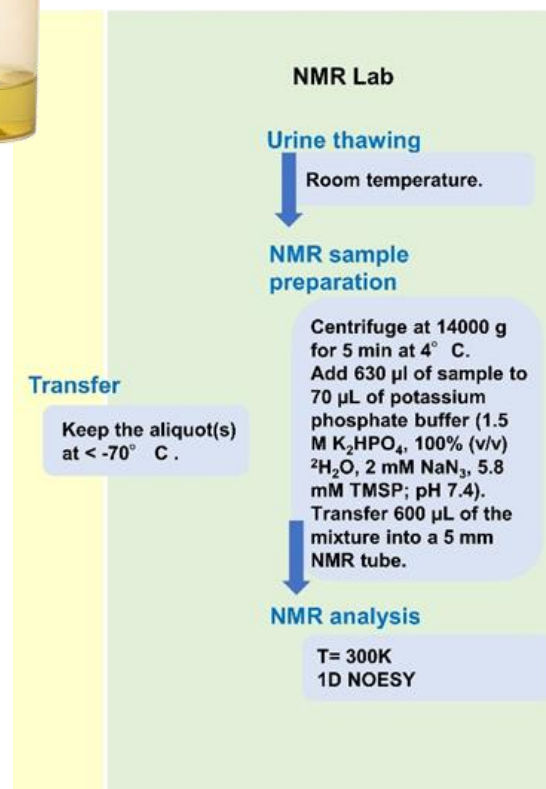
**All the samples of your dataset should be treated in the same way.**
For new type of samples: make your own SOPs for specific types of samples and/or metabolites your are interested to characterize. Simulate and validate all the procedures to identify possible “critical” points

.

Collect data on patient treatment, if possible
Collect the sample before patient treatment, if possible (i.e. anesthesia)
Perform the processing procedures as soon as possible from sample collection
Keep the samples at 4° C before the processing
Store at -80°C the processed samples as soon as possible
Use a refrigerate sample changer for automatic acquisition
Reduce the oxygen exposure
Avoid freezing/thawing cycle (divide the samples into different small volume aliquots)

# NMR-Sample preparation

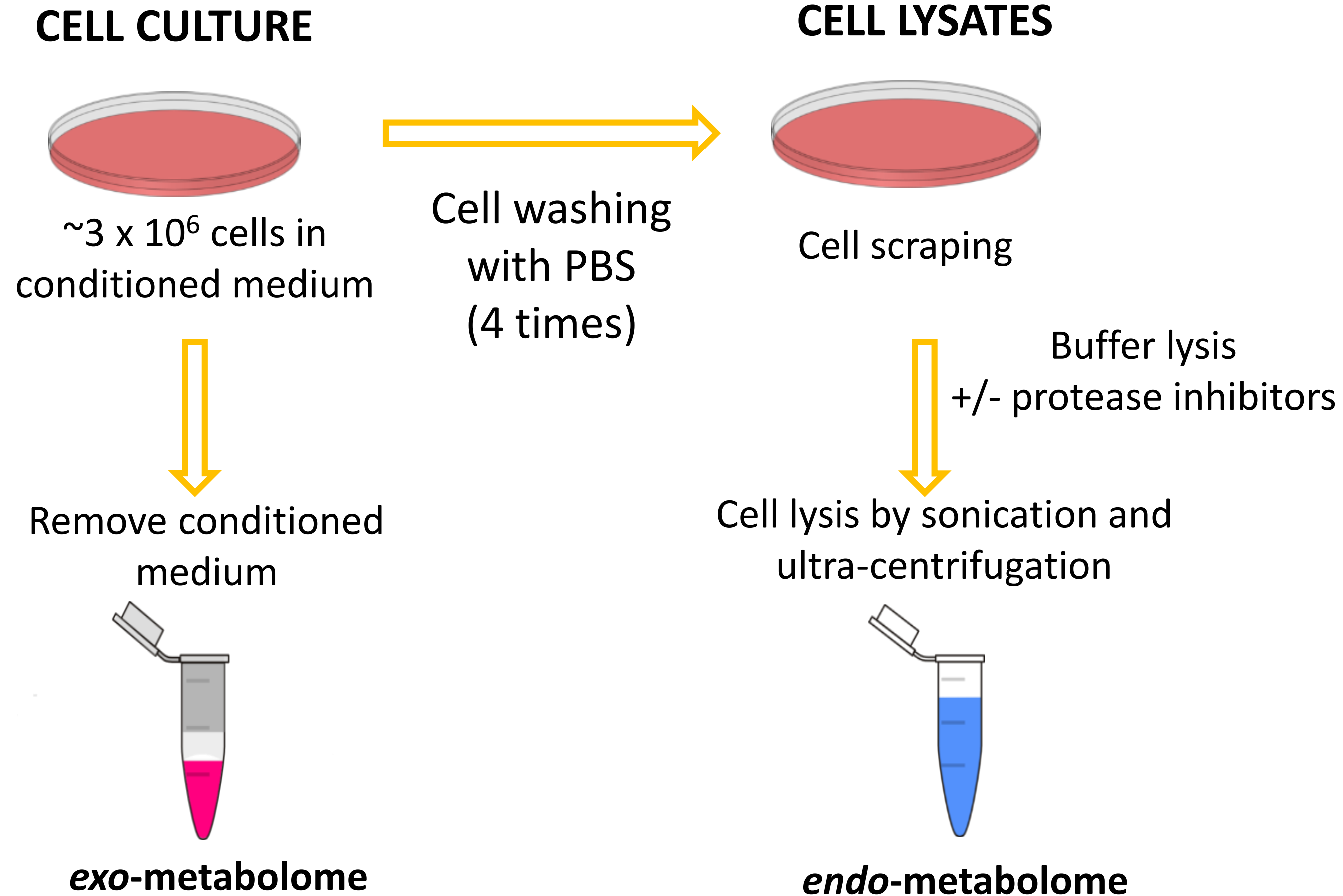





Angew Chem Int Ed Engl. 2019 Jan 21;58(4):968-994. doi: 10.1002/anie.201804736

# Cell lysates and growing media

*Cell culture procedure suitable for NMR analysis*



# Minimal sample handling

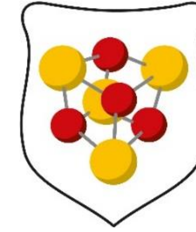
| Type of sample | Experimental procedure | NMR analysis |
| --- | --- | --- |
| Urine | • Thawing at room temperature and shaking<br>• Centrifugation at 14 000g for 5 min<br>• Dilution 1:9 with potassium phosphate buffer (1.5 M $K_2HPO_4$, 100% (v/v) $^2H_2O$, 10 mM sodium TMSP; pH 7.4)<br>• 600 µL of each mixture was transferred into a 5 mm NMR tube for the analysis. | • NOESY experiment<br>• J-resolved experiment |
| Serum/Plasma | • Thawing at room temperature and shaking<br>• Dilution 1:1 with sodium phosphate buffer (10.05 g $Na_2HPO_4{\cdot}7H_2O$; 0.2 g $NaN_3$; 0.4 g sodium TMSP in 500 mL of $H_2O$ with 20% (v/v) $^2H_2O$; pH 7.4)<br>• 600 µL of each mixture was transferred into a 5 mm NMR tube for the analysis. | • NOESY experiment<br>• CPMG experiment<br>• Diffusion-edited experiment<br>• J-resolved experiment |
| Saliva | • Thawing at room temperature and shaking<br>• Centrifugation at 14 000g for 5 min<br>• Dilution 1:9 with potassium phosphate buffer (1.5 M $K_2HPO_4$, 100% (v/v) $^2H_2O$, 10 mM sodium TMSP pH 7.4).<br>• 600 µL of each mixture was transferred into a 5 mm NMR tube for the analysis. | • NOESY experiment<br>• J-resolved experiment |

# Minimal sample handling

| Type of sample | Experimental procedure | NMR analysis |
| --- | --- | --- |
| Cerebrospinal fluid | • Thawing at room temperature and shaking<br>• Dilution 1:1 with sodium phosphate buffer (10.05 g $Na_2HPO_4 \cdot 7H_2O$; 0.2 g $NaN_3$; 0.4 g sodium TMSP in 500 mL of $H_2O$ with 20% (v/v) $^2H_2O$; pH 7.4).<br>• 600 μL of each mixture was transferred into a 5 mm NMR tube for the analysis. | • NOESY experiment<br>• CPMG experiment |
| Cell lysates | • Cells were plated in 100 mm dishes and were grown until they reached 70% confluence.<br>• Dishes were placed onto ice and cells were rinsed twice with Phosphate-Buffered Saline (DPBS). Cells were scraped and collected with DPBS supplemented with 1% Protease Inhibitor Cocktail and 1% Phosphatase Inhibitor Cocktail.<br>• Cellular lysates were sonicated on ice and centrifuged.<br>• Supernatants were collected and stored at –80 °C.<br>• Thawing in ice and shaking<br>• Adding 10% in volume of $^2H_2O$<br>• 600 μL of each mixture was transferred into a 5 mm NMR tube for the analysis. | • NOESY experiment<br>• CPMG experiment<br>• J-resolved experiment |
| Tissues | • Frozen tissue samples were trimmed (10–15 mg) to fit rotor insert capacity<br>• The insert was filled with a solution of TMSP in $^2H_2O$.<br>• Rotor inserts were covered with plug and plug-restraining screw and inserted into the 4 mm rotor for HR-MAS. | • NOESY experiment<br>• CPMG experiment |

**HR-MAS NMR**

# Thanks to… and all of you for your attention!

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Regione Toscana

Regione Toscana, project COMETA (CUP: B95F21002080003), Bando COVID-19 (https://www.regione.toscana.it/-/bando-ricerca-covid-19-toscana).

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