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Anne-Lise Royer, Yann Guitton, Gaud Dervilly, Jean-Philippe Antignac, Antoine Escourrou, Nora Cesbron, Karinne Pouponneau, Fabrice Monteau, Bruno Le Bizec

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OVERVIEW OF METABOLOMICS DIRECTED SAMPLE PREPARATION STRATEGIES FOR A RANGE OF BIOLOGICAL MATRICES

A.-L. ROYER, Y. GUITTON, G. DERVILLY-PINEL, J.-P. ANTIGNAC, A. ESCOURROU, N. CESBRON, K. POUPONNEAU, F. MONTEAU, B. LE BIZEC



LABORATOIRE D'ÉTUDE DES RÉSIDUS ET CONTAMINANTS DANS LES ALIMENTS (LABERCA)
USC INRA 1329, Oniris, LUNAM Université, BP 50707, 44307 Nantes Cedex 3, France
Fax : +33 (0)2 40 68 78 78 - Tél : +33 (0)2 40 68 78 80
laberca@oniris-nantes.fr



Metabolomic approach can be summarized in the form of a generic workflow from experimental design, sample preparation, generation of metabolic profiles, reprocessing of raw data and statistical analysis to the biological interpretation of data.

In a metabolomic analysis, research question, choice of the matrix are studied however it's also important to examine the detail of the process; the collection technique, the type of tube, etc. These parameters are essential because they can be source of variability and interpretation errors.

My Checklist Experimental design :

- ✓ Research question ?
- ✓ Number of samples :
Ensuring statistical power, anticipating realization of a pre-test
- ✓ Choice of biological matrix :
Depending on the research question
Invasiveness or non-invasiveness
- ✓ Conditions of experimentation :
Animals locale
Food management, ...
To collect the physicochemical parameters, behaviour, disease, ...
- ✓ Collection and storage of samples :
Collection technique, homogeneity of collection
Materials used for collection: contamination, ...
Make of aliquots : allows limit thawing and freezing cycles
Quenching ?
Storage at -20 °C or at -80 °C

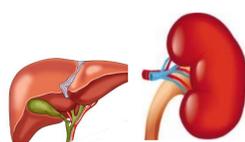
My Checklist Sample preparation :

- Targeted or non-targeted ?
. Compromise between the overall view of metabolism and quality of data
- ✓ . What fraction of interest?
- . Type of preparation :
Filtration
Extraction with an organic solvent
SPE (Solid Phase Extraction)
- ✓ Conditions of preparation :
Control the thawing step
Glass, plastic container
Use of filter cones for sampling
Keeping temperature close to zero during sample preparation
Preparation of the samples according to the injection sequences
- ✓ Ensuring data quality :
. Quality Control (QC)
. Internal standard and/or external standard
. Blank sample

Specificity according to matrices



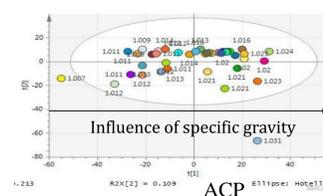
Blood requires collection of anticoagulant such as EDTA, which can increase the analytical variability by transformation of the blood matrix into plasma matrix.^[1]



A solid matrix implies adding a homogenization step of the sample. For this purpose, a grinding device (Precellys, Retsch or other manufacturers) is regularly used before extraction.

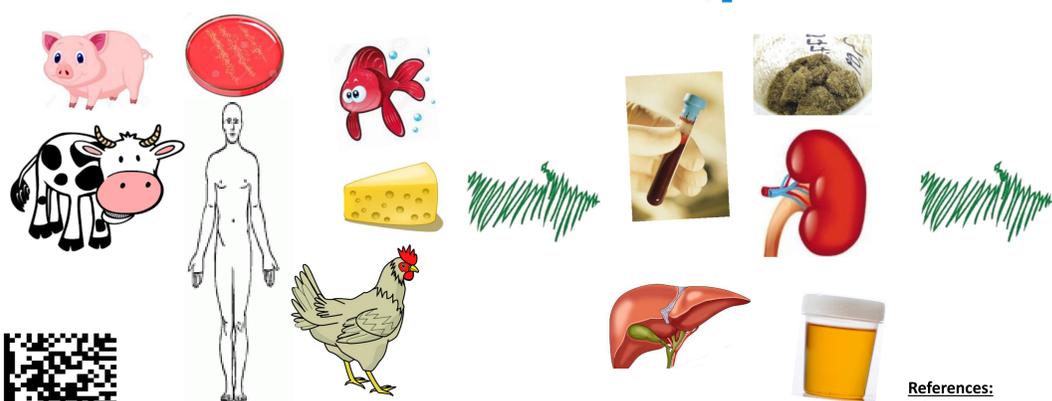


Urine matrix is strongly impacted by the consumption of water and the physiological factors which can cause a more or less dilution of the urine^[2]. In statistical analyzes, this phenomenon may involve separation according to dilution and not according to the research question.



In order to minimize this effect, a standardization step of samples is regularly performed, freeze-drying or specific gravity.

And, what do we analyze in our platform



Extraction for Metabolomic and Lipidomic analysis

- + 190 µL MeOH (vortex 20s)^[3]
- + 380 µL Chloroform (vortex 20s)
- + 120 µL Water (vortex 20s)
- Centrifugation (8000g, 10 min, T 10°C)

Extraction for Lipidomic analysis

- + 220 µL MeOH (vortex 10s)
- + 750 µL MTBE (vortex 10s)
- + 188 µL Water (vortex 20s)
- Centrifugation (1400g, 2 min, T 10°C)

Extraction for Metabolomic analysis

- + 1400 µL MeOH (vortex 15s)
- Centrifugation (15800g, 20 min, T 10°C)

References:

- [1] Dunn, W.B., et al., Nature Protocol, 2011, 1060-1083.
- [2] Royer, A.L. et al, Spectra Analyse, 2017, n°314,
- [3] Bligh, E.G., and Dyer, W.J., Can. J. Biochem. Physiol., 1959, 37(8):911-7.