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Urine sample preparation for untargeted MS-based metabolomic V.1

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We are still developing and optimizing this protocol

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Abstract

The following protocol describes the preparation of urine samples for non-targeted analysis by mass spectrometry coupled to liquid chromatography as proposed by the PFEM (MetaboHUB-Clermont). A pre-analytical normalisation step is carried out based on the osmolality measurement determined for each sample.

The steps cover the sample preparation and quality control.

The expected outcome of this protocol is a list of urine samples ready for analysis by LCMS.

Materials

Model 2020 Osmometer (Advanced instrument)

Sigma 3-16PK centrifuge (Fischer Bioblock Scientific)

Protocol materials

 MilliQ water

 MilliQ water



Before start

Randomise the order in which samples are prepared and organize them according to this order.

Two aliquots per sample to be analysed are required. The first will be used to determine the osmolality measurement and the second for metabolomic analysis by LCMS.

Osmolality measurement

- 1 Thaw an aliquot of each  urine sample at  Room temperature
- 2 Centrifuge samples  15493 x g, 4°C, 00:10:00
- 3 Measure the osmolality value of each  urine sample using an osmometer.
This setting determines the dilution factor that will be carried out on each  urine sample analysed by LCMS.



Model 2020 Osmometer

- 3.1 Calibrate the device using the following three solutions (once per month)
 - 50 mOsm/kg (Advanced Instruments Reference :3MA005)
 - 850 mOsm/kg (Advanced Instruments Reference :3MA085)
 - 2000 mOsm/kg (Advanced Instruments Reference :3LA201)
- 3.2 Each day of use, carry out a quality control measurement (clinitrol) with a value of 290 mOsm/kg. If the measured value differs from the expected value, recalibrate the device. 
- 4 Measure the osmolality value of each  urine sample . If it exceeds 2000 mOsm/kg, the  urine sample must be diluted in  MilliQ water and analysed again. 

Sample preparation



- 5 Thaw the second aliquot of each  urine sample at  Room temperature
- 6 Centrifuge  urine sample  15493 x g, 4°C, 00:10:00
- 7 Add  100 µL of  urine sample in Eppendorf tubes
- 8 Dilute each  urine sample in  MilliQ water so that each diluted sample has an osmolality value of around 100mOsm/kg
- 9 A pooled QC sample is prepared by mixing  10 µL from each diluted samples
- 10 Each diluted  urine sample is divided into three aliquots : one is reserved for LCMS analysis in positive mode, another is dedicated to analysis in negative mode, and the last is a backup sample.
- 11 Store samples for LCMS analysis at  -20 °C