SOP for urine processing for NMR metabolomics

**Following urine collection, the sample should be expeditiously handled as follows:**

Immediately following sample acquisition place the specimen cup at 4°C and protect from light (e.g., wrap in foil). Within 3 h of collection, transfer 8mL of urine into a prepared BD Vacutainer® urinalysis tube (gray stopper, with added NaN3) using the integrated transfer device in the lid of the specimen cup. Make sure there is sufficient volume (to minimum fill line) in the tube. The specimen cup can be tilted to accomplish this. Invert the tube eight times. Label the tube with the patient’s study number, the date and the time of collection; also record this information in the data collection form. Then follow the steps outlined below:

1. Centrifuge (4°C, 2000 x *g*, 10 min) to precipitate sediment.
2. Transfer the supernatant into a sterile 15mL conical tube taking care not to disturb the bottom of the tube whether there is a visible pellet or not. Discard the pellet and ~500 μl of sample above it.
3. Measure and record pH.
4. Accurately measure (using a clean graduated cylinder) the remaining total sample volume in the 15mL tube. Record the volume.
5. Return the sample to the 15mL conical tube and add a volume of Chenomx standard solution (IS-1 Chenomx internal standard- DSS with added imidazole[[1]](#footnote-1)) equivalent to 10% of the final sample volume (e.g., if the sample volume is 7mL then add 778µL of IS).
6. Following the addition of IS, cap the 15mL conical tube and invert 4X to thoroughly mix.
7. Keep the sample on ice and use a pH meter to check the sample’s pH. Record the pH.
8. Use NaOH (1M) to bring the sample to a pH of 7.0+0.25. This will likely require a small volume (< 5-10µL). Record the pH.
9. Keep the sample on ice and transfer 1mL to a microcentrifuge tube (1.5 mL)
10. Dipstick this 1mL volume of urine with a Chemstrip 10 MD[[2]](#footnote-2). This requires the pipetting of the 1mL sample from the microcentrifuge tube onto the dipstick. Check and record the Chemstrip results. Discard the dipstick and the dipstick sample.
11. Keeping tubes and the sample on ice generate sample aliquots by allocating 1mL of urine into a labeled sterile microcentrifuge tubes (1.5 mL); generate up to 6 aliquots/patient.
12. Freeze (-80°C) aliquots.

On day of assay:

1. Remove samples from dry ice upon receipt.
2. Store in -80ºC freezer until day of measurement.
3. On day of measurement, thaw samples at room temperature.
4. Check and record pH.
5. Place 750 μl of sample into clean glass NMR tube and carry out spectral collection.

1. <http://www.chenomx.com/support/support.php?pageID=69>; this is a special order- contact Chenomx for this request [↑](#footnote-ref-1)
2. <https://www.poc.roche.com/poc/rewrite/generalContent/en_US/article/POC_general_article_70.htm>; catalog no.: 03260763160 [↑](#footnote-ref-2)