

PROCEDURES FOR URINE SAMPLE PROCESSING

Processing urine samples in this way allows urine to be used for several proteomic applications including 1D and 2D-PAGE and SELDI/MALDI profiling. The effect of addition of protease inhibitor tablets is currently being evaluated in matched samples (+/- PI) from patients with either renal cancer, post-renal transplant or healthy controls, stored for various periods of time. If necessary the protocol may be subsequently modified. The neutralisation of samples minimises precipitation during storage.

1. Following the obtaining of consent from the patient, a mid-stream urine sample should be collected directly into a sterile urine pot. Take immediately to the lab.
2. Place the urine sample on ice immediately upon arrival in the lab. Record sample details on the sample forms (patient id, date, time of sample), allocating the sample the next available number (if bloods are received from that patient at the same time, use the same sample number for both). Write the sample number on any paper work received.
3. Measure and record the volume of urine using a disposable pipette.
4. Label a sterile universal with the patient's details (name, hospital number and date of sample) and transfer 5ml of urine into the universal. Complete Chemical Pathology Request form, place with the universal in a sample bag and take over to Clinical Biochemistry during the centrifugation step for protein and creatinine measurements
5. Test the main urine sample for blood and protein using a Dipstix (follow instructions on the container) and record results.
6. Fill a 50 ml tube with urine (all the urine if <50mls) and discard the remainder (mix discard with equal volume of 2% Virkon and leave to stand overnight before disposal). Add and dissolve 1 Roche Mini protease inhibitor cocktail tablet (stored in the fridge) per 25mls of urine. These are toxic and should be added to the urine pots in the lab.
7. Check and record the urine pH using the handheld pH meter (calibrate the meter first then rinse in dH₂O and dry on a tissue) – do not hold the meter whilst measuring as this alters the reading.
8. Adjust the pH to 7.0 +/- 0.1 using 1M HCl or 1M NaOH as appropriate. Thoroughly rinse the pH meter with deionised H₂O and dry before storing.
9. Remove particulate matter by filtering the urine through a 100 µm cell strainer into a fresh 50 ml falcon tube.
10. Centrifuge the sieved urine at 2,000g at 4°C for 10 minutes.
11. Using the label maker, label 10 x 0.5ml eppendorfs and 2 x universals (label the tube and lid) with the sample number suffixed by U.

12. Aliquot the centrifuged urine into the labelled tubes. Fill (but don't overfill and split the rest between the 2 universals taking care not to disturb any pellet. Record aliquot details on the sample sheet.
13. Log sample locations and store upright at -80°C . Record time of freezing.