

## **Sample Preservation – urine**

Urine is a great way of obtaining information about someone in a non-invasive manner and with regularity. Obviously there are a number of factors which need to be considered before approaching collection, which includes accounting for renal function itself. With that said though this can be a valuable source of, for example, biomarkers. Below are discussed some issues that need to be thought about with regard to collection, processing and storage of samples, some of which echo my last article.

Type of collection – will you need a random specimen, first morning specimen, midstream clean catch, timed collection, catheter collection, suprapubic aspiration or is it a paediatric specimen? These parameters will dictate analyte concentrations, cellular content, sterility and diurnal variation.

Timing and Handling – are you looking for something that is expressed at a particular time of day, e.g. hormones?

How stable is what you're looking for? Some metabolites such as porphyrins and urobilinogen are light sensitive and so need to be collected into amber-coloured containers. It is recommended for certain clinical tests that urine is analysed within 2 hours of collection, but this may not be practical. If this is the case then what is the best way to store your specimen? For longer timed collections refrigeration is recommended. Some common 24-hour preservatives are hydrochloric acid, boric acid, acetic acid, tartaric acid, formaldehyde, thymol and toluene and these may allow urine to be kept at room temperature as opposed to refrigerated. Generally, the length of preservation capacity ranges from 24 to 72 hours. The most common preservative used for culture and sensitivity is boric acid. There is clinical evidence to suggest that non-buffered boric acid may be harmful to certain organisms and that buffered boric acid preservatives can reduce the harmful effects of the preservative on the organisms. It is important that the proper specimen-to-additive ratio must be maintained when using a chemical preservative to ensure accurate test results. Maintaining the correct ratio is especially important when transferring samples into a preservative tube. Use the indicated fill lines on the tube to ensure proper fill. Underfilling the tube will leave a high concentration of preservative in the specimen, while overfilling the tube will overly dilute the preservative. In either case, the function of the preservative may be compromised. Another alternative preservative are protease inhibitors including aprotinin, pepstatin, antipain, leupeptin, benzamidine, PMSF but it must be remembered that these are toxic to living cells.

On the subject of cells it is possible to culture cells from urine which exhibit an epithelial-like phenotype. The derivation of these cells is sometimes debatable but both urothelial and proximal tubular epithelial cells have been identified. The cells present are a useful source of samples for cytology. Traditionally these cells have been fixed with ethanol (500 ml/l) but the addition of 20g/l PEG improves preservation. A further product from urine has been suggested recently in terms of biomarkers. These are small 'nanovesicles' termed exosomes. Urinary exosomes contain apical membrane and intracellular fluid and are normally secreted into the urine from all nephron segments. These may carry protein markers of renal dysfunction and structural injury. Exosomes are a notable feature of malignancy, with elevated exosome secretion and tumour-antigen enrichment of exosomes associated with cancer cells. The physiological importance of cancer exosomes remains unclear. It has been shown that exosomes are best when protease inhibitors are present.

Processing – this has been a much more widely appreciated area recently, partly due to the guidelines put in place by the Human Tissue Act. As urine can often contain cellular material its collection comes under the regulation of the Act. Even one cell contained within the sample constitutes licensable material. Cells can either be spun down in a centrifuge and the urine then aliquoted and stored or it can be filtered using standard syringe or bottle top filters.

Storage – As with any sample if we are unsure of what we may be using it for in the future then we need to keep it in conditions that are as good as we can possibly manage. It has been routine to store aliquots of urine at -20°C but it has been shown that this temperature causes a major loss in urinary exosomes compared to freshly collected urine. In contrast, recovery after freezing at -80°C was almost complete. More studies are now making small aliquots and storing at low temperature to future-proof their collections.

One thing is clear from all of this is that there needs to be adherence to established, standardised protocols to decrease variability between samples and increase the validity of biomarker determinations.

Dr Julie Williams