

SEED Urinalysis

Sysmex Educational Enhancement and Development
February 2012

Preanalytical aspects in urinalysis

In the recent past there has been an increasing interest in preanalytical variables which might affect urinalysis results. The methodologies used in urinalysis show more or less imprecise results and many of them – with the exception of the recommended reference procedure for the enumeration of particles in urine – provide merely semi-quantitative results. Sysmex fluorescence flow cytometers enable laboratories to analyse urine precisely and accurately to a great extent. To obtain realistic quantitative results preanalytical variables increasingly matter and the control of the preanalytical steps should gain indeed more importance.

Preanalysis comprises several areas with different variables starting from the point of sample collection until the urine sample reaches the laboratory.

The following areas will be addressed

- type of the urine sample
- proper collection of a clean urine sample
- age of the urine sample



Fig. 1 Preanalytical aspects did not matter when the uroscopy wheel served as guideline [1]

The required type of urine sample depends on the type of analysis to be performed in the laboratory. For quantitative, clinical chemistry urinalysis like creatinine clearance, usually 24 hour collected urine is used. For this it is important to instruct the patients exactly concerning the duration of the collection period and the handling of the sample (mixing, keeping of the receptacle). It is also of importance to add a suitable preservative to the receptacle at the very beginning. Due to the many influences it may be desirable to shorten the collection period or totally do without collected urine and instead to modify the related laboratory parameters accordingly (excretion in concentrations, related to creatinine, etc.).

The most frequently used sample type is the first morning urine, because it is relatively concentrated and therefore rich in formed elements, which increases the sensitivity of particle analysis in urine. The longer stay in the bladder has a positive effect on the detection of proteinuria and bacteriuria (an incubation of 4–8 hours is needed). Especially the nitrite test on urine test strips shows less false-negative results than when using spontaneous urine from a later micturition, because bacteria could produce enough nitrite overnight, as long as the metabolism could provide enough nitrate as substrate. Only glucosuria should be diagnosed in postprandial urine, because bacteria metabolise the glucose present. There is a growing discussion to use the second morning urine (passed in the course of the morning) for the cellular analysis, because the long length of stay of the first morning urine in the bladder causes morphological changes or even lysis of the cells. Also glucosuria may be detected in such urine. Urines from patients with excessive diuresis are less suitable as these samples are correspondingly strongly diluted and therefore raise the detection limit. In addition, cells are more likely to be lysed in the diluted urine because of its low density. Moreover, it should be considered if the patient has a raised body temperature or was physically active during the last 12 hours. Both circumstances lead

to increased physiological proteinuria and excretion of hyaline casts.

Proper collection of a clean urine sample

A normal routine analysis of formed elements in urine is usually based on midstream urine from spontaneous micturition. It should be refrained from simple spontaneous urine, as it is, especially from female patients, very often contaminated with cellular particles (like bacteria from the rectum, vaginal flow components). Also midstream urine can be contaminated, if collected without sufficient hygiene.

A typical characteristic of urine not collected properly is an increased bacteria count without elevated white blood cell excretion together with a higher number of epithelial cells, especially squamous epithelial cells.

If there is a suspicion of infections in the urethra the first portion of the midstream urine, which is usually discarded, will be of special diagnostic interest. It should be collected separately from the actual midstream urine. A comparison of both urine portions allows localising the focus of infection (bladder or urethra).

The collection of sterile urine is essential for specific, bacteriological questions. Besides the sterile urinary catheter the percutaneous, suprapubic bladder puncture is the method of choice since the contamination of the collected urine by bacteria from the lower and outer urogenital tract can be circumvented.

With babies and infants usually plastic bags are used, which are fixed around the freshly washed genitals. If no micturition has occurred, the bag should be removed after 30 minutes and replaced with a fresh one after repeated washing. Contaminations with traces of faeces or bacteria can only be eliminated with a suprapubic bladder puncture. If urine collection bags, for example from indwelling catheters, are emptied, it is of special importance to mix the urine before filling it into tubes to counteract the natural sedimentation of the corpuscular particles and to obtain realistic analysis results.

For proper collection of urine samples it should go without saying that only clean receptacles without residues are used for collection and transport of the urine. Ideally, lockable

disposable bottles should be used, which are hygienically impeccable or even sterile, to avoid any contamination of the urine.

Age of the urine sample

Special focus has to lie on the age of the urine sample: it is very important to analyse as soon as possible after collection. If the analysis is not performed with fresh urine, the instability of this material causes changes and/or breakdown of the formed elements and the dissolved substances (oxidative, photolytic and hydrolytic processes). In consequence, the analysis results may vary significantly from the in vivo state of the urine sample. Ideally, there should be a time period of 30–45 minutes between collection and analysis, although 1–1.5 hours are acceptable.

If this time period is not achievable, the urine sample should be stored at 4°C. Up to 24 hours the bacteria count remains stable; a longer cooling time should be avoided, because the formed particles would be disfigured. Before analysis, the sample has to be warmed up to room temperature, for example to dissolve precipitates that appeared due to coldness.

The use of chemical preservatives is not recommended for the determination of the urine status and for particle analysis, as they might influence the components morphologically and chemically.

Morphological analysis of the cellular components of the urine, like red blood cell morphology, has to be completed within 1 hour after micturition. Otherwise, shrinking or swelling of the cells (depending on the osmolality and the pH-value of the urine) and the associated morphological changes (like crenated red cells, echinocytes) would falsify the result.

Also alkaline urines, which develop after several hours with uncontrolled bacteria growth (due to ammonia being produced by the bacteria) may cause problems: casts and also uric acid crystals are not stable in this medium and cannot be detected after a short while. In addition, amorphous phosphates may form precipitates which complicate or prevent analysis of the remaining cellular components of the urine by covering them up.

Measures for standardisation

To exhaust the possibilities which high quality modern analytical methods offer, laboratories should endeavour to be aware of the preanalytical steps and to improve them wherever possible. This requires constructive cooperation between the laboratory, the clinicians and the wards the urine samples are coming from.



Fig. 2 Uroscopy has been replaced by more scientific approaches.

The following topics are useful to be discussed

- the type of urine coming into the laboratory for certain analyses, for example the second morning urine for analysis of formed elements
- education of patients how to properly collect clean midstream urine (cleaning and washing, clean-catch technique for collection, receptacles) and, if necessary, a corresponding hand-out to the patients
- reduction of the time period between micturition and analysis (for example <2 h, <4 h at the very most)
- cold storage and refrigerated transport if the time period cannot be shortened

The definition of a certain type of urine is not without meaning. Prof. Györy states that for a safe exclusion (or prediction) of an abnormality in renal histology after kidney biopsy, only the morning urine is appropriate. This urine must then be free of casts and red blood cells. Spontaneous urines collected over the day deliver less reliable results.

Workgroups for the standardisation of urinalysis

- Finnish recommendations for basic urinalysis and urine cultures (1983; English version published 1990)
- NCCLS – National Committee for Clinical Laboratory Standards: Approved Guideline Document GP16-A (1995)
- ECLM – European Council of Legal Medicine (European Urinalysis Group): European Urinalysis Guidelines (2000)
- ISLH – International Society for Laboratory Hematology (Task Force for Automated Urinalysis): Recommended Reference Procedure for the Enumeration of Particles in Urine (published 2003)

References

Kouri T., Györy A. Rowan R.M. (2003): ISLH Recommended Reference Procedure for the Enumeration of Particles in Urine. *Laboratory Hematology* 9: 58.

European Urinalysis Group of the ECLM (2000): *European Urinalysis Guidelines* (eds. Kouri et al); *Scand J Clin Lab Invest*, 60 (suppl. 231): 1.

Delanghe J.R., Kouri T.T., Huber A.R., Hannemann-Pohl K., Guder W.G., Lun A., Sinha P., Stamminger G., Beier L. (2000): The Role of Automated Urine Particle Flow Cytometry in Clinical Practice; *Clinica Chimica Acta* 301: 1.

Györy A. Z. (1999): Urine Microscopy Analysis. *Lab Medica Int*: 23.

The National Committee for Clinical Laboratory Standards (1995): *Urinalysis and Collection, Transportation, and Preservation of Urine Specimens, Approved Guideline, NCCLS Document GP16-A*, Vol. 15, No. 15.

Colombo J.P. (Publisher) (1994): *Klinisch-chemische Urindiagnostik*, Work group 'Urin' of the Swiss society for clinical chemistry, LABOLIFE Verlagsgesellschaft, CH-Rotkreuz.

Fogazzi G.B., Passerini P., Ponticelli C., Ritz E., Cameron J.S. (1994): *The Urinary Sediment, English Edition*, Chapman and Hall Medical, London.

Voswinckel P. (1993): *Der schwarze Urin*, Blackwell Wissenschafts-Verlag Berlin.

Heintz R., Althof S. (1993): *Das Harnsediment*, 5th edition, Georg Thieme Verlag Stuttgart, New York.

Zimmermann-Spinnler M. (1991): *Urinlabor*; Publisher Medical Laboratory Consulting AG, CH-Liestal.

Koivula T. et al (1990): Basic urinalysis and urine culture, Finnish recommendations from the working group on clean midstream specimens, *Scand J Clin Lab Invest*, 50 (suppl. 200): 26.

[1] *Almanack, Tabula festorum, mobilium ab anno 1364 usque annum domini (1462)*: York, 1364. MS 1004/29. Rosenbach Museum and Library, Philadelphia

