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Review

Preanalytics in urinalysis

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ABSTRACT

Urine contains an enormous amount of information. Well-standardized procedures for collection, transport, sample preparation and analysis should become the basis of an effective diagnostic strategy for urinalysis. As reproducibility of urinalysis has been greatly improved due to recent technological progress, preanalytical requirements of urinalysis have gained importance and have become stricter. Since the patients themselves often collect urine specimens, urinalysis is very susceptible to preanalytical issues. Various collection methods and inappropriate specimen transport can cause important preanalytical errors. In addition to the insurance of correct collection, the clinical laboratory should optimize transport and sample preservation. Errors due to variation in diuresis may be corrected by recalculating the results using dilution parameters (e.g. osmolality, creatinine, conductivity, urine density). Next to the use of a primary urine container, it is recommended to split the original urine sample into various smaller aliquots for morphological, microbiological and chemical analyses, decreasing the risk of contamination. The use of preservatives may be helpful for particular analytes. A universal urine preservative however does not exist. Preanalytical aspects are also of major importance for newer urinalysis applications (e.g. metabolomics).

Contents

1. Introduction

Urinalysis is a major diagnostic screening test in the clinical laboratory [1–4]. Until recently, microscopic urine sediment analysis was the standard. However, this methodology is associated with large errors [4]. The introduction of new automated technologies has improved the accuracy and precision of the process [5,6]. On the other hand, consolidation of laboratories has increased the physical distance between patient and laboratory. Both tendencies create a major preanalytical challenge. In the clinical laboratory, total quality could be defined as the guarantee of a correctly performed activity throughout the total testing process, providing valuable medical diagnosis and efficient patient care. Despite the improvements in standardization, the lion share of errors in urinalysis falls outside the analytical phase; in particular preanalytical steps are much more vulnerable [7].

A number of subphases have been distinguished in the preanalytical phase of urinalysis. Specimen collection and transport of the specimen

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to the laboratory, receipt of the sample by the laboratory and preparation and transport to the proper laboratory section for testing can imply potential sources of error [8]. Despite the availability of clear collection instructions, the proportion of samples that need to be rejected because of poor preanalytics remains high [9]. As more effort needs to be spent in the preanalytical phase for the further reduction of errors [10], preanalytical processes became the center of international recommendations [11,12]. Preanalytical issues have become the limiting factor in the organization of urinalysis services [13]. In the present paper we give a review of the preanalytical challenges of urinalysis.

2. Patient preparation and sampling procedures

The clinical laboratory is responsible for providing the correct information regarding optimal patient preparation and best collection procedure [2]. Informing the patient goes far beyond only explaining the practical aspects of urine collection. The effects of possible confounding factors (e.g. diuresis, dietary intake, physical exercise, …) should be stressed. If necessary illustrated instructions for sampling may be provided [11]. This may include information about first morning urine, washing of the outer genitals with water and time of collection, if timed urine is to be collected.

2.1. Which precautions should be implemented?

Minimizing contamination is already achievable by implementation of simple precautionary measures. Washing the glans penis of men or the introitus of women results in a 20% reduction of false positive urine cultures [12,14,15]. The use of antiseptics or soap cannot be recommended due to the influence on the viability of bacteria [11,16].

When deciding the best procedure, the patient's characteristics (suspected microorganisms or presence of a urinary catheter) should be taken into consideration. Sample quality can only be warranted if standardized instructions for urine collection are available. Midstream portions or clean-catch urine of first morning urine samples collected in a sterile container are the most commonly obtained specimens in clinical practice. However, overnight bacterial growth in the bladder may affect casts and cells [11,14]. Morphological studies could demonstrate a better reproducibility if incubation time was ±1–2 h. Using second morning urine specimens is sometimes recommended (urine samples voided 2–4 h after the first morning urine) because of a better reproducibility in morphologic studies [17]. Midstream urine is likely to be the most appropriate sample, since the presence of contaminating elements (e.g. bacteria, analytes and formed particles) are minimized [11].

In a multicenter study [18], results obtained from first-voided and midstream urine samples of healthy subjects were compared using dipstick analyzers and particle analyzers. In the first-voided samples, counts of leukocytes, erythrocytes and epithelial cells, but not for casts, appeared to be higher in the first-voided samples. Higher counts of epithelial cells, erythrocytes and leukocytes were also observed between males and females in first-voided specimens, whereas no significant difference could be observed in mid-stream specimens. The European Confederation of Laboratory Medicine (ECLM) has proposed a classification of reference measurement procedures for urine measurements. Different levels of accuracy have been defined (levels 1–4, Table 1) [19].

Table 1
Classification of reference measurement procedures for urine measurements.

Level	Description	Remark
Level 1	Rapid microscopic methods	Non-standardized urine sediment is not recommended because of wide uncertainty of results and reduced sensitivity.
Level 2	Routine identification methods	Standardized urine sediment under a coverslip is recommended as a routine visual procedure in examination for kidney-related urine particles.
Level 3	Comparison methods	Automated instruments have improved precision, which increases the pre-analytical needs.
Level 4	Reference methods for urine microscopy	Centrifugation step with removal of supernatant is a major tool for concentration of the specimens, but is also a major source of errors.

2.2. Transport and storage of urine samples

Time between sampling and performance of the examination procedure is critical for the reliability of urine results. Changes in concentration of urine constituents can appear, making the measured result useless. Most parameters critically depend on the time window between sampling and analysis. In particular in automated urinalysis, the importance of adherence to early time points in urinalysis (within 90 min) has been stressed [20]. Some accreditation bodies advocate for a time window of two hours (IQMH) [21] and other studies have shown stability up to six hours and longer (depending on the type of analysis). A standardized organization of transport and storage time is needed as well as documentation of storage temperature. Stabilization and adequate timing of transport are of particular importance. Stability data are known for most usual urine examinations [22].

2.3. Use of preservatives (Table 2)

Table 2 summarizes a number of common preservatives and their applications. Low osmolality, low relative density and alkaline pH can induce a rapid lysis of some urine particles after collection [23]. Addition of stabilizers usually prevents metabolic changes of urine analytes and overgrowth of bacteria. The value of preservatives for semiquantitative and qualitative assessment of urine cultures gets especially important when the sample transport times exceed 2 h [24]. However, preservatives may affect some chemical properties and alter the appearance of particles. Unfortunately, a universal preservative that allows a complete urinalysis does not (yet) exist. An appropriate label carrying a hazard symbol should give information dealing with any preservative [11,14,22]. The correct preservative to specimen ratio should be respected when samples are preserved for transport and analysis [8]. Lyophilized formulations are to be preferred as there is no risk of sample dilution or spillage. Also, containers supplemented with boric acid alone or in combination with formic acid or other stabilizing media are used [11,25]. White cells, casts, epithelial cells and bacteria are well preserved, whereas red cells tend to shrink and are less stable [2]. As no preservative seems ideal for all tests required from one sample, the fact that 24-h urine is only rarely needed, helps to solve this problem. Thus spot urine in the morning is of equal value when proteinuria is differentiated. This is possible by correcting concentration to creatinine [11].

Flow cytometry is sensitive to non-dissolved preservative remnants [26]. Boric acid is considered to be a good preservative for flow cytometric urinalysis [27]. For urinary proteomics analysis, the urine should be centrifuged to remove cell debris and kept at 4 °C. The addition of

Table 2
Common urine preservatives and their interferences.

Preservative	Possible interference
Boric acid	Initial pH values are changed; borate may inhibit growth of *Pseudomonas* spp. The use of boric acid affects a number of test strip reactions [26].
Sodium azide	Recommended for preventing bacterial overgrowth [25]
Formaldehyde	False positive leukocyte esterase, peroxidase reaction and urobilinogen on strips, lowers pH [11]
Mercury salts	Negative leukocyte esterase reaction
Chloral hexidine	
Addition of polyethylene glycol (20 g/L) to the ethanol fixative (Saccomanno's fixative) (Schuman)	Cellular analysis

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