

The EFLM European Urinalysis Guideline, Update 2023

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Abstract

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Background: The EFLM Task and Finish Group Urinalysis has updated the ECLM European Urinalysis Guidelines (2000) on urinalysis and urine bacterial culture, to improve accuracy of these examinations in European clinical laboratories, and to support diagnostic industry to develop new technologies.

Recommendations: Graded recommendations were built in the following areas:

Medical needs and test requisition: Strategies of urine testing are described to patients with complicated or uncomplicated urinary tract infection (UTI), and high or low-risk to kidney disease.

Specimen collection: Patient preparation, and urine collection are supported with two quality indicators: contamination rate (cultures), and density of urine (chemistry, particles).

Chemistry: Measurements of both urine albumin and α 1-microglobulin are recommended for sensitive detection of kidney disease in high-risk patients. Performance specifications are given for urine protein measurements and quality control of multiproperty strip tests.

Particles: Procedures for microscopy are reviewed for diagnostic urine particles, including urine bacteria. Technologies in automated particle counting and visual microscopy are updated with advice how to verify new instruments with the reference microscopy.

Bacteriology: Chromogenic agar is recommended as primary medium in urine cultures. Limits of significant growth are reviewed, with an optimised workflow for routine specimens, using leukocyturia to reduce less important antimicrobial susceptibility testing. Automation in bacteriology is encouraged to shorten turn-around times. Matrix assisted laser desorption ionization time-of-flight mass spectrometry is applicable for rapid identification of uropathogens.

Aerococcus urinae, *A. sanguinicola* and *Actinotignum schaalii* are taken into the list of uropathogens. A reference examination procedure was developed for urine bacterial cultures.

Key words: bacteriological techniques; kidney diseases; practice guideline; reference measurement procedures; urinalysis; urinary tract infections.

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Introduction and Summary

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Introduction and Summary

The current document is compiled by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Task and Finish Group Urinalysis (TFG-U) to become a Type 1a Guideline document of the EFLM Procedures. It represents an update to the European Urinalysis Guidelines published under the European Confederation of Laboratory Medicine (ECLM) with a Working Party from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in 2000 [1, 2].

Scope

The driving force for a continued co-operation in urinalysis and bacterial culture among professionals in clinical chemistry and clinical microbiology is the shared, most frequent urine specimen, requested and collected from the same micturition, but analysed variably at points-of-care, in primary laboratories, or in specialised laboratories of clinical chemistry or microbiology according to local organisation of health care. The diagnostic focus is most often urinary tract infection (UTI) or a non-infectious disease of kidneys or urinary tract. Results of different laboratory tests may be used to guide laboratory workflows or interpretations. Finally, all of the results are interpreted by clinicians as a combined “urinalysis”. Standardisation, verified quality, preanalytical organisation with clinical customers, and proven cost containment both in automated and manual examinations are a shared professional task.

The terms “Urinalysis” and “Urine analysis” are used in these guidelines synonymously, and also include urine bacterial culture. The major scope remains the diagnostic of urinary tract infections, and detection and follow-up of common non-infectious diseases of kidneys and urinary tract from urine specimens, limiting the diagnostics to the most often requested examinations. Medical indications to request urinalysis tests remain a major starting point, followed by detailed descriptions of preanalytical procedures. At the other end, reviews on some new technologies were written to provide future perspectives, without giving recommendations before clinical experience.

Target audiences

The updated EFLM European Urinalysis Guidelines are aimed mainly at laboratory professionals in small and general laboratories and at points-of-care. Special features from both clinical chemistry and microbiology are included in the appropriate sections. Scarcity of laboratory test or examination procedure-related guidelines is evident in many common and old laboratory tests, as compared to clinical practice-related guidelines that discuss clinical use of these examinations, i.e., customer-interface.

List of abbreviations in Introduction

CKD	Chronic kidney disease
ECLM	European Confederation of Laboratory Medicine
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ISO	International organisation for standardization
IVD	In vitro diagnostic (medical) device
IVDR	In vitro diagnostic medical device regulation
KDIGO	Kidney Disease Improving Global Outcomes (initiative)
UTI	Urinary tract infection

Structure of the Guideline document

To make the reading understandable, the guideline is divided into Chapters that follow routine workflow in clinical laboratories.

The first three chapters discuss the clinical and preanalytical interface between laboratories and their clinical units. In Chapter 1, Medical indications of common urine tests and their requisition are reviewed. These are a major topic in strategic planning between clinicians, laboratories and health care administration. Chapter 2 raises the role of patients as stakeholders of their diagnostics and treatment in Preparation to laboratory tests, supporting healthcare professionals to empower and commit their patients to prepare themselves for urine tests. The technical detail of Specimen collection and preservation is reviewed in Chapter 3, to advise professionals in details with their patients. We all want to achieve reasonable quality of urine specimens, as a prerequisite to high-quality results and proper treatments.

The analytical chapters start with definition of levels of accuracy in examination procedures of urinalysis and urine bacterial culture in Chapter 4. This theoretical text intends to provide background for laboratory and other professionals, how to classify their procedures, and how to compare them with relevant references to verify their sufficient accuracy.

The major analytical Chapters 5 to 7 discuss Chemistry, Particle analysis and Bacteriology of the urine tests with a similar structure: diagnostic significance, measurement procedures and analytical performance specifications as amenable. Some specific examinations are included in each of these analytical chapters based on their connection to the primary examinations. Some future perspectives are also given without clinical use at the moment, to envision developmental paths.

Evidence and recommendations

Rating the evidence

In the end of each chapter, novel recommendations were given following the GRADE principles with levels of evidence (A-D) and strengths of recommendations (1-2) [3], considering guidance for diagnostic tests [4]. Possibilities to specialty-related interpretations were compared with those given by the nephrologists in the KDIGO guideline for chronic kidney disease (CKD) [5], and by the European urologists in their guideline [6]. A description for reporting well-designed studies on diagnostic accuracy is available (STARD = Standards for Reporting of Diagnostic Accuracy Studies) [7]. The EFLM-COLABIOCLI guideline for venous blood sampling was also compared [8]. The description of the used evidence rating is shown in the **Table** below.

The style of writing may be felt too verbose. The purpose was to allow judgment of the provided evidence by reviewing available key publications.

Table. The Levels of Evidence (LoE).

modified from the GRADE principles [3]

A	High quality: Consistent evidence from well performed controlled studies or overwhelming evidence of some other form. <i>Further research is unlikely to change our confidence in the estimate of effect.</i>
B	Moderate quality: Evidence from controlled studies with important limitations (inconsistent results, methodologic flaws, indirect or imprecise evidence), or very strong evidence of some other research design. <i>Further research is likely to change the estimate of effect.</i>
C	Low quality: Evidence from observational or limited studies, or from controlled trials with serious flaws. <i>Further research is very likely to change the estimate of effect.</i>
D ^a	Very low quality: Consensus of expert panels, position statement by scientific societies, surveys, or case reports.

^a The category D level of evidence (consensus) was not used.**Rating the recommendations**

Strengths of recommendations were based on consensus risk assessment of the authors guided by the following examples by the EFLM for laboratory examinations:

Benefits: improvement of turn-around time, analytical performance, diagnostic performance, clinical outcome, or cost-effectiveness.

Harms: unacceptable analytical error, unnecessary diagnostic, or therapeutic intervention due to false positive result, inappropriate or lack of treatment due to false negative result, high cost or waste of resource, or major impediment to implementation, including comparisons to the European legislation, such as the Regulation 2017/746 on In Vitro Diagnostic Medical Devices, IVDR 2017/746 [9].

The Strengths of Recommendations (SoR)

The following rating for the Strengths was used:

(1) **Strong recommendation** for using a procedure was given when the estimated benefits were remarkable against harms or costs. Strong recommendation for avoiding a procedure was given when the opposite was true, i.e., when the estimated harms or costs were remarkable against the expected benefits.

(2) **Weak recommendation** for using a procedure was given when the estimated benefits appear to outweigh or may be controversial against created harms or costs.

No recommendation was given if the estimated harms or costs appear to outweigh the benefits, or balance between harms or costs against the benefits cannot be determined.

Some proposed analytical performance specifications are suggestions for diagnostic use of clinical urine specimens. The provisional performance specifications were tailored separately for chemical measurements, particle counting, and bacterial cultures. These are intended to help European medical laboratories to describe their own performance, e.g., when needed for accreditation of examination procedures at the laboratories, as required by the ISO 15189:2022 standard [10].

Guideline process

The literature search was started with 960 citations on chemical urinalysis, urine particle counting, and bacterial cultures, as compiled together with the informaticist at the Library of Medical Faculty of the University of Helsinki in 2019-2020. The relevant publications were supplemented with separate citations on detailed topics, as collected by the professionals of the EFLM Task and Finish Group Urinalysis during the writing process. The Group was divided into subgroups for reading and writing the updated texts in 2021-2022, based on professional knowledge on Chemistry, Particles or Bacteriology. Preanalytics was shared by all subgroups. The draft chapters were discussed mostly in distant meetings, also encouraged by the COVID-19 pandemic. A new reference procedure was carefully developed for urine bacterial cultures to allow verification of routine procedures and new automated instruments in clinical bacteriology.

The financing of the project was organised in the initial meeting with the IVD sponsors at the EuroMedLab Barcelona 2019. Due to the COVID-19 events in Europe, the first draft of the updated guideline text was available during the summer 2022. Each of the four sections was given to 1-2 distinguished reviewers for primary corrections during July-November 2022. The modified GRADE system of rating evidence and recommendations was developed by the TFG Urinalysis together with the Chair of the Committee of Science of the EFLM, Michel Langlois. The final draft was given to the Chair for official review in December 2022, and distributed to the National Society members according to EFLM Procedure Manual for Type 1a documents. In parallel, the draft document was given to the Guidelines Subcommittee of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for Public Consultation and possible endorsement according to the ESCMID Guidelines.

During February – June 2023, the EFLM TFG Urinalysis received a total of 245 comments or suggestions for improvement from the EFLM National Societies, ESCMID Public reviewers, French Society of Microbiology (Société Française de Microbiologie), Danish Study Group for Urine Bacteriology, and representatives of the supporting IVD industries that were met at the EuroMedLab Rome 2023 to provide information on the progress. During April – November 2023, the responses, corrections and amendments have been prepared and agreed within the working group. The revised version of the EFLM European Urinalysis Guideline was presented for voting among the EFLM National Societies. In addition, ESCMID Guidelines Subcommittee sent an AGREE II Global Rating Scale (GRS) form for its reviewers, providing us with six ratings to the draft document from the ESCMID reviewers and one from the French Society of Microbiology. The lists of the received comments and responses to them, as well as the AGREE GRS ratings of the draft guideline, are delivered as a background information of this document to the EFLM and ESCMID organisations, and interested professionals as electronic supplemental material.

When having received the revised guideline version, the National Societies voted on the acceptance of the document.

The result of the voting was as follows:

The decision of the ESCMID Guidelines Subcommittee was as follows:

The EFLM TFG Urinalysis plans to publish the EFLM European Urinalysis Guideline in Clinical Chemistry and Laboratory Medicine as an open access document, according to the rules of the Society.

Declaration of conflicts of interest

None of the members of the group declares a conflict of interest that would interfere with the scientific contents of this guideline. Neither the organization of the EFLM nor the educational support by the diagnostic companies had a commercial influence to this document.

Funding sources

This guideline work of the EFLM TFG Urinalysis was supported for travel and lodging by the eight diagnostic companies listed in the alphabetical order below. The money was transparently collected into the EFLM bank account, and used under the supervision of the EFLM Office and Treasurer, according to the EFLM Procedures.

77 Elektronika Kft

A. Menarini Diagnostics

BD Life Sciences

Beckman Coulter

ROCHE Diagnostics GmbH

GREINER Bio-One

Sarstedt AG & Co

SYSMEX EUROPE GmbH

Contributors to the EFLM European Urinalysis Guidelines

The members of the EFLM Task and Finish Group Urinalysis shared the work of planning, reading literature, writing, and reviewing the text according to their expertise in the subtopics of urinalysis, as shown in the Table below.

Table. Members of the EFLM Task and Finish Group (TFG) Urinalysis 2018-2023

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Walter Hofmann	Synlab MVZ, Augsburg and Dachau, Germany	Munich, Germany	Chemistry
Rosanna Falbo	University Department of Laboratory Medicine, ASST Brianza, Pio XI Hospital, Desio (MB)	Brianza, Italy	Particles
Matthijs Oyaert	Department of Laboratory Medicine University Hospital Ghent	Ghent, Belgium	Particles
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Primary reviewers

Selected distinguished professionals accepted the invitation to review the draft contents based on their professional knowledge before submitting the text into the official process of the EFLM for Type 1a documents, and Public Consultation for endorsement under ESCMID Guidelines Subcommittee. The primary reviewers are listed below.

Prenalytics: Joris Delanghe, Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

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The text does not necessarily reflect the detailed opinions of any of the contributors or sponsors, since it is the product of a consensus process or based on written evidence.

Implementation

This guideline was primarily written to clinical laboratories, to improve accuracy of preanalytical and analytical processes in urinalysis and urine bacterial culture, also required by the ISO 15189:2022 standard for medical laboratories. The first three chapters discuss medical indications, patient preparation, and specimen collection for urinalysis tests, to help laboratories and their clinical units in designing targeted diagnostics, and to encourage them to avoid waste in processes with usually restricted resource. Three key levels of implementation may be visualised:

Local level: Each clinical laboratory organization performing urinalysis tests should review the recommendations related to verification and implementation of their analytical procedures. In particular, a new suggestion for reference examinations is given to microbiology laboratories. In addition, several quality improvements are suggested to preanalytical phases of urinalysis that are easily overlooked, resulting in low-quality, or misleading specimens. ISO 15189:2022 standard already contains requirements of controlling non-conformities of preanalytical phase as well.

National level: Several procedures and shared practices are to be decided at a national level, in addition to harmonising units for urinalysis and urine bacterial culture. That is why national professional societies and professionals of accredited laboratories have a role in initiating discussions, and deciding on national adaptations of the laboratory procedures described at a general level in this guideline.

Industrial level: The diagnostic IVD industry develops new technologies for preanalytical or analytical phase of urinalysis. Descriptions of medically needed analytes (measurands), the given reference procedures, and provided performance specifications are intended to support evaluation of diagnostic and analytic accuracy of new devices when developed.

Suggested format of citation

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Executive summary of the EFLM European Urinalysis Guideline 2023

Timo T. Kouri, Walter Hofmann, Rosanna Falbo, Matthijs Oyaert, Sören Schubert, Jan Berg Gertsen, Audrey Merens, and Martine Pestel-Caron, on Behalf of the Task and Finish Group for Urinalysis (TFG-U), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

Background: The EFLM Task and Finish Group Urinalysis has updated the previous ECLM European Urinalysis Guidelines (Scand J Clin Lab Invest, Suppl 231, 2000) on laboratory procedures in urinalysis and urine bacterial culture. We aim to improve accuracy of urine examinations in European clinical laboratories, and to support diagnostic industry to develop new technologies.

Recommendations: Graded recommendations based on the GRADE evaluation on the levels of evidence were built in different areas of urinalysis and urine bacterial culture. Examinations are classified into *Level 1* (ordinal scale procedures), *Level 2* (quantitative, routine procedures), and to *Level 3* (highest, reference, or advanced comparison procedures), based on the accuracy of the examination, and applied also for identification of particles and bacterial species.

Medical needs and test requisition: Strategies of urine testing were described to patients with complicated and uncomplicated urinary tract infections (UTI), and to those with low and high risk for chronic kidney disease. Electronic requisition is recommended to support exchange of clinical information between clinicians and laboratories, and to avoid errors in patient or specimen identification.

Patient preparation: Interaction with patients and professionals should be improved, and supported with culturally adopted materials, to improve quality of mid-stream urine collections.

Specimen collection: High-quality urine collection and preservation are supported with two quality indicators: contamination rate (cultures), and density of urine (chemistry, particles). Cleansing before mid-stream urine collections is recommended for large and variable patient populations, despite not necessarily needed in collections by skillful young patients. Single catheter urine or suprapubic aspiration specimen is recommended to establish the diagnosis of UTI in children or older patients without urinary control. Preservation requirements and verification of preservatives in the collection containers were updated to all examinations discussed.

Chemistry: Measurements of both urine albumin and $\alpha 1$ -microglobulin are recommended for sensitive detection of kidney disease in high-risk patients (with diabetes and cardiovascular diseases with known renal complications). Albuminuria screening is recommended for detection of cardiovascular disease in patients with chronic kidney disease. Performance specifications for urine protein measurements (*Level 2*) and quality control of multiproperty strip tests (*Level 1*) were given. Urine concentration is recommended to be reported together with all chemical examinations from single-voided specimens, understanding the biochemical limits of each measure of volume rate. Analytical performance specification was given to the measurement of urine albumin.

Particles: Procedures for particle counting and detection are reviewed for clinically significant urine particles. Health-associated upper reference limits for leukocytes and erythrocytes were given, and estimates of diagnostic cut-off limits for most common particles. Laboratories should clearly describe and follow their routine quantitative procedure (*Level 2*) in patient results, endorsing application of the IFCC-IUPAC recommended SI unit, particles $\times 10^6/L$. An operating procedure is suggested for classification of dysmorphic erythrocytes in urine. They are also recommended to follow the frame of the given reference visual microscopic procedure (*Level 3*) for instrument verification. Verification of automated particle analysers is supported with statistical modelling and analytical performance specifications.

Bacteriology: Chromogenic agar is recommended as primary medium in urine cultures, because of rapid and cheap recognition of *E.coli* on the plates. A new optimised workflow for routine specimens is given, by using leukocyturia and limits of significant growth to reduce less important antimicrobial susceptibility testing. Automation in bacteriology is encouraged to shorten turn-around times. Matrix assisted laser desorption ionization time-of-flight mass spectrometry is applicable for rapid identification of uropathogens, and recommended to middle-sized and large bacteriology laboratories. *Aerococcus urinae*, *A. sanguinicola* and *Actinotignum schaalii* were taken into the list of uropathogens. Moreover, a novel reference examination procedure was carefully developed for urine bacterial cultures to support verification of performance of automated instruments, or aid in focussed assessing of routine procedures of bacteria detection and isolation, as included in the ISO 15189:2022 requirements.

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In Europe, a national source of EN ISO standards is recommended.

The EFLM European Urinalysis Guideline Update 2023

Chapter 1. Medical needs and requisition

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Background

Development of medicine and increasing needs in human populations challenge the relevance of different investigations of urine, similar to other laboratory examinations used in health care. Cost-benefit analyses, or even economic analyses of gained quality-adjusted life years (QALY) should guide the implementation of all laboratory examinations for various patient populations [1, 2, 3].

Clinical symptoms are essential in guiding the use of tests related to urinary tract infection (UTI), since asymptomatic bacteriuria is frequent due to the presence of microbiota in the urinary tract of even healthy individuals [4, 5, 6]. To detect kidney disease, urine tests are recommended in addition to estimation of glomerular filtration rate (GFR) [7]. The epidemiology of target diseases should be considered: screening and intensified treatment of nephropathy in patients with diabetes mellitus is recommended world-wide [8], as well as prevention of cardiovascular disease in chronic kidney patients [9]. On the other hand, non-invasive urine specimens are becoming successful in screening for specific microbes but the approaches usually differ in endemic areas from areas with low prevalence [10]. Examples of common indications for urine tests for diseases of kidneys and urinary tract are given in **Table 1-1**.

Table 1-1. Frequent medical indications for urine tests in diseases of kidneys and urinary tract ^a.

Suspicion or symptoms suggesting the possibility of urinary tract infection (UTI)
Screening for asymptomatic bacteriuria in specific patient groups only (Chapter 1.2.2)
Suspicion of renal disease, either primary or secondary to systemic diseases, such as diabetes mellitus, hypertension, rheumatic diseases, toxæmia of pregnancy, or to the adverse effects of drugs
Suspicion or follow-up of post-renal disease
Detection of glycosuria, ketonuria or urine pH from specified patient groups only (Chapter 5.2.1.5)

^a If understood widely, urine quantities are measured in diagnostics of several endocrine, metabolic and inherited diseases, pregnancy, drugs of abuse, etc., most of which were not discussed in this guideline that focuses on diseases of kidneys and urinary tract.

Clinical presentations vary widely from asymptomatic ambulatory patients to high-risk immunosuppressed individuals with life-threatening complications. No age range is exempt. Clinical need may dictate an urgent examination with a turn-around time less than 2 hours, rather than a confirmatory examination that is reported too late for decision-making. The repertoire of local laboratory or point-of-care environments will also influence the selection of requested laboratory examinations.

RECOMMENDATION: Epidemiology and clinical symptoms of the target diseases, as well as diagnostic and prognostic significance of the chosen tests are recommended to guide the clinical use of urinalysis tests.(SoR 1, LoE B) ^a

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

1.1. Examinations for general patient populations

Most of the costs arising from screening programmes result from confirmation of positive findings. That is why screening of completely unselected individuals, i.e., at general epidemiological level, is discouraged except for research purposes. A focussed strategic planning includes health economical assessment of technologies for improving quality of patients' life.

Selected asymptomatic individuals may be investigated if justified by cost/benefit analyses, e.g., in screening of asymptomatic bacteriuria (see **Chapter 1.2.2**) or for patients with high-risk for chronic kidney disease [11]. Examinations for diseases in kidneys and urinary tract can be recommended for many clinical populations, i.e., patients attending health care services in hospitals or at ambulatory clinics because of their symptoms or diseases, but not for all patients. Even the use of urine test strips shall be associated with diagnostic significance [12].

A *multiple (multiproperty) test strip* measurement, or a quantitative *urine particle analysis* may be used to detect laboratory findings shown in **Figure 1-1**. In addition to the shown minimum measurands and clinical findings, acute cases or specific patient groups may incidentally benefit from measurements of *urinary glucose, ketone bodies, or pH*. Specific diagnostics for diabetes mellitus or diabetic ketosis no more relies on measurements of urine analytes. Clinicians should remain sensitive to individual needs based on patient data. A multiple test strip investigation was designed to improve general efficiency of urinalysis among routine patient populations, and to help in emergency cases. When a rapid urine test (strip test or particle counting) remains negative, the clinician should consider other diagnostics based on clinical presentation (**Figure 1-1**).

Detailed discussion on laboratory tests requested for detection of UTI is in **Chapter 1.2** and that for detection of non-infectious kidney and urinary tract diseases in **Chapter 1.3**.

RECOMMENDATION: Urinalysis tests should be requested based on assessment of risk or presence of severe or complicated disease. Specific test planning between laboratories and clinics is recommended to balance benefits against resource. (1, C)

RECOMMENDATION: General screening strategies for low-risk and routine patients (work-flow optimisation) are to be separated from targeted diagnostics for high-risk or complicated patients or specific specimens. (1, C)

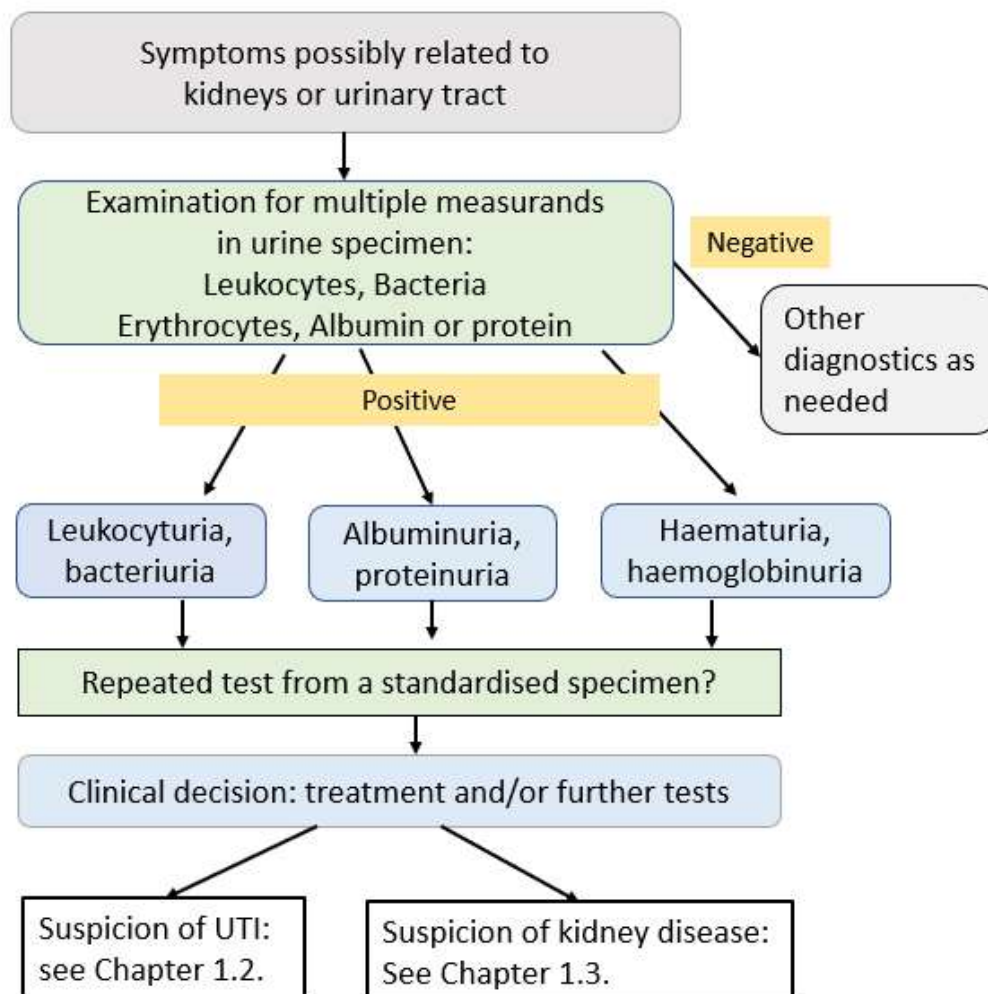


Figure 1-1. Urinalysis examinations with a sieving strategy for patients with general symptoms, possibly related to kidneys or urinary tract.

The actual measurements may be carried out by strip tests, or particle counting, depending on the location, availability and patient profile in a health care unit. The term “rapid test” means analytically point-of-care and similar tests (**Chapter 4.1**), but clinically also tests provided by the laboratories at the emergency hours, as compared to reporting after 1-2 days from a request. In addition to the measurands (analytes) related to diseases of the kidneys and the urinary tract, other incidental analytes, such as urine glucose, ketone bodies, or pH on a test strip, may be useful in specific purposes.

1.2. Examinations for detection of urinary tract infection

The suggested sieving strategy aims to limit the number of bacterial cultures to patients who need a bacterial culture for their correct diagnosis and treatment (**Figure 1-2**).

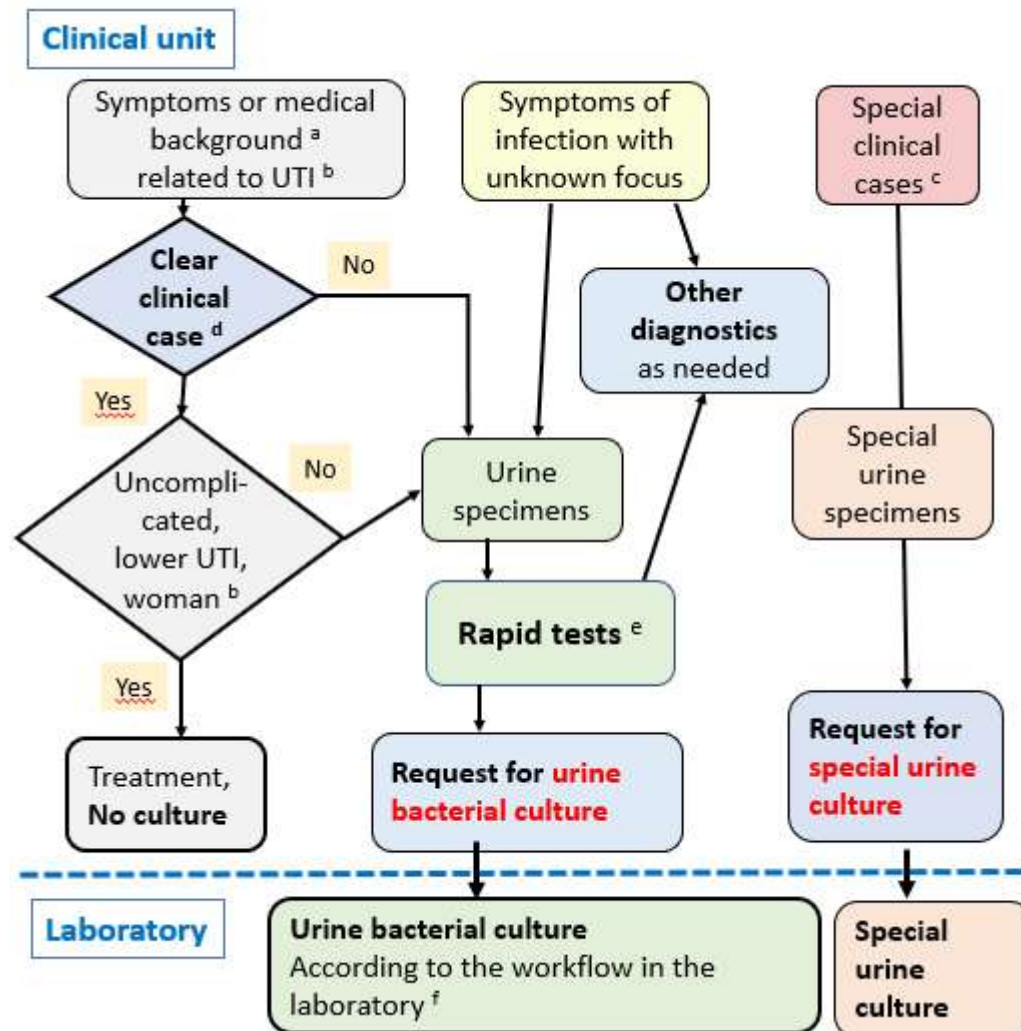


Figure 1-2. Urine examinations in suspicions of urinary tract infection

The figure divides the activities in a clinical unit and in a laboratory. Rapid tests may be organised locally in several ways depending on health care setting. Explanations to the footnotes:

^a Medical record known to predispose UTI (urinary tract infection)

^b Patient groups, see **Chapter 7.1.2** and **Table 7-2**

^c Special cases, see **Chapter 1.2.1.2**

^d Application of the Acute Cystitis Symptoms Score, see **Chapter 1.2.1.1** and **Chapter 7.1.1**

^e Rapid tests to detect leukocytes and bacteria to increase the probability of UTI, see **Chapter 7.3.2**

^f Routine workflow in bacterial culture, see **Chapter 7.5.2**, and **Figure 7-2**

Cultures from clearly uncomplicated patients (**Chapter 1.2.1.1**) are not needed [13]. In emergency patients, the balance between sufficient rapid diagnostics and inappropriate routine requisition of urine bacterial cultures is important. Requests of urine tests may be markedly reduced in co-operative planning with responsible professionals working at frontline [14].

The diagnostic strategy to detect UTI shall consider problems with specificity such as contamination and false positive reactions, and those with sensitivity, i.e., false negatives in detection of uropathogenic bacteria. Despite a strategy to reduce traditional cultures, health care professionals should remain sensitive to needs of problematic or specific cases.

1.2.1. Symptomatic patients

1.2.1.1. Uncomplicated UTI

Uncomplicated UTI is defined as “acute, sporadic or recurrent lower (uncomplicated cystitis) and/or upper (uncomplicated pyelonephritis) UTI, limited to non-pregnant women with no known relevant anatomical and functional abnormalities within the urinary tract or comorbidities”. [15]. Within the uncomplicated UTI patients, an uncomplicated lower UTI (cystitis) in otherwise healthy non-pregnant females without vaginal irritation makes an exception. These female patients have a low risk for recurrency or serious course of infection. Their lower UTI may be diagnosed without laboratory tests by using a focussed questionnaire, called ACSS (Acute Cystitis Symptoms Score) as validated already for several languages [16, 17, 18]. The sensitivity of ACSS is reported to be 94 % with a specificity of 90 % in patients with typical symptoms. See **Chapter 7.1.1**.

Follow-up of uncomplicated patients: If no symptoms remain after treatment, no further examination is needed. If symptoms persist, urine bacterial culture with antimicrobial susceptibility testing is warranted (see **Chapter 7.1.3**).

Epidemiology of uropathogens: The prerequisite for treatment of urinary tract infection without bacterial cultures is an epidemiological knowledge of uropathogens and their antimicrobial susceptibilities within a local community. Co-operation with networking laboratories of the European Antimicrobial Resistance Surveillance Network (EARS-Net) [19], and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) is encouraged [20].

1.2.1.2. Other patients suspected for UTI

Urine cultures are needed for other patient groups with symptoms related to lower or upper UTI, including males, children, patients with atypical or recurrent symptoms, patients with abnormalities or various devices in their urinary tract, and those who do not respond to antimicrobial treatment, see **Figure 1-2** and **Chapter 7.1.2**.

Acute cases benefit from results of rapid diagnostics, since a clearly positive result from urine strip test or particle analysis may support a clinical diagnosis of UTI in unclear cases. The specific result from urine bacterial culture serves to finalise the classification of disease after 1-2 days. Empirical treatment can be justified with known local epidemiology. Symptomatic cases that remain negative on a rapid examination should still be treated after urine collection (false negative

cases). If necessary, the antibiotic treatment must be adjusted based on the results of the urine bacterial culture. In doubtful cases, a standardised morning specimen should be requested for re-investigation, considering also other diagnostic possibilities and tests.

Special cases and specimens needing for special urine cultures (Figure 1-2) may include those from patients with selected urological diseases or procedures, such as differentiating chronic bacterial prostatitis from non-bacterial pelvic syndromes, with Meares and Stamey procedure for urine collection (**Chapter 3.2.9**) [15], patients with suspected fastidious bacterial infections, or specimens with leukocyturia but a negative routine urine culture. Arrangements for test requisition, preanalytical details, and specific culture conditions for these cases should be agreed locally. See **Chapter 7.4.1** for specific culture conditions.

1.2.2. Asymptomatic bacteriuria

1.2.2.1. Definition of asymptomatic bacteriuria

Asymptomatic bacteriuria (ASB) is defined as the presence of 1 or 2 species of growth at 10^5 colony-forming units (CFU)/mL – or 10^8 colony-forming bacteria (CFB)/L – or more in culture of a properly collected mid-stream specimen, irrespective of the presence of pyuria (leukocyturia), in the absence of signs or symptoms attributable to urinary tract infection (UTI) [21, 15].

In women, ASB should be present in two consecutive samples, usually within 2 weeks, and result in growth of the same bacterial species, because between 10 % and 60% of healthy females do not have persistent bacteriuria in the repeated specimen after being initially positive [21]. In men, one mid-stream specimen is sufficient for ASB diagnosis [22]. As an exception, a single positive specimen for Group B streptococci (*Streptococcus agalactiae*) is recommended to allow for ASB diagnosis in pregnant women because of the risk of neonatal infection [23].

1.2.2.2. Prevalence of asymptomatic bacteriuria

ASB represents colonisation of bacteria in the urinary tract without causing symptoms. In most cases, ASB will not predispose patients to urinary tract infection [24]. Prevalence of ASB is 1-5% in healthy premenopausal women, 5-10% in pregnant women, 8.5% in patients hospitalized for acute care, and 50% in elderly residents of long term care [21]. The prevalence of asymptomatic bacteriuria in individuals with long-term indwelling urinary catheter is close to 100% [25].

1.2.2.3. Clinical management of asymptomatic bacteriuria

In general, ASB does not require antimicrobial treatment because

- it is not associated with adverse outcomes
- antimicrobials are intended to treat infection, not to eradicate microbiome that might even protect from symptomatic infections [26]
- unnecessary antimicrobial use increases antibiotic consumption and contributes to evolution and spreading of multi-resistant bacteria from urobiome

Exceptions of ASB to be treated include

- pregnant women: During pregnancy, bacteriuria is treated to prevent symptomatic infection and premature birth. [21, 15, 27, 28].
- patients undergoing invasive urogenital surgery when the surgery is going to disrupt the mucosal barrier [15]. Preoperative mixed flora in urine culture obtained before urogynaecological operations is probably not a risk for postoperative complications [29]. This applies for orthopaedic operations as well.

Screening or treatment for ASB is NOT recommended for the following patient groups: renal transplant patients after 1 month of the transplantation, recipients of other solid organ transplants, patients living with urologic devices, cognitively impaired patients, patients with diabetes in good homeostatic control, and patients with spinal cord injury causing impaired voiding. In particular, residents in long-term care facilities, and patients with long-term indwelling urethral catheter should not be treated for their ASB. It is also recommended NOT to screen nor treat ASB in patients with recurrent UTI and in patients prior arthroplastic surgery [21, 15]. For specific groups, such as neutropenic patients, and renal transplant patients within one month of transplantation, no consensus exists for their ASB screening.

RECOMMENDATION: Asymptomatic bacteriuria must generally not be treated with antimicrobials in order to avoid unnecessary treatments and selection of multi-resistant uropathogens. Exceptions include pregnant women and patients undergoing invasive urological operations. (1, A)

1.3. Examinations for detection of kidney disease

Clinical indication to look for a disease in kidneys or urinary tract may derive from symptoms related to the urinary tract, such as haematuria, dysuria, or localised pain. The need to screen for a kidney disease may also arise from a background disease with a *high risk* for kidney damage, such as diabetes or hypertension, without symptoms directly related to kidneys or urinary tract [7], or from an *increased risk* to cardiovascular disease (CVD) among patients with chronic kidney disease (CKD) [9] (**Figure 1-3**). A detailed discussion is presented in **Chapter 5.3**.

1.3.1. Examinations of proteinuria

Transient proteinuria is a common finding among acutely ill patients [30] even at higher than the conventional limit of 100 mg/L albumin concentration (or 200 mg concentration of total protein) in test strips. To avoid inappropriate additional investigations, transient proteinuria is excluded by repeated measurements and review of anamnestic data.

Quantitative specific protein measurements may follow a positive albumin strip result but shall be used as primary investigations for detection or follow-up of kidney disease in high-risk patients (**Figure 1-3**). A sensitive albumin or protein quantitation is recommended from a single-voided specimen as albumin-to-creatinine or total protein-to-creatinine ratio instead of measuring 24-hour protein excretion rate.

Orthostatic (postural) proteinuria is identified by separate day and overnight collections. Conventional sieving of specimens by means of traditional strip tests for urine microscopy [31] or kidney disease is not sensitive enough in high-risk patients (see **Chapters 5.2.2**). For high-risk patients, immunoglobulin free light chain or other specific determinations may additionally be important in differentiation of patient's disease, in addition to albumin and α 1-microglobulin measurements (See **Chapter 5.3.1**).

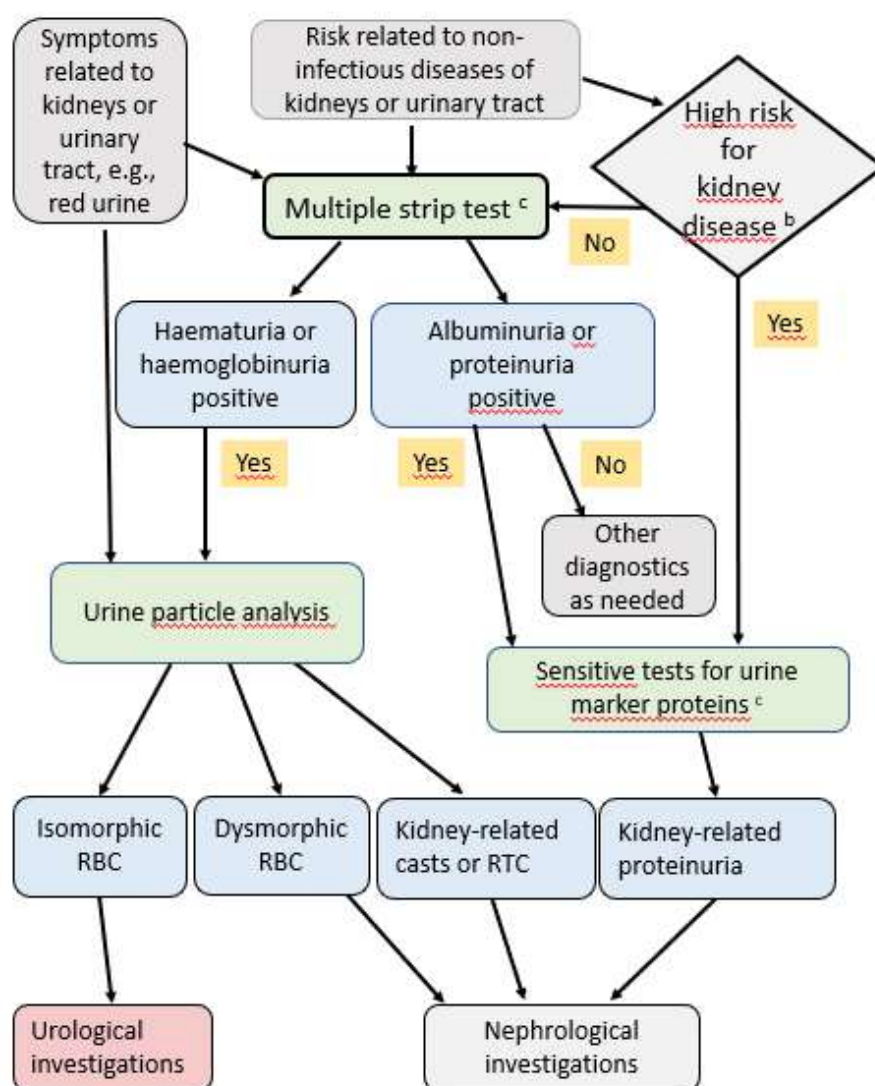


Figure 1-3. Urine examinations in suspicions of non-infectious disease of kidneys or urinary tract ^a

The examinations differ depending on the presence of symptoms, and the level of risk to kidney disease in asymptomatic individuals. Explanations to the footnotes:

^a Urine specimens should be examined for the presence of non-infectious diseases of kidneys or urinary tract only after exclusion of a urinary tract infection to allow for correct interpretation of leukocyturia and haematuria

^b Patients with increased risk for chronic kidney disease include at least patients with diabetes and cardiovascular diseases, see **Chapter 5.3.1**

^c Details of multiple strip tests, see **Chapter 5.2**, those of protein markers, see **Chapter 5.3**

Concentrations of proteinuria markers should be given together with a measurand of volume rate, e.g., urine relative density together with a strip test result, or urine creatinine concentration with quantitative albumin or other specific protein measurement, see **Chapter 5.4**

RECOMMENDATION: Quantitative specific protein measurements are recommended as primary investigations to high-risk patients for detection and follow-up of kidney disease. (1, A)

1.3.2. Examinations of haematuria and renal particles

Suspicion of a disease in kidneys or urinary tract may be initiated by the patient noticing red urine. Other coloured substances (red beets, porphyria, drugs, **Chapter 5.1, Table 5-1**) should be at first excluded. Particle analysis is needed to obtain a count of *red blood cell (RBC)* excretion from a standardised specimen (see **Chapter 6**). Urine particle analysis is also needed to detect renal elements in patient's urine. The RBC should be assessed for isomorphism and dysmorphism if no proteinuria is present. Isomorphic RBC indicate bleeding from the urinary tract, whereas dysmorphic RBC suggest glomerular bleeding [32, 33] (**Figure 1-3**).

Urine particles (casts, renal tubular epithelial cells) typically confirm the presence or differentiate the type of renal damage. They may also provide prognostic information [34, 35]. Automated particle counting possesses higher precision than visual urine sediment examination, with increasing sensitivity to detect renal particles with technical development [36]. Either advanced automated counting or visual microscopy is recommended to detect specifically a renal disease for patients with a high-risk for renal disease, in addition to proteinuria measurements.

An alternative *chemistry approach to haematuria* is the differentiation of the bleeding site based on urinary IgG/albumin and α 2-macroglobulin/ albumin ratios [37] (see **Chapter 5.3.1**).

RECOMMENDATION: *Either advanced automated counting or visual microscopy of urine particles is recommended to detect specifically a renal disease in low and high-risk patients with proteinuria.*
(1, B)

1.4. Essential information in urinalysis requests

The formats of requests and reports of urinalysis are influenced by the site of examination: at points-of-care, specimen collection and analysis results can be documented directly into the patient record, whereas a remote laboratory always needs a written (paper or computerised) request. The request reaching the laboratory may initiate a stepwise procedure if agreed locally for a particular patient group. Pre-determined strategies aim to maximise diagnostic yield while maintaining cost-efficiency.

The importance of adequate clinical and specimen related information is generally underestimated. Coded information is needed for correct selection of examination procedures and interpretation of results. Sufficient detail is seldom documented for urinalysis specimens. The minimum information is proposed in **Chapter 1.4.1**, to be adopted locally on available electronic requisition platforms and interfaces of clinical and laboratory information systems.

1.4.1. Specimen identification and patient data

The list below compiles key areas of information needed for clinical urine diagnostics. If no information is given, a minimum level of investigations should be applied as agreed locally based on patient populations. On specimen containers, the information is best transferred using waterproof labels (see **Chapter 3.4.4**) providing barcoded specimen ID that is connected to detailed patient data and specific information of each request in laboratory information systems.

- **Patient identification**

- Full name
- Gender (female, male, other)
- Personal ID code (recommended if nationally available)
- Date of birth (if not included in the personal ID code)
- Requesting unit (where patient is being treated)
- Return and billing addresses (to whom laboratory report and invoice should be sent)
- Responsible physician / nurse (to be contacted if consultation is needed)
- Concurrent antimicrobial therapy (if bacterial or yeast culture is requested)
- Additional clinical information for specific specimens (signs, symptoms, or a specific clinical question)

- **Specimen details**

- Specimen identification (ID) code (barcode, if used)
- Date and time of voiding (final real time)
- Way of collection (Mid-Stream Urine, Single Catheter Urine, Indwelling Catheter Urine, Suprapubic Aspiration of urine, Bag Specimen of Urine; Other)
- Storage temperature of the specimen (if different from laboratory's advice)

- **Success in patient preparation**

- Success code of collection (single-voided specimens): qualified specimen or defective collection

(such as untimed collection, urgency, difficulties in technique, etc.; classified by health care personnel when known)

Success in following specific diets before timed collection, e.g., in specific hormone tests

- **Results from rapid examinations** (if performed at point-of-care)

1.4.2. Requesting urinalysis examinations

Locally applied stepwise strategies should be translated into practical requisition routines together with laboratories and clinical units, as agreed locally, and based on expertise, patient populations, and equipment. Adaptation of computerised interfaces between electronic patient records and laboratory information systems with their computerised middleware to analytical devices is highly recommended to improve transfer of patient-specific clinical information and diagnostic reports between clinicians and laboratories [38, 39, 40]. They also support structured patient identification and help to minimise specimen mislabelling [41]. In clinical requisition, decision trees need to be organised locally to support mutually agreed workflows.

Considerable savings usually result if a *sieve principle* replaces manual work, e.g., visual microscopy or bacterial culture is performed only for specimens positive with a sieving examination, such as a multiple strip or an automated particle count. The size of a laboratory and its level of automation have major impacts on the optimisation of workflow in various healthcare systems. In agreed cases, *sensitive* bacterial culture, protein measurements, or visual microscopy should be requested independently of the general workflow optimisation.

RECOMMENDATION: Requisition and reporting of urinalysis tests using electronic interfaces is encouraged, with local diagnostic algorithms. Electronic transfer improves exchange of systematic information between clinicians and laboratories, including specimen details. (1, B)

1.5. RECOMMENDATIONS FOR MEDICAL NEEDS

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
1	Epidemiology and clinical symptoms of the target diseases, as well as diagnostic and prognostic significance of the chosen tests are recommended to guide the clinical use of urinalysis tests.	1, B	1
2	Urinalysis tests should be requested based on assessment of risk or presence of severe or complicated disease. Specific test planning between laboratories and clinics is recommended to balance benefits against resource.	1, C	1.1
3	General screening strategies for low-risk and routine patients (work-flow optimisation) is to be separated from targeted diagnostics for high-risk or complicated patients or specific specimens.	1, C	1.1 – 1.3
4	Asymptomatic bacteriuria must not generally be sought to avoid unnecessary antimicrobials and multiresistant strains of uropathogens. Exceptions include pregnant women, and patients undergoing some invasive urological operations.	1, A	1.2.2
5	Quantitative specific protein measurements are recommended as primary investigations for detection and follow-up of kidney disease in high-risk patients.	1, A	1.3.1
6	Either advanced automated counting or visual microscopy of urine particles is recommended to detect specifically a renal disease in low and high-risk proteinuria patients.	1, B	1.3.2
7	Requisition and reporting of urinalysis tests using electronic interfaces is encouraged, with local diagnostic algorithms. Electronic transfer improves exchange of systematic information between clinicians and laboratories, including specimen details.	1, B	1.4.2

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction [42].

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The EFLM European Urinalysis Guideline Update 2023

Chapter 2. Patient Preparation

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2.1. Patient preparation before specimen collection

2.1.1. Patient as the owner of her/his case

The patient should be treated as the key player and responsible owner of her/his diagnostic investigations, to motivate her/him to learn carefully the procedure of urine specimen collection. The obtained laboratory results do have a direct impact on her/his treatment.

Elderly citizens may particularly think that they cannot discuss and decide upon their diagnostics and treatment options with their doctors [1]. Thus, the health care personnel needs to learn how to empower their patients, rather than treating them as objects of their activity. The **premise** is saving in lost time and money, repeated testing due to non-diagnostic results.

RECOMMENDATION: Interaction with patients shall be improved to invite patients to become active in decision-making on their disease. This would encourage them to learn how to collect a mid-stream urine (MSU) specimen in a best achievable way, in order to minimise contamination during collection. (1, C) ^a

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

The patient must be told why her/his urine specimen needs to be tested. She/he also needs to be given instructions on how it should be collected. Ideally, the instructions should be given both orally and in written form accompanied by illustrations where possible, to ensure uniformity of the collection procedure (see **Annex I.1.1**). Because the same specimen is often shared both for microbiological and chemical measurements, the instructions should combine both requirements. Use of electronic media in editing, storage, and presentation to patients is encouraged.

Success in patient preparation is suggested to be monitored in clinical urine collections. Since an excessive contamination rate of mid-stream urine specimens above the level of physiological microbiota is usual, a quality indicator, QI, is recommended at the laboratory level, to be adjusted after considering the types of specimens and patient populations received by the laboratory. See **Chapter 3.2** for detailed discussion.

RECOMMENDATION: Laboratories shall maintain educational material banks and enforce routine co-operation with their clinical units in order to improve preanalytical processes, including preparation of patients for delivering their urine specimens. (1, C)

2.1.2. Transmission of pre-analytical information

Adequacy of patient preparation, type of urine specimen, and way of collection can be coded at requisition, and followed on the waterproof label adhered onto the specimen container after the collection. The final success may be documented in the laboratory information system (LIS) when receiving the specimen.

After organising the process, this preanalytical information may ultimately be available in the electronic patient record together with the results of examinations to increase the reliability of medical interpretation. An example coding may be, e.g., “qualified” vs. “random”, or “standard” vs. “non-standard” specimen, with additional details of voiding time, bladder incubation time, and way of collection. At least, the verified “standard” mid-stream collections are useful in the laboratory to support investigation of low colony counts. See Chapter **2.2.3**.

2.2. Biological factors affecting results

Biological (*in vivo*) factors, changing the true concentration of a measured component, cause problems in the interpretation of laboratory results although the measurement process itself is correct. In laboratory medicine, these physiological factors are called influence factors (discussed in this chapter).

In addition, other factors may technically interfere with the analytical method applied (called interference factors). These are particularly important with non-specific analytical methods, such as those used in traditional test strip fields (see **Chapter 5.2.2**), but also in other measurements from urine, including drugs [2].

2.2.1. Volume rate (diuresis) and fasting

Many urine constituents change in concentration when the rate of water excretion (diuresis = urine volume rate) alters due to variation in fluid intake, reduction of renal concentrating ability, or ingestion of diuretic substances. The measurand reflecting urine volume rate may be creatinine, osmolality, relative density (old term: specific gravity), or conductivity of the specimen. Measurement procedures are described in detail in **Chapter 5.2.2** (test strips) and **Chapter 5.4.2** (quantitative measurements).

If sensitive screening is needed, a low volume rate (20-50 mL/h or 500-1000 mL/day) is desirable to produce concentrated specimens. This is best achieved in morning urine after an overnight limitation of water intake. A high water intake results in a high volume rate (up to 200-500 mL/h), and dilute specimens with false negative results. The osmolality of human urine may vary from 50 to 1200 mOsm/kgH₂O, an isotonic urine corresponding to about 300 mOsm/kgH₂O [3]. Among healthy adult volunteers, restriction of water to one litre/day created a fluctuation of 600 to 900 mOsm/kgH₂O in urine osmolality, while ingestion of 2.5 L water/day was followed by a fluctuation of urine osmolality from 200 to 500 mOsm/kgH₂O [4].

Starvation decreases urinary constituents provided by diet (e.g., salt and phosphate), but increases the excretion of metabolites associated with catabolism, e.g., ketone bodies and ammonia [5]. In general, fasting for urinalysis is intended to reduce diuresis only. Abstinence of food intake is not needed if water intake is restricted when preparing to collect morning urine. The preparation of patients for standardised fasting blood specimens may, however, be combined with specimens for standard urinalysis if no urgency symptoms are present.

Chemical measurands in urine: Documentation of the urine concentration improves interpretation of results of all chemical measurements in single-voided urine specimens. This has been used most often for measuring albuminuria reported as albumin-to-creatinine ratio, to minimise the intra-individual biological variation [6, 7]. A comparison to a reference measurement also allows better follow-up and classification of patients with albuminuria [8]. See more details in **Chapters 3.1.5** and **5.3**. Diagnostic classifications of other chemical analytes, such as hormones or rare elements in urine, need also an adjustment of urine volume rate if measured from single-voided specimens [9, 10].

Urine particles: Concentrations of urine particles have traditionally been reported without relating them to urine concentration, despite comparing higher concentrations in disease to lower health-

related concentrations. Development of automated particle analysis has reduced imprecision of low counts from those obtained with visual microscopy [11]. The improved accuracy now allows a better classification of leukocyturia and haematuria and justifies the use of diagnostic limits with more precise 3 to 5-fold grey zones. The particle concentrations can be compared to those in standardised morning specimens if the measured urine osmolality is $> 300 \text{ mOsm/kgH}_2\text{O}$ (estimated from urine conductivity), or urine density (by refractometry) is > 1.015 . With dilute urine, a false negative case is possible. The principle of reporting urine concentration always with chemical and particle analyses from single-voided urine specimens was first introduced in the Italian urinalysis guidelines to support clinical interpretation [12].

Urine bacteria: Correlating urine bacteria counts to diuresis is more complex, since bacteria counts in urine depends on the measurement principle of a particle analyser, growth rate of detected bacterial species, incubation time in the bladder, contamination during mid-stream collection, and colonisation of lower urinary tract. As a result, a 10-fold or higher grey zone exists in the cut-off of significant counts in bacteria *counting*, and a 100-fold or higher range may appear in the cut-off of significant colony counts in bacterial *culture* associated with UTI, representing a cut-off from 10^2 CFU/mL to 10^5 CFU/mL (or 10^5 CFB/L - 10^8 CFB/L) in culture [13]. Because of this wide range of significant growth, the variability related to diuresis does not influence the otherwise large uncertainty caused by other factors.

RECOMMENDATION: Interpretation of chemical measurements and particle counts from single-voided urine specimens is improved by reporting concentration of urine (related to diuresis). Chemical measurands are recommended to be reported as measurand-to-reference ratios, e.g., albumin-to-creatinine ratio. Particle counts should be accompanied with results of urine relative density, conductivity, or osmolality. (1, B)

2.2.2. Exercise and body posture

Wide biological variation in urine composition is related to physical activity and body posture. Examination of the morning urine and avoidance of strenuous physical exercise minimises these influences. Exercise may increase the amount of body constituents excreted into urine by increasing glomerular filtration, or other mechanisms. Transient albuminuria or haematuria after exercise are common [14]. On the other hand, urinary calcium excretion increases more than twofold on immobilization of a patient into bed rest [15].

If an orthostatic proteinuria needs to be investigated, the correct clinical interpretation is ensured by specific requests for the overnight and daytime collections. See **Chapter 5.3.2** for detailed interpretation.

Timed overnight urine is collected by emptying the bladder just before going to bed, noting the time (hours and minutes), and then collecting all urine portions during the bed-rest period. At the end of the period, the last portion is collected, the time (hours and minutes) recorded, and the total volume of overnight urine noted. The specimen or a representative aliquot is then sent to the laboratory for calculation of excretion rate of requested analytes.

2.2.3. Incubation time in the bladder

To demonstrate reliable bacterial growth, classical advice is to allow bacteria a log phase of growth by incubating urine in the bladder for 4-8 hours [16]. Urine is a good culture medium for many bacteria. The classical Griess's examination (the nitrite field on a test strip) is more sensitive in detecting asymptomatic bacteriuria among pregnant women from the first morning urine than from a later specimens [17]. Incubation time for at least 4 hours in the bladder before collection improves the sensitivity, and decreases the number of false negative results [16]. If urgency of micturition, or pollakisuria associated with acute lower UTI will not permit sufficient bladder incubation time, interpretation of significant growth needs to be performed at lower colony counts [13]. The bladder incubation time is then useful for interpretation of colony counts in culture. For chemical analyses, incubation time is not necessary.

In studies on urine particle morphology, the best results are obtained after a short incubation time for 1-2 hours because of preserved morphological detail, provided that a high diuresis does not lead to false negative results. Rare particles are seen more often in concentrated urine specimens. For patients, advice to limit water intake to allow longer bladder incubation time, and recording of that time, are highly recommended to reach the highest sensitivities in detection of bacteriuria, and to communicate interpretation correctly.

RECOMMENDATION: Reporting bladder incubation time is recommended to improve interpretation of significance of low bacterial counts, or fragile particles in urine. Urgency or dilute urine is present if the bladder incubation time < 4 hours. (2, C)

2.2.4. Contamination

The detailed discussion is in **Chapter 3.2.**

2.3. RECOMMENDATIONS FOR PATIENT PREPARATION

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
8	Interaction with patients shall be improved to invite patients to become active in decision-making on their disease. This would encourage them to learn how to collect a mid-stream urine (MSU) specimen in a best achievable way, in order to minimise contamination during collection.	1, C	2.1.1
9	Laboratories shall maintain educational material banks and enforce routine co-operation with their clinical units in order to improve preanalytical processes, including preparation of patients for delivering their urine specimens.	1, C	2.1.1
10	Interpretation of chemical measurements and particle counts from single-voided urine specimens is improved by reporting concentration of urine (related to diuresis). Chemical measurands are recommended to be reported as measurand-to-reference ratios, e.g., albumin-to-creatinine ratio. Particle counts should be accompanied with results of urine relative density, conductivity, or osmolality.	1, B	2.2.1
11	Reporting bladder incubation time is recommended to improve interpretation of significance of low bacterial counts, or fragile particles in urine. An urgency, or dilute urine is present if the bladder incubation time < 4 hours.	2, C	2.2.3

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction.

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Chapter 3. Specimen collection and preservation

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3.1. Urine specimens based on timing

The following timing types of urine specimens were modified from classical definitions quoted in textbooks [1, 2] or earlier European guidelines [3, 4]. The actual time of specimen collection should be transferred from the examination request to the examination report to aid in the correct interpretation of findings.

3.1.1. Random urine

Random urine is a portion of single voided urine without defining the volume, time of the day, or detail of patient preparation. Random urine specimen is usually unavoidable in acute situations with dysuria or other emergency symptoms. Random urine specimens are associated with many false negative and some false positive results. These can be reduced if the volume rate (diuresis) is adjusted with a reference measurement. Interpretation of significance of lower bacteria counts in urine culture is correlated with a bladder incubation time less than 4 hours (see **Chapter 3.1.2** for standard bladder storage times before urine collection).

3.1.2. First morning urine

First morning urine is the specimen voided immediately after an overnight bed rest before breakfast and other activities. This is also called early morning urine. If needed, it is recommended that the early morning urine be voided after an 8-hour period of recumbency, and after not less than 4 hours storage time in the urinary bladder (even if the bladder was emptied earlier during the night) [2]. This has been traditionally recommended as the standard specimen for urinalysis and urine bacterial culture, because it is more concentrated than the day urine and allows time for possible bacterial growth in the urinary bladder, and improves sensitivity of nitrite test on the strip for detection of bacteriuria (**Chapter 5.2.2**). This specimen is most easily collected from hospitalised patients but may be collected even at the patient's home if compliance and rapid transportation or preservation of measurands to the laboratory can be organised. In patients with emergency symptoms or dysuria, the first morning urine is usually not possible.

3.1.3. Second morning urine

Second morning urine is a single specimen voided 2-4 hours after the first morning urine. In contrast to the first morning urine, its composition may be affected by prior ingestion of food and fluids and by movement in upright position. However, it may be more practical for ambulatory patients, both for chemical and microbiological analysis. To increase the sensitivities of bacterial culture and particle counting, the quality of the second morning urine should be improved by allowing ingestion of maximum of one glass of water (200 mL) after 22:00 on the previous evening and extending this abstinence up to the time of specimen collection. A bladder incubation time exceeding four hours is possible with this fluid restriction. Postural proteinuria cannot, however, be prevented and should be further investigated by comparing results to those from a first morning urine sample if necessary. If these standardised collection instructions have not been followed for various reasons, the second morning urine is classed as a "random" specimen.

RECOMMENDATION: *The first morning urine is recommended to be collected after an 8-hour period of recumbency, and after an incubation of 4-8 hours in the bladder. The second morning urine is suggested be considered in ambulatory patients, and a random urine in emergency patients if needed. (1, B) ^a*

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

3.1.4. Timed collection of urine

Timed urine is collected at a specified time in relation to another activity e.g., therapy, meals, daytime or bed rest. A *24-hour urine collection* contains all portions voided over 24 hours. A timed 24-hour collection can be started at any time of the day by emptying the bladder and noting the time. All urine during the next 24 hours is then collected and preserved as appropriate for each analyte.

Despite being the tradition, the biological intra-individual variation in excretion of physiological substances to 24-hour collections in healthy individuals is remarkable, and in diseased individuals even higher. Three separate collections are a possibility for epidemiological studies aiming at detailed classification of patient populations [5]. In epidemiological studies, urine creatinine measurements may be utilised to confirm completeness of urine collections. In addition to self-reporting, uses of developed equations [6], or anthropometric reference intervals [7] have been suggested.

Efforts should be made to decrease the frequency of nonconformities in timed urine collections, starting from audits on current local practices, and mutually designed educational events to the healthcare personnel that advises the patients. Both defined quality indicators (**Chapter 3.2**) and availability of counselling for patients remain a continuous need [8].

3.1.5. Measurand-to-creatinine ratios in urine

Assessments of *measurand-to-creatinine ratios* (to compensate diuresis) in single voided specimens have replaced most of the timed collections in the diagnostics or follow-up of patients with proteinuria or some metabolic conditions. Clinically sufficient prediction of 24-hour collection by spot urine measurand-to-creatinine ratio should be confirmed for each new measurand and patient population when single-voided samples are applied for diagnostic classifications. In clinical routine, increase of biological intra-individual variation related to diseases needs to be remembered, as shown for albuminuria in diabetic children with a coefficient of variation (CV) of 61% compared to 19% in healthy children [9].

Applicability of measurand-to-creatinine ratios has been studied, e.g., in orthostatic proteinuria in children [10], alpha-1-microglobulinuria studies [11], or patients with kidney disease [12, 13, 14]. Concerns have been reported for patients with systemic lupus erythematosus when measuring total protein in urine [15].

In pregnant women with suspected pre-eclampsia, ruling-out of proteinuria at 300 mg/24 h seems to be possible, but mid-range excretion was difficult to predict from single-voided samples [16]. An area under curve of 0.69 was detected in ROC analysis of protein-to-creatinine ratios to detect preeclampsia in a systematic review [17]. It is to be reminded that excretion of total protein in 24-h urine may be affected by variable success of completeness of urine collection [8].

Albumin-creatinine ratio at a calculated optimum cut-off of 8 mg/mmol (sensitivity 96% with a specificity 57%) in a single-voided urine sample was the most cost-effective option in health economic assessment of management for severe pre-eclampsia in the U.K., while the receiver-operating characteristics (ROC) curve of albumin-creatinine ratio were similar to those of total protein-creatinine ratio in spot samples of 959 pregnant women [18]. Collection of 24-hour sample was not better over single voided samples in women with hypertension of pregnancy. Quantitative measurements of maximum proteinuria or a rise in proteinuria showed no advantage in the prediction of severe pre-eclampsia or adverse perinatal events [18].

We recommend using albumin-to-creatinine ratio measured from single-voided samples as the primary measurement of renal disease like KDIGO Chronic Kidney Disease Guideline 2012 [19], because (1) the measurement is better standardised than that of total protein in urine, and (2) single-voided samples are practically easier than timed collections, resulting in low incidence of non-conformities in urine collections. Timed collections should be used in primary verification, and occasional confirmations of detected findings. Details on measurements of total protein and different specific proteins are described in **Chapter 5.3**.

RECOMMENDATION: Measurand-to-reference ratios, e.g., relating measurands to creatinine concentrations in urine, from single-voided specimens are recommended to replace timed urine collections for chemical measurements because of the lower incidence of non-conformities. Verification of the intended measurand to a new patient group is needed before clinical application. (1, A)

3.2. Procedures to collect single voided specimens

Urinalysis may be requested on specimens obtained by voiding (micturition), by catheterisation, needle puncture, through a post-operative urostomy, or by using different collection vessels, such as bags or special receptacles for bed-bound patients. The most often obtained specimen is the mid-stream urine (MSU). To benefit from improved accuracy and sensitivity of examination procedures, steps of the preanalytical phase should be reviewed regionally, and standardised [20, 21].

Sexual intercourse should be avoided for one day before specimen collection because of the resulting increased amounts of proteins and cells. Urine from males is usually contaminated with small amounts of secretory products from the prostate. Seminal fluid may contaminate urine after normal ejaculation and in diseases with retrograde ejaculation to urinary bladder. Vaginal secretions or menstrual blood may contaminate urine from females. This may be minimized by tamponing the vagina if acute symptoms necessitate examination of urine during a menstrual period.

The term *contamination* was decided to be kept in this guideline, because at the laboratory level it is difficult to differentiate contamination with skin or urogenital commensal microbes

during collection from those derived from microbes residing in the urinary bladder of asymptomatic individual based on a single specimen (see **Chapter 7.2.1.3**). The microbiological requirements usually determine the details of collection of single-voided urine specimens because urine intended for microbiological examination is frequently requested together with chemical examinations. Then, most single voided samples must avoid or minimise

- contamination of specimens by commensal micro-organisms,
- growth of bacteria following specimen collection,
- damage or death of diagnostically relevant bacteria, and
- disintegration of diagnostic formed elements (microbes, cells, other particles).

No single marker for a contaminated urine specimen exists. The presence of commensal microbes from skin and external genitalia (health-related microbiota), or low-count uropathogens, presence of polymicrobial growth (mixed culture), and numerous squamous epithelial cells in a single-voided urine specimen have been used to indicate contamination during urine collection [22]. A health-related physiological level of urogenital microbiota must be considered. An excessive frequency of contaminated urine specimens received by a laboratory suggests problems in urine collection or preservation before analysis (see **Chapter 7.2.1** for detailed discussion on contamination and health-related urinary microbiota).

Suggested Quality Indicator (QI)

Preamalytical quality indicators (QI) are a developing area in laboratory medicine, encouraging measures for continuous quality improvement [23]. The ISO 15189:2022 requires laboratories to establish and monitor quality indicators to demonstrate performance of their pre-analytical, and post-analytical phases, in addition to analytical quality [21]. To be motivated and used consistently, the developed key performance indicators must adhere to key outcomes of the applied laboratory tests, be easy to measure continuously with defined intervals, e.g., from data in laboratory information systems, have a defined threshold for an acceptable value, and be comparable between different laboratory environments [24].

It is advisable to use defined QI for clinical urine specimens in a way similar to blood specimens, and to describe operating procedures for nonconformities. A plausible QI for urine specimens is contamination rate of single-voided urine collections, as suggested already by the IFCC-Working Group on Laboratory Errors and Patient Safety, WG-LEPS [23]. College of American Pathologists (CAP) followed contamination rates of outpatient urine specimens received by their customer laboratories, expressed as polymicrobial growth (> 2 isolated species) in their external quality assessment surveys, called Q-Probes studies. In their repeated Q-Probes questionnaire to 127 U.S. or Canadian laboratories in 2008, the median rate of polymicrobial growth was 15% at 10^4 CFU/mL (or 10^7 CFB/L) among outpatient specimens, with a 10 – 90% percentile interval 1 – 42% [25]. Refrigeration and instructions given to patients were associated with reduced contamination rates.

By taking the median as a tentative cut-off, a QI is defined as a maximum frequency of 15% polymicrobial growth at 10^4 CFU/mL (or 10^7 CFB/L) in routine cultures of a laboratory. A frequency above that would suggest a need to improve the local processes in single-voided urine collections by the laboratory. A Finnish experience was 12% polymicrobial growth among 56426 routine specimens (53% females) in the Helsinki and Uusimaa regional laboratory service with 300 000 urine specimens cultured annually [26], indicating that a fraction less than 15% is

achievable. A quality indicator is a tool for continuous quality improvement. It should be adapted and followed regularly to reduce the amount of non-diagnostic urine specimens received by a laboratory.

RECOMMENDATION: A quality indicator, QI, is recommended for continuous improvement of routine (or mid-stream) urine specimens. A recommended target for assessment is a maximum rate of 15% polymicrobial growth at 10^4 CFU/mL (or 10^7 CFB/L) in urine culture, unless otherwise estimated at a laboratory level. (1, C)

3.2.1. Mid-Stream Urine (MSU)

Mid-stream urine (MSU) characterises the middle portion of a voided specimen, also called clean-catch urine). Since clean-catch may be confused with cleansing of external genitalia before specimen collection, the precise term mid-stream is preferred. The procedure implies that first portion of urine is not collected, to minimise contamination by commensal skin, genital and urethral microbiota in both sexes (when the specimen should represent bladder urine). The first portion also contains higher counts of squamous epithelial cells, RBC and WBC than the mid-stream portion of urine specimen [27].

Minimising contamination requires detailed patient advice and co-operation when collecting a mid-stream specimen [28, 29], in particular from emergency patients [30], or in infants under 2 years of age [31]. Contamination may reduce the diagnostic value of 40-50 % of mid-stream collections.

3.2.1.1. Importance of cleansing

Washing the introitus around the urethra in females, and the glans penis in males with water only, before micturition, was originally reported to reduce false-positive urine cultures by 20% or more [32]. Later, a study both on non-toilet trained and toilet-trained children showed -25% reduction of false positive strip test results by cleansing [33]. The guideline of Infectious Diseases Society of America (IDSA) [34] still endorses the American Society of Microbiology (ASM) guideline [35] for cleansing because “specimens obtained without skin cleansing routinely contain mixed flora, and yield high numbers of one or more potential pathogens on culture”.

Three systematic reviews on mid-stream (MS) collections vs cleansing before mid-stream urine collection (MSCC) exist with fairly small, partially also same patient groups and various definitions for contamination (commensal species, polymicrobial growth or presence of squamous epithelial cells) [22, 36, 37]. A reduction of contamination rate (defined as polymicrobial growth) by washing was not confirmed in specimens from 165 young symptomatic female outpatients with 27% of polymicrobial growth at 10^3 CFU/mL in culture using MSCC collection compared to 26% in collection of 77 patients with no specific advice [38]. In urine specimens of 113 asymptomatic pregnant women, leukocyte esterase strip test was positive in 50% of cases, and 33-39% contained skin microbiota, but only 1 specimen with polymicrobial growth was seen in 112 MS procedure and 3 specimens in 111 MSCC procedure at 10^4 CFU/mL in culture [39]. Among 158 symptomatic non-pregnant females, 1/93 patients had mixed growth after cleansing and 1/65 patients without

cleansing prior MS collection [40]. These studies remind of the possibility that cleansing is not always needed for a good specimen, but they do not represent the excessive rates of polymicrobial growth seen in large mixed patient populations, perhaps because of selection of the tested individuals for the controlled study. A polymicrobial growth of about 30% at $\geq 10^3$ CFU/mL seems to reflect an average contamination rate in MS collections of advised young females. It corresponds to about 10-15% contamination rate at 10^4 CFU/mL.

Clinical patient populations demonstrate a risk for considerably high contamination rates. Two Scandinavian studies reported a contamination rate (polymicrobial growth) of 38-63% against 10^3 CFU/mL or more in routine urine cultures [41, 42]. On the contrary, after a routine practice with cleansing before MSCC in Finland, the average contamination rate is below 30% at 10^3 CFU/mL, or below 15% at 10^4 CFU/mL in large regional patient populations consisting of 70-80% MSU specimens [26].

As a conclusion, this guideline endorses the American recommendations to carry out midstream collection with cleansing (MSCC) [34, 22]. The MSCC collection may not be needed with all premenopausal women who can expose their urethral orifice by spreading the labia, and collect a proper MS fraction of voiding successfully, and as shown in the quoted studies. MSCC may not be applicable to all women or men with difficulties in the detailed procedure for various reasons, requiring adjustments or professional collections to minimise contamination during collection.

The use of antiseptics, such as benzalkonium or hexachlorophene - or soaps (with variable additives) - is not recommended when washing the outer genitalia to provide a mid-stream urine specimen as this may sterilise the urine [2]. The last portion is also left over after collecting 50-100 mL of urine into the container. Detailed instructions for MSCC with relevant figures are included in **ANNEX I.1**. The use of different sterile devices may help women to urinate more easily into a collection container [43].

RECOMMENDATION: Mid-stream collections are strongly recommended for single voided urine specimens, because of the lower level of contaminants as compared to first-stream specimens. (1, B)

RECOMMENDATION: Cleansing before mid-stream collection is recommended based on practical evidence on increased polymicrobial growth without cleansing among large patient populations. The use of antiseptics is not recommended. By skillful patients, mid-stream urine collection without cleansing may, however, satisfy the diagnostic need. (2, C)

3.2.2. First-void urine

First-void urine specimen is the first portion of urine voided at the beginning of micturition. It is the optimal sample for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and other sexually transmitted bacteria causing urethritis. It is NOT suitable for diagnostics of UTI.

3.2.3. Single Catheter Urine (in-and-out catheterization)

Single Catheter Urine is collected after inserting a sterile catheter into the bladder through the urethra (straight or “in-and-out” catheterisation). For children without urinary control, this is one of the methods to confirm or exclude the presence of urinary tract infection, although contamination rate is higher than that of suprapubic aspiration (SPA) specimen [44]. Single catheterisation is also used by patients with urinary retention or neurogenic bladders. Meticulous technique can reduce contamination with urethral microbiota.

Two practical steps should be implemented: (1) the first few milliliters obtained by catheter should be discarded, i.e., allowed to fall outside of the collecting container, and only the subsequent urine cultured; and (2) if the attempt of catheterisation is unsuccessful, a new, clean catheter should be used; in girls additionally, by leaving the initial catheter in place as a marker [45, 46].

In non-toilet trained infants, it is common to obtain a spontaneously voided or pad / bag urine specimen to screen for possible leukocyturia or bacteriuria. In case of positive rapid test, a Single Catheter Urine or a SPA urine is recommended to confirm the diagnosis.

RECOMMENDATION: Single catheter urine or suprapubic aspiration (SPA) specimen is recommended to establish the diagnosis of UTI in children or older patients without urinary control. (1, B)

3.2.4. Indwelling Catheter Urine

To diagnose catheter-associated urinary tract infection (CA-UTI) from a specimen representing bladder urine, a specimen should ideally be collected after removing the old catheter and taking the sample through the new catheter because of rapid development of bacterial biofilm in urine catheters [47], or within 48 hours after catheter removal as a mid-stream specimen. Pyuria is not diagnostic for CA-UTI [4]. Urine specimens must not be taken from the collection bag of a permanent indwelling catheter. In doubtful cases, a suprapubic aspiration specimen is needed [48].

RECOMMENDATION: Urine specimens must NOT be taken from the collection bag of a permanent indwelling catheter. A specimen shall be collected after removing the old catheter and taking the sample through the new catheter. (1, B)

3.2.5. SupraPubic Aspiration (SPA) urine

SupraPubic Aspiration (SPA) urine is usually collected by sterile aspiration of urine through the abdominal wall from a distended bladder. The benefit of this technique is that it allows a clear-cut decision on the presence or absence of signs related to urinary tract infection with 1% contamination rate, while a single in-and-out catheterisation has a contamination rate of 10% [44, 49].

Indications for SPA include the following clinical situations [50]:

- Urinalysis or urine culture in neonates or children younger than 2 years
- Urinary retention
(e.g., prostate hyperplasia or cancer, gynecologic malignancy, or spinal cord injury)
- Phimosis
- Chronic infection of the urethra or periurethral glands
- Urethral stricture or trauma

Detailed instructions are provided in **ANNEX I.1**. The risk of bladder colonisation by suprapubic aspiration is lower than that by in-and-out catheterisation. Miniaturisation of measurement techniques by laboratories may allow several different examinations from a 5-mL specimen of urine obtained typically by suprapubic aspiration.

3.2.6. Bag or pad urine

Urine bags are often adopted to collect urine from small infants, but they carry high probability of contamination with skin organisms. The entire genital region should be washed carefully with water. A sterile collection bag is applied, and the urine flow checked frequently. Specific collection pads have been developed for urine collection from infants to minimise skin irritation by adhesive tapes. Rapid tests for screening for leukocytes (esterase), erythrocytes (haemoglobin), nitrite or protein by test strips are possible. Collection pads suffer from contamination of urine bacterial cultures like bags, as well as reduced particle counts because they adhere to pad fibres [51, 52].

Diapers or nappies are sometimes suggested as collecting tools for clinical specimens from babies [53]. Due to high variability in the fibre construction of different brands of nappies and inability to measure the urine volumes voided, non-standard diapers are not recommended because specific collection pads are available.

The collection bag should be in place and observed for specimen continuously for a maximum of 30 min, possibly replaced with a new bag, and removed immediately after the observed first void, because of the high probability of contamination [54]. Bag urine becomes easily diagnostically useless due to improper collection. Detected false-positive growth creates problems particularly in the follow-up of infants after a UTI [54]. Negative culture results may be used to exclude UTI. Borderline results need to be re-investigated from a suprapubic aspiration or single catheterised urine specimen. Every positive sample in bag or pad urine should be confirmed by single catheter or SPA urine [55].

RECOMMENDATION: Urine specimens from specific collection pads or bags may be used to exclude UTI in small infants, but they become easily contaminated. Consider spontaneously voided specimens. Non-standard diapers are not recommended. Positive growth should be confirmed by single catheter or SPA urine collection. (1, B)

3.2.7. Spontaneously voided urine

Probability to obtain a spontaneous urine specimen from pre-continent babies is improved by using suprapubic cutaneous stimulation with gauze soaked in cold fluid, called Quick-Wee method, with an increase in the yield from 12% to 31% after 5 minutes [56], despite non-significant difference on contamination rate (27% compared to 45 % without stimulation).

3.2.8. Urostomy

After bladder surgery, urine specimens from *ileal conduits* are frequently obtained through urostomy opening. Paediatric and adult patients with dilated ureters may be given bilateral ureterostomies. Chronic infection and bleeding at the site of the stoma are common. Cleansing the stoma and discarding the first portion of urine obtained through a sterile disposable catheter of suitable size ensures specimen quality.

3.2.9. Segmented urine collection (Meares and Stamey procedure)

The collection of specific segments of urine flow may help in defining abnormal areas of the urinary tract that may need urological attention. Traditional Meares and Stamey collection method [57] remains to be recommended by the European Association of Urology Guidelines to localise male urological infections, such as chronic bacterial prostatitis [4]. Detailed instructions are given in the **Annex I.1.2**. An alternative procedure with collection of two specimens has also been described by researchers of chronic prostatitis [58]. Detailed diagnostics of prostatitis infections are beyond the scope of this guideline.

A dialogue between the requesting clinician and the bacteriology laboratory is essential before requesting any specific culture procedure in order to guarantee the following:

- origin of each specimen (left/right ureter, bladder, or another anatomical site),
- need to identify any bacteria to as low levels as 10^2 CFU/mL (corresponding to 10^5 CFB/L),
- use of large inocula of urine (100 μ L) to ensure accurate plate counts, and
- request of antimicrobial sensitivity testing of any bacteria grown.

3.3. Preservation and transport

The time elapsing between voiding and examination of urine is a major obstacle to diagnostic accuracy in most laboratories. Investigations performed at point-of-care are not subject to this delay but may suffer from analytical problems. Precise collection times must be documented and delays exceeding the specified limits should be stated on reports.

RECOMMENDATION: The actual time of urine collection is recommended to be documented and informed to the analytical site together with the specimen, to allow assessment of acceptability of the specimen after the preanalytical delay and storage conditions before analysis. (1, B)

Test strips: Many chemical constituents examined with test strips do not need preservatives provided the analysis is performed within 24 hours and the tube has been refrigerated. If the specimen contains bacteria and has not been refrigerated, false positive nitrite or protein results may be obtained using multiple test strips. In practice, strip examination should be performed on-site when rapid or refrigerated transportation is not possible. Preservation is important for longer delays. Also, the type of preservation may be critical since some preservatives interfere with enzymatic measurements (**Annex I.2, Table I-2**).

Quantitative chemical measurements: It is known that several specific proteins are unstable in urine, but preservatives can inhibit their degradation [59, 60, 61]. In the present guidelines, the list of preservatives and temperatures acceptable for chemical measurands is limited essentially to urinary proteins and measurands needed for renal stone formers [62, 63] (**Annex I.2, Table I-3**). For a variety of measurands from urine, end-users of different measurement procedures should confirm the primary preservative and storage from their subcontracting laboratory [21], with possible alternatives [64]. Laboratories testing themselves urine analytes are recommended to clarify their primary preservation procedure for each analyte, and possible alternate preservatives against the procedure they are using. A practical two-step assessment protocol to preservation for measurements in ratio scale, including urine specimens, has been suggested by the Extra-Analytical Quality Commission of the Spanish Society of Laboratory Medicine (SEQCML) [65].

Particle examinations: The specimen for particle counting should be refrigerated if not examined within 2-6 hours, despite that precipitation of urates and phosphates will occur in some specimens. If precipitation disturbs interpretation, a new specimen should be kept and examined at $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ to avoid artefactual generation of precipitates. The longer the delay, the more likely are elements to lyse, especially when the urinary pH is alkaline and the relative density is low, as often true with children producing large diuresis [66]. The WBC counts may be questionable after 2-4 hours, even with refrigeration [67]. Traditionally, ethanol (50% volume fraction) was used to preserve the cells but this prevents lysis of red and white blood cells only partially. To avoid shrinkage, polyethylene glycol (2% mass fraction, low molecular mass such as Carbowax[®]) was suggested to be included in the fixative, called Saccomanno's fixative [68]. On mixing equal parts of sample and fixative, the particles should then be stable for 2 weeks. Alternative fixatives also exist [69]. Commercial preservatives, such as buffered boric acid and formate-based solutions are also available [70]. Fixation of urine particles is interesting when planning centralised use of

automated systems. Fixatives may be adapted after verification with a new technology (**Annex I.2, Table I-2**).

Bacterial cultures: Specimen requiring bacteriology investigation must be collected in a clean container and examined in the laboratory within 2-6 hours. They should be refrigerated at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ without preservative if a delay > 2 hours is expected. Then, they should be examined within 24 hours [2, 22]. If the delay is unavoidable and a refrigeration is not possible, containers pre-filled with preservative, e.g., boric acid alone [71] or in combination with formate or other stabilising media [72, 73] must be used. Boric acid will stabilise white cell number and bacterial concentration in urine held at $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 24 hours. It should be noted, however, that borate may inhibit particularly growth of *Pseudomonas* spp. [74]. Since boric acid concentration may be critical for successful preservation, containers containing boric acid shall be filled to the indicated line to achieve the correct borate concentration. (**Annex I.2, Table I-2**).

Biobanks: Urine specimens collected for biobanking have different specifications, depending on the intended analytics. Many analytes are stable in urine specimens maintained at $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 24 h before cryopreservation; however, sensitive analytes exist, e.g., in metabolomics [75].

RECOMMENDATION: Preservation of urine specimens is obligatory if the sample is not analysed within 2-6 hours after voiding. Consider refrigeration if applicable. Guidance to criteria of successful preservation of most common measurements from urine is given in this guideline. (1, B)

3.3.1. Criteria to successful preservation

According to the ISO 15189:2022, Chapter 6.6.3 [21], consumables that can affect the quality of examinations often need a verification of performance with relevant clinical specimens despite validation by the manufacturers, before placing them into use. Many common urine analytes exhibit exponential changes in their concentrations in disease, while some components show linear changes in disease. Criteria for a preserved specimen are suggested accordingly.

3.3.1.1. Chemical measurements

For chemical measurands, exponential changes occur, e.g., in albumin excretion into urine, starting from below 3 mg/mmol creatinine up to 30 mg/mmol creatinine or more in nephropathies (corresponding to 30 to 300 mg/g creatinine, respectively). Excretion of electrolytes and several metabolites, such as sodium, urate or citrate increases or decreases usually in a linear way in diseases. For linear changes, a 90% preservation of original concentration is suggested to be desirable (a minimum is 80%), to remain negligible as compared to intra-individual biological variation (changes in diuresis or diet).

3.3.1.2. Particle counting

WBC and RBC counts vary from below $10 \times 10^6/\text{L}$ to 3-4 exponentials higher counts, and urine bacteria from $10 \times 10^6/\text{L}$ up to 4-5 exponentials higher counts in clinical specimens. The preservation of a particle component in the original urine specimen is suggested to be defined as a maximum of a two-fold change (a maximum loss of -50% or increase of +100%) from the original concentration of urine WBC, RBC or other cells, to remain negligible as compared to an exponentially defined classifications of disease [76, 70].

3.3.1.3. Bacterial culture

Significant limits of bacterial colony counts in culture vary from 10^2 to 10^5 CFU/mL (or 10^5 to 10^8 CFB/L). Less than a three-fold ($0.5 \times \log_{10} = \sqrt{10} = 3.1$) change (increase or decrease) in bacterial concentration in the index urine sample is suggested to be a criterion for successful preservation as compared to the refrigerated control urine sample.

Validation of new types or principles of preservation containers

The following procedure is suggested to be used for validation of new types of preservation containers for bacterial culture, as modified from a published example [77], and preservation studies on particle counting.

Specimens. For assessment of a potential preservation, a representative selection of ATCC or equivalent strains, such as *E. coli* 25922, *E. faecalis* 29219, *P. aeruginosa* 27853 and *S. pneumonia* 6305 is recommended (see **Table 7-5, Chapter 7.4.4.4**). These should be spiked into sterile-filtrated urine from healthy donors not receiving antimicrobial treatment. Also, clinical specimens should be collected, targeting representative uropathogens, and polymicrobial growth at clinically relevant colony counts, and negative specimens in culture (less than 30% of the total amount of specimens).

Procedure. Aliquots of the tested samples are first inoculated into the routine bacterial culture before preservation (time point T_0). For testing a preservation system, the samples are kept at room temperature for 24 hours (time point T_{24}) - and optionally for 48 hours (T_{48}) before inoculating the follow-up bacterial cultures if needed to verify for routine practice (e.g., due to transportation delay). In parallel, positive control (kept at room temperature without preservation) and a reference procedure (refrigerated at $+5^\circ\text{C} \pm 3^\circ\text{C}$ without preservation) tubes of the same samples are cultured immediately, and after the same follow-up periods. Colony counts at T_{24} , and optionally at T_{48} , are compared to the original counts (T_0) within each preservation system or against reference preservation at $+5^\circ\text{C}$, using a contingency table with locally adjusted categories of significant growth. The given example table (**Table 3-1**) may be modified as needed.

Evaluation. Colony counts obtained from a tested preservation system at T_{24} should not differ significantly from those obtained from T_0 specimens and those with the reference system (at refrigerated temperature) at T_{24} . Statistical significance of differences should preferably be assessed using ordinal scale tests (see **Chapter 5.2.3.3** and **7.4.4.5**), comparing sizes of differences from the diagonal agreement. The calculation with the Pearson's chi-square test (= goodness of fit test) is possible if distance from the agreement values is not important. Polymicrobial growth

(mixed flora) is excluded from the frequencies if their colonies are not counted. Assessment of clinical significance should accompany with statistical evaluation.

Table 3-1. Example comparison in preservation of bacterial growth

Preservation system tested or controls		Original growth (T_0), lower limit of each category of colony counts, CFU/mL					
		Negative	10^2	10^3	10^4	10^5	Mixed flora
Follow-up growth (T_{24}), lower limit of each category, CFU/mL	Negative						
	10^2						
	10^3						
	10^4						
	10^5						
	Mixed flora						

Verification of preservation containers before use

A recent study emphasizes the importance to evaluate even commercially available preservation containers in clinical laboratories, to ensure relevant performance characteristics before use [78].

The preservation system may be tested by using *spiked pooled urine* made from sterile filtrated urine obtained from healthy donors not receiving antimicrobial treatment. A few relevant reference strains or equivalent may be sufficient to show the applicability (see **Chapter 7.2.2, Table 7-3**). A specification of less than a three-fold ($0.5 \times \log_{10} = \sqrt{10} = 3.1$) difference should be verified between the tested preserved samples at 18 ± 2 °C and the refrigerated control samples at $+5^\circ\text{C} \pm 3^\circ\text{C}$ after 24 hours. A method for quantitation is recommended in **Chapter 7.4.4.4**.

3.4. Collection containers

Sterile sampling of urine is important for microbial tests from urine but it may influence some chemical measurements, too. Sterility of collection vessels means that the interior of the unopened and unused container is free from interfering microbial contaminants. Container manufacturers must document their product's compliance with the intended clinical use (see **Chapter 7.5.2** for significance limits of bacterial growth). It is to be remembered that a particle analyser or a nuclear amplification method will detect even non-revivable bacteria. Furthermore, since waste is an increasingly important problem globally, the development of environmentally safe materials is encouraged for all disposable containers.

Containers and test tubes (receptacles) that contain or preserve urine specimens after collection shall comply with *the European Regulation 2017/746 on In Vitro Diagnostic Medical Devices* (IVDR, instruments and their accessories), according to the given Definitions of medical devices in the Article 2 [79]. They belong to *class A devices* according to the Classification Rule 5c of the Annex VIII of the IVDR regulation. *The European Regulation 2017/745 on Medical Devices* (MDR) covers some devices used for primary specimen collection, classified either as non-invasive class I devices (e.g., bags), or *class IIa-IIb invasive devices* (e.g., urine catheters) by the Rules 1 and 5-6 in the Annex VIII of the MDR regulation [80].

The following subchapters contain practical details considered to be important for collection, transportation and analytical containers used for urinalysis tests, including urine bacterial cultures.

RECOMMENDATION: Technical features of urine collection containers given in this guideline are recommended to be followed by the manufacturers to improve the quality of clinical urine specimens. The given specifications are open for revisions after technical or clinical evidence. (1, B)

3.4.1. Collection containers for different types of specimens

Single-voided specimens: The design of collection containers should enable detection of uropathogenic bacteria even in special situations, i.e., at as low as 10^1 CFU/mL level (equivalent to 10^4 CFB/L) [2]. The primary collection container should be clean and have a capacity of at least 50-100 mL with an opening of at least 5-cm diameter to allow easy collection of urine by both men and women. The container should have a wide base to avoid accidental spillage and should be capped so that it can be transported and stored without leakage of its contents. The container and its cap should be free from interfering substances and should not absorb nor change the urine constituents to be examined. Those parts of the container and its cap, which come into contact with the urine specimen, should not contribute to microbial contamination after specimen collection.

Timed collections: For many chemical constituents, quantitative excretion rates are important. A container designed for a 24-hour or overnight urine collection should have a capacity of 2-3 L. The container should be constructed from materials that prevent

- adherence of urine constituents,
- exposure of urine to direct light that might alter clinically significant metabolites,

- contamination from the exterior when closed.

Stabilizers usually prevent metabolic and other changes of urine constituents. The container should allow for use of recommended preservatives in **ANNEX I.2, Table I-3**.

3.4.2. Transport, storage and analytical containers

Secondary containers (for basic urinalysis and bacterial culture, usually examination tubes) should be easily filled from the primary container without risk of spillage or contamination. The tube should be translucent to allow a clear view of the sample. Moreover, the possible adverse effect of vacuum aspiration on particle concentrations should be minimised, since a high suction power through a small gauge syringe may destroy particles during aspiration into the secondary containers [81, 82].

Urine specimen should preferably be divided into aliquots according to their preservation needs before transportation. A range of needed volume is usually 1 – 10 mL for chemical and morphological investigations, and occasionally up to 100 mL for special chemistries. For microbiological analysis, 1-3 mL of urine in a *clean* container is sufficient. For large laboratories, a standardised vessel with a volume of 3-10 mL is essential for automated analytical systems.

Examination tubes for *test strip measurement, particle counting, or urine bacterial culture* should keep the specimen suitable for analysis at $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ or at $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ (tubes without preservatives in bacteria investigations) as specified for at least 24 hours, preferably for 72 hours (over the weekend may not be applicable for urine culture). Specifications to assess preservation are in **Chapter 3.3** and collected details on allowable preservation times are compiled in **ANNEX I.2, Table I-2**.

For *urine particle analysis* and *bacterial culture*, uncentrifuged specimens are primarily recommended. Investigation of concentrated urine sediment (by centrifugation) is needed for low-concentrations of renal particles only. Traditional urinalysis tubes have been conical to allow decanting of supernatant to concentrate the specimen after centrifugation. A more accurate sediment volume and concentration of particles is obtained by suction of the supernatant, followed by gentle re-suspension of the sediment into an accurate volume. In addition to automated tracks a round-bottom tube works better than a conical tube, and should be considered when counting urine particles after centrifugation [83]. The examination procedures for particle counting are described in **Chapter 6.2.3** (routine), and **Chapter 6.2.2** (reference procedure).

The examination tubes used for specimens for *quantitative chemical analysis* should keep the specimen intact and the cap should remain closed upon freezing and during centrifugation up to $3000 \times g$ (relative centrifugal force, RCF) for 15 min. The size, structure, and length of the secondary container vary depending on the needs of the diagnostic procedures. Preservation of specimens for investigations related to kidneys and urinary tract are described in **ANNEX I.2, Table I-3**.

3.4.3. Order-of-draw – from primary container into secondary containers

Order of draw from the primary container to be used in filling the secondary containers is proposed to be:

- (1) Initial 1 tube without preservatives to test the practice of filling (e.g., tube for strip test or albumin-to-creatinine ratio) if given to a patient without previous experience in filling of secondary containers
- (2) Tubes for microbial tests – first tubes without preservatives, then tubes with preservatives
- (3) Tubes for chemistry tests – possible non-preservative tubes if not used in step (1), then preservative tubes

The suggested order-of-draw reflects an assessment by the working group that the risk of interference with chemistry test results by additives of microbiology tubes in cases of carry-over is less likely than the risk of contamination of preservative-containing microbiology tubes with skin bacteria of the patient if filling all non-preservative tubes first (including tubes for chemistry tests).

3.4.4. Labelling

All clinical specimen containers must be labelled with a waterproof tag that remains adherent during refrigeration and when frozen. Labels with SPREC codes [84] are recommended for biobanking purposes when details of preanalytical steps need to be included. Otherwise, the label created by hospital or laboratory information system should include a bar code that is traceable to details of the requested sample: a code of the examination requested, patient identification and requesting unit, provisional or recorded collection time, way of collection, and any additional pre-analytical information in coded form.

Detail of possible preservatives should be shown on a separate label, including any appropriate hazard symbol. Labelling should not prevent a clear view of the specimen. The label must be placed on the container, not on the cap.

3.4.5. Packaging

When body fluids are mailed to a distant laboratory, additional biohazard labels should be added. The packages should comply with requirements for the Category A of infectious substance (UN 2814) or category B (UN 3373) for possible infectious substance that not meet the criteria for inclusion in Category A. Prepared packages shall be transported as dangerous goods according to United Nations recommendations on the Transport of Dangerous Goods [85].

3.5. RECOMMENDATIONS FOR COLLECTION AND PRESERVATION OF SPECIMENS

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
12	The first morning urine is recommended to be collected after an 8-hour period of recumbency, and after an incubation of 4-8 hours in the bladder. The second morning urine is suggested be considered in ambulatory patients, and a random urine in emergency patients as needed.	1, B	3.1.1 – 3.1.3
13	Measurand-to-reference ratios, e.g., relating measurands to creatinine concentrations in urine, from single-voided specimens are recommended to replace timed urine collections for chemical measurements because of the lower incidence of non-conformities. Verification of the intended measurand to a new patient group is needed before clinical application.	1, A	3.1.5
14	A quality indicator, QI, is recommended for continuous improvement of mid-stream urine specimens. A provisional target for assessment is a maximum rate < 15% of polymicrobial growth at 10 ⁴ CFU/mL (or 10 ⁷ CFB/L) in urine culture, unless otherwise calculated at a laboratory level.	1, C	3.2
15	Mid-stream collections are strongly recommended for single voided urine specimens, because of the lower level of contaminants as compared to first-stream specimens.	1, B	3.2.1
16	Cleansing before mid-stream collection is recommended based on practical evidence on increased polymicrobial growth among large patient populations without cleansing. The use of antiseptics is not recommended to avoid inhibition of growth. By skillful patients, mid-stream urine collection without cleansing may, however, satisfy the diagnostic need.	2, C	3.2.1
17	Single catheter urine or suprapubic aspiration specimen is recommended to establish the diagnosis of UTI in children or older patients without urinary control.	1, B	3.2.3
18	Urine specimens must NOT be taken from the collection bag of a permanent indwelling catheter. A specimen shall be collected after removing the old catheter and taking the sample through the new catheter.	1, B	3.2.4

19	Urine specimens from specific collection pads or bags may be used to exclude UTI in small infants, but they become easily contaminated. Consider spontaneously voided specimens. Non-standard diapers are not recommended. Positive growth is recommended to be confirmed by single catheter or SPA urine collection.	1, B	3.2.6
20	The actual time of urine collection is recommended to be documented and informed to the analytical site together with the specimen, to allow assessment of acceptability of the specimen after the preanalytical delay and storage conditions before analysis.	1, B	3.3
21	Preservation of urine specimens is obligatory if the sample is not analysed within 2-6 hours after voiding. Consider refrigeration if applicable. Guidance to criteria of successful preservation of most common measurements from urine is given in this guideline.	1, B	3.3 and ANNEX I.2
22	Technical features of urine collection containers given in this guideline are recommended to be followed by the manufacturers to improve the quality of clinical urine specimens. The given specifications are open for revisions after technical or clinical evidence.	1, B	3.4

^a Strengths of recommendations are: 1= strong, 2 = weak recommendation. Levels of evidence are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating [86] is described in the Introduction.

3.6. References, Chapter 3

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Chapter 4. Accuracy levels of urinalysis examinations

Timo Kouri

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4.1. Terminology used to describe accuracy of examinations

In urinalysis, a general term “examination” is formally used instead of the term “measurement” that refers to assessment of quantities, since clinically investigation of urine also detects and differentiates urine particles or bacterial species, i.e., qualitative or nominal properties [1]. Urinalysis tests and urine bacterial cultures traditionally consist of visual microscopy, chemical strip tests, and manual cultures of bacteria with various uncertainties of results. The term *nominal scale examination* is used to describe a procedure for detection and identification of nominal properties.

Before clinical use of new devices and technologies, a comparison of a new examination procedure to the old assay for the same analyte (measurand) is needed. To avoid comparisons of mismatched pairs (like comparing “apples” with “oranges”), an examination procedure with a higher order of accuracy is then required, to allow estimation of accuracy of both the new and the old field procedure. In metrology, accuracy is defined as “closeness of agreement between a measured quantity value and a true quantity value of a measurand” [1]. A traceability chain is formed from a sequence of calibrations from a highest available reference system to the actual routine measuring system. Measurement uncertainty increases, and accuracy decreases along the sequence of calibrations from the primary reference measurements to the routine field measurements [2].

In the clinical accreditation practice, a new examination procedure shall be validated for its analytical performance, and verified by the end-user laboratory before its intended use [3]. Consequently, a reference procedure is required for any new procedure for the purpose of clinical use, as reminded in the accreditation standard.

For purposes of assessment, the examination methods in urinalysis and urine bacterial culture are classified into the following three levels of accuracy (**Table 4-1**).

Table 4-1. Levels of accuracy of urinalysis examination methods

Level 1. Rapid methods, used in emergency or point-of-care needs
Level 2. Field methods, used in standardised routine examinations
Level 3. Advanced comparison methods, used as references for field methods

Because of simplicity and robustness in non-laboratory and emergency use, most rapid tests (**Level 1**) apply either ordinal scale or single cut-off values of a ratio scale in reporting. Because of their measurement technology, the obtained results typically contain inherent inaccuracy. In the assessment, they should be compared to a higher order method, i.e., to a quantitative field method (**Level 2**) for the same measurand. The quantitative field methods in clinical chemistry are usually reported in ratio scale and traced back to a higher order reference procedure (**Level 3**) with available calibrators that support their accuracy.

The standardised counting of urine particles (visual microscopy or automated counting) and routine bacterial culture of urine specimens represent *Level 2* examination procedures, but a traceability to a higher order reference procedure has not been a rule. The EFLM European urinalysis guideline stresses the importance of advanced reference procedures, or comparison methods (*Level 3*) for these examinations as well.

RECOMMENDATION: Clinical laboratories are recommended to express clearly, which level of analytical performance (Levels 1-3) is the target, when they establish their urine examination procedures, including nominal scale examinations. (1, B)

4.2. Level 1: Rapid methods

Rapid methods are used to provide fast, sufficiently accurate results to emergency needs in clinical service. Many instruments are portable and designed for easy handling and quality control, to be applicable to points-of-care. Single or a few diagnostic cut-off values for positive results are typically applied in reporting. In larger laboratories, parallel advanced technologies and devices have been developed to manage larger workflows of rapid tests.

A traditional example of rapid tests discussed in this guideline is the multiple test strip of chemical analytes. Results from the strip pads have been reported in ranks, officially “ordinal scale quantity values”, classified as “negative”, “1+”, “2+” or “3+”, or with arbitrary concentrations, due to the uncertainty of the chemical reactions on the pads. The term “semi-quantitative” is no more recommended to describe ordinal scale (See **Chapter 5.2.2** for details of measurements with multiple strip tests).

NOTE: Urine particle counting is occasionally also called as “rapid method” in the context of bacteriology if used to detect bacteriuria and leukocyturia in urinary tract infections, before results from urine bacterial culture are available. The principle of quantitative urine particle counting belongs, however, to Level 2 procedures.

4.3. Level 2: Routine or field methods

Clinical laboratories report most of their results “quantitatively”, i.e., in ratio scale because of clinical need for patient follow-up or classification. Advanced technology and human assessment require experienced laboratory personnel, which leads into centralised testing and automated methods. The routine or field methods have been developed by optimising performance and speed, to satisfy requirements of turn-around time. Their accuracy is classified into *Level 2* in this guideline. The accuracy of routine quantitative methods is better than that of *Level 1* methods, because the measurement procedures have been confirmed by reference procedures and materials.

Urinalysis tests belonging to *Level 2* include quantitations of chemical analytes and routine counting of urine particles. Quantitative measurements of proteinuria are discussed in **Chapter 5.3.2** and those of volume rate (diuresis) in **Chapter 5.4.2**. Routine counting of urine particles is described in **Chapters 6.2** and **6.3**. Routine bacterial culture of urine specimens also belongs to this category as discussed in **Chapter 7.4**.

4.4. Level 3: Advanced comparison methods

Reference examination procedures (*Level 3*) are needed to obtain results with higher accuracy than with routine methods (*Level 2*), to allow advanced comparisons to higher order references. Typically, advanced comparison methods are not applicable for routine use, because they need extra resource or time.

The official description of a reference procedure is as follows: “Reference measurement procedure is a measurement procedure accepted as providing measurement results fit for their intended use in assessing measurement trueness of measured quantity values obtained from other measurement procedures for quantities of the same kind, in calibration, or in characterizing reference materials” [1].

For nominal scale examinations, the trueness may be expressed as misclassification rates of identified properties (such as categories of counted urine particles or bacterial species in culture), or by probabilities of confidence for reported classifications (such as confidence index for mass spectra).

The analyte in urine particle counting is complex (mixture of particles with variable clumps, sizes or shapes in counting), and that in bacterial culture is both complex and biologically evolving (colonies of variable microbes and their variants in culture). That is why the proposed *Level 3* procedures are needed to provide improved accuracy of examinations, but they are not as accurate as the reference procedures described to stable molecules, e.g., for blood haemoglobin A_{1c} [4] or plasma creatinine [5].

The advanced reference procedure, called advanced comparison method for urine particle counting is discussed in **Chapter 6.2.3** and that for urine bacterial culture in **Chapter 7.4.4**.

4.5. RECOMMENDATIONS FOR CLASSIFICATION OF EXAMINATIONS

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
23	Clinical laboratories are recommended to express clearly, which level of analytical performance (Levels 1-3) is the target, when they establish their urine examination procedures, including nominal scale examinations.	1, B	4

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction.

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Chapter 5. Chemistry

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List of abbreviations, Chapter 5

ACR	Albumin-to-creatinine ratio
AKI	Acute kidney injury
APS	Analytical performance specification
CFB	Colony-forming bacteria
CFU	Colony-forming unit
CKD	Chronic kidney disease
CRM	Certified reference material
CV	Coefficient of variation
CVD	Cardiovascular disease
EAU	European Association of Urology
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
eGFR	Estimated glomerular filtration rate
EQA	External Quality Assessment
ERA	European Renal Association
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESRD	End-stage renal disease
FN	False negative
FP	False positive
GFR	Glomerular filtration rate
ICSH	International Committee for Standardization in Hematology
IDMS	Isotope-dilution mass spectrometry
ISO	International Organisation for Standardization
IVDR	In vitro diagnostic medical device regulation
JCGM	Joint Committee for Guides in Metrology
KDIGO	Kidney Disease Improving Global Outcomes (initiative)
LoC	Confirmation limit
LoD	Detection limit
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight (mass spectrometry)
MDR	Medical device regulation
MS	Mass spectrometry
NGAL	Neutrophil gelatinase-associated lipocalin
NIST	National Institute of Standards and Technology (U.S. Department of Commerce)
RBC	Red blood cell(s), erythrocyte
SI	International system of unit
URL	Upper reference limit
UTI	Urinary tract infection
VIM	International vocabulary of metrological terms
WBC	White blood cell(s), leukocyte

5.1. Visual inspection and odour of urine

The most traditional urinalysis was based on human senses. Abnormal colour or odour of urine is often reported by the patient, and may occasionally provide clues to an underlying disease. Since these are related to sense perceptions of urine colour or odour, no standard differentiation is expected from the laboratories. Some traditionally reported causes for abnormal colour or turbidity of urine are given in the **Table 5-1**, to be considered as background hints for clinical inquiries [1, 2, 3]. The normal urine is generally mild in odour. Abnormal colour or odour of urine is harmless if related to ingested food or drugs. Infected urine may be ammoniacal or fetid. Some metabolic diseases have characteristic urine odours (**Table 5-2**).

Patient-oriented information on abnormal colour of urine is provided on-line, e.g., by the MedlinePlus website, a service of the National Library of Medicine (NLM), which is part of the National Institutes of Health (NIH) [4]. Patient-oriented lists of abnormal odours of urine are provided, e.g., by the Mayo Clinic [5] and by the National Health Service, NHS, in the U.K. [6].

Table 5-1. Characteristic appearances of urine.

Modified from references [1, 2, 3].

Appearance	Cause	Remarks
Colourless	Dilute urine	polyuria, non-fasting specimen
Cloudy, turbid	Phosphates, bicarbonates, urates	
	Leukocytes, RBC, bacteria, yeasts, spermatozoa, mucin, crystals, pus, tissue, faecal contamination, radiographic dye	may indicate UTI rectovesical fistula possible
Milky	Pyuria	infection
	Chyluria	lymphatic obstruction
	Paraffin	vaginal cream
Blue-green	Biliverdin	
	Pseudomonas infection	small intestine infections
	Drugs: Methylene blue, occasional drugs possible	mouth deodorants
Yellow	Flavines (acriflavine, riboflavine)	vitamin B ingestion

Yellow-orange	Concentrated urine	yellow foam
	Urobilin, bilirubin	
	Rhubarb, senna	
	Drugs: Salazosulfapyridine, phenacetin, pyridine derivatives, rifampicin	alkaline pH
Yellow-green	Bilirubin-biliverdin	yellow foam
	Riboflavin	
	Thymol	
Yellow-brown	Bilirubin-biliverdin	beer brown
	Drugs: Nitrofurantoin	
Red or Brown	Haemoglobin, RBC	positive strip result, menstruation
	Myoglobin	positive strip also; muscle injury
	Methaemoglobin	acid pH
	Bilifuscin	result of unstable haemoglobin
	Urobilin	
	Porphyrin	may be colourless
	Beets, rhubarb, carotene	alkaline pH
	Fuchsine, aniline derivatives	foods, candy
	Drugs: Aminophenazone, aminopyrine, antipyrine, bromsulphthalein, cascara, chinine, chloroquine, chrysarubin, hydroquinone, L-Dopa, naphthole, phenytoin, metronidazole, nitrite, nitrofurantoin, phenacetin, phenolphthalein, phenothiazine, salazosulfapyridine, senna, thymol	
Red-pink	Urate	may be associated with (massive) crystalluria
Red-orange	Drug: Rifampicin	
Red-purple	Porphyrins	may be colourless
Brown	see above	
Brown-black	Methaemoglobin	blood, acid pH
	Homogentisic acid	alkaptonuria (alkaline pH)
	Melanin/melanogen	rare

Darkening upon standing	Porphyrin, homogentisic acid, melanogen, serotonin
	Drugs: cascara, chlorpromazine, methyldopa, metronidazole, phenacetin, imipenem

Table 5-2. Characteristic odours of metabolic diseases
Obtained from reference [1].

Odour	Disease
Sweaty feet	Isovaleric acidemia and glutaric acidemia
Maple syrup	Maple syrup urine disease
Cabbage, hops	Methionine malabsorption
Mousy	Phenylketonuria
Rotting fish	Trimethylaminuria
Rancid	Tyrosinemia

5.2. Multiproperty test strips

Because many urinary tract diseases present acutely, there is a need for rapid diagnostics, frequently at points-of-care. Then, the first urinalysis measurement is often performed by using a test strip (dipstick) at an ordinal scale, now officially “ordinal quantity-value” scale [7], and historically a “semi-quantitative” scale. In addition to points-of-care, the strip tests may be performed in laboratories, together with other examinations of urine.

5.2.1. Diagnostic significance of test strips

The aim of the classical multiple test strip is to perform routine chemical analysis in one single operation, with an increased yield of diagnostic or prognostic information. Multiple test strips, officially called “multiproperty” strips, have been designed to detect several of the following components: leukocytes (white blood cells, WBC), bacteria (nitrite), erythrocytes (red blood cells, RBC), protein (albumin), glucose, ketone bodies, pH, relative density, bilirubin, urobilinogen, and ascorbic acid. A minimum combination depends on the intended use and health-care setting. A maximum of 11 test areas needs instrumental analysis, a visual reading is not recommended. High false-positive rates emphasize the need for laboratory confirmation of positive results.

RECOMMENDATION: Multiple (multiproperty) test strips are still recommended as screening tools for routine patient populations because of their cost-efficiency. Conventional strip tests are NOT sensitive enough for diagnostics of patients with high-risk to kidney disease (patients with diabetes or cardiovascular diseases), or complicated UTI patients. (SoR 1, LoE A)^a

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

5.2.1.1. Urinary tract infections (UTI): bacteriuria and pyuria

Detection of UTI, adults

Rapid screening for urinary tract infection (UTI) by using a test strip (dipstick) measurement from a urine specimen, by counting of urinary leukocytes, bacteria (and erythrocytes), or as a combined “urinalysis”, is needed in clinical practice [8, 9, 10]. No laboratory tests are needed for otherwise healthy non-pregnant female patients with sporadic symptoms of uncomplicated lower UTI, who may be treated based on symptoms as confirmed with a questionnaire providing an Acute Cystitis Symptoms Score, ACSS. Recurrent lower UTI, and other patient groups need laboratory investigations (see **Chapter 1.2** and **Chapter 7.1.2**).

Diagnostic performance of test strips in detecting bacterial UTI must be interpreted carefully, since the selected cut-off for significant colony counts in culture affects the performance. Also, definition of UTI may or may not include presence of both pyuria (leukocytes in urine) and clinical assessment, changing the comparison of performance [11, 12].

The combination of either nitrite or leukocyte result positive is generally most useful in screening, since the nitrite test has a high specificity, while the leukocyte esterase test improves the sensitivity. In a meta-analysis of 72 studies, a combined sensitivity of either leukocyte esterase or nitrite result was 80 % against bacterial growth at $\geq 10^5$ CFU/mL (10^8 CFB/L) in culture, but it was decreased to 67 % against $\geq 10^4$ CFU/mL (10^7 CFB/L) in culture, and down to 45 %, when assessed at $\geq 10^3$ CFU/mL (10^6 CFB/L) [13]. The studied patient population affected the performance, the sensitivity of 88% being highest in ambulatory care close to patient data (family practice). The combined strip tests were more efficient in ruling out than ruling in patients with UTI, indicating a need for additional diagnostics [13].

Patient's symptoms and signs raise the pretest probability for UTI. In a meta-analysis of 16 studies on uncomplicated UTI of non-pregnant women, symptoms of dysuria, frequency or urgency of micturition provided a sensitivity of 62-88% with a specificity of 21-51% in detecting UTI, while symptoms of haematuria had a specificity of 87% as compared to bacterial growth at $\geq 10^3$ CFU/mL (10^6 CFB/L) [14]. Vaginal discharge decreased the probability of UTI. Nitrite and erythrocyte fields in multiple strips may help in ruling in UTI among patients with acute infection-related symptoms. Leukocyte field is generally used to confirm the presence of pyuria, or to rule out UTI in symptomatic patients [13]. The post-test probability of uncomplicated UTI in women with either dysuria, urgency or frequency was 78 - 81% after a positive leukocyte or nitrite strip result, but 18-20 % after a negative leukocyte or nitrite strip result against bacterial culture at $\geq 10^3$ CFU/mL (10^6 CFB/L) [14]. Diagnostics of recurrent or complicated UTI is NOT recommended with test strips only.

Suspicious of upper UTI and catheter-associated UTI

Fever with flank pain indicates an upper urinary tract infection with renal involvement (pyelonephritis). These patients should be investigated thoroughly as hospital emergencies, including urine particle analysis (leukocytes and bacteria, and possible renal particles), bacterial culture and clarification of possible proteinuria, and estimation of glomerular filtration rate (GFR), with locally verified diagnostic algorithms [15].

Use of test strips for pyuria is not recommended to diagnose for potential catheter-associated UTI [16]. Institutionalised elderly citizens have often asymptomatic bacteriuria or pyuria that is not necessarily associated with generic symptoms or falling of the residents [17]. Laboratory tests should be requested from the elderly patients after a clinical intention to treat only, to avoid misleading results (leukocyturia and bacteriuria without symptoms of UTI) and unnecessary antimicrobial treatments.

Detection of UTI, children

Diagnosis of UTI in infants at 2-24 months of age is based on the presence of both pyuria and bacteriuria at least $\geq 5 \times 10^4$ CFU/mL (5×10^7 CFB/L) of a single uropathogenic organism in an appropriately collected specimen of urine. Results in the range of $10^3 - 10^4$ CFU/mL ($10^6 - 10^7$ CFB/L) need an assessment of the context, such as symptoms, quality of specimen, and urinalysis findings [10].

In detection of paediatric and adult UTI, the diagnostic performance of both leukocyte esterase and leukocyte counts varies by urine concentration. It is particularly important that a patient, particularly an infant, is not drinking too much to initiate micturition before collecting the specimen. A measurement of urine concentration (e.g., relative density with a strip test, conductivity of a particle analyser, or osmolality in intensive care if needed) is recommended for interpretation of urinalysis results of paediatric patients, to avoid false negative diagnostics from dilute specimens [18, 19].

RECOMMENDATION: No laboratory tests are recommended for otherwise healthy non-pregnant female patients with sporadic symptoms of uncomplicated lower UTI. (1, A)

RECOMMENDATION: Rapid tests to detect UTI should include tests for detection of both leukocytes and bacteria. (1, A)

RECOMMENDATION: Rapid tests are recommended be requested from elderly patients after a clinical intention to treat only because of a high prevalence of asymptomatic bacteriuria. (1, A)

RECOMMENDATION: Concentration of urine is valuable in interpretation of urine specimens of paediatric patients, to alert of dilute specimens. (2, B)

5.2.1.2. Haematuria

Haematuria, i.e., increased amount of blood or haemoglobin in urine, is a common finding with a prevalence in the range of 4-5%. Causes can be classified into prerenal, renal and postrenal groups (**Table 5-3**). Haemoglobin without RBC may be detected in haemolytic states, and in patients with haematuria if the cells have been destroyed (either *in vivo* or *in vitro*) due to a delay in investigation.

In differential diagnostics, causes of haematuria related to specimen collection and artefacts (reddish colour without haemoglobin) should be considered as well. Myoglobin in urine creates a positive test strip result for RBC because it contains also a haem moiety that exhibits pseudoperoxidase activity. Myoglobin is demonstrated in urine of patients with muscle necrosis, rhabdomyolysis or polymyositis, or myopathies, such as caused by statins used for hypercholesterolaemic patients. Specific measurement of myoglobin or creatine kinase in plasma or serum may confirm the presence of myoglobinuria.

Differentiation of haematuria based on urinary protein measurements is discussed in **Chapter 5.3.1, Table 5-13** and **Table 5-14**.

Table 5-3. Causes of haematuria

Classification	Examples
<i>Prerenal haematuria</i>	Bleeding tendency
Systemic diseases	Haemolysis (causing haemoglobinuria)
<i>Renal haematuria</i>	Glomerulonephritis, such as IgA nephropathy
Renal disease	Renal infections, such as tuberculosis, epidemic nephritis
	Renal tumours
	Ischaemic disease of renal vessels, acute kidney injury
	Strenuous exercise
<i>Postrenal Haematuria</i>	Ureteral stone
Diseases of the lower urinary tract	Tumours of the urinary tract
	Urinary tract infection
	Operation or catheterisation of the urinary tract
	(Prostate disease rarely)
<i>Specimen-related causes</i>	Menstrual bleeding
	Gynaecological disease
	Intensive genital washing before collection (children, elderly patients)
<i>Artefacts</i>	Urate precipitate (infants with diapers)
Reddish colour without haemoglobin	Beets in the diet
	Drugs, e.g., nitrofurantoin, ibuprofen (see also Table 5-1)

5.2.1.3. Proteinuria

Prevalence of proteinuria, as detected with a test strip at about 200 mg/L (corresponding to about 100 mg/L albuminuria), is globally around 2% among adult populations, being somewhat higher in Japan as compared to U.S. The observed prevalence increases with age up to 5% at 80 years due to vascular diseases and diabetes in older individuals [20]. In Japan, proteinuria and haematuria have been screened with a test strip from all school children and adults ≥ 40 years of age because of the highest national prevalence of end-stage renal disease (ESRD) in the world. Proteinuria assessed with a traditional strip test has predicted ESRD better than elevated plasma creatinine

concentration. The screening has been considered cost-effective in Japan, finding 68% of the new IgA nephropathy cases [20].

Sensitive albuminuria screening at 5-10 mg/L is not warranted for total populations because of costs created by management of consequent investigations when as high as 12-18% tested individuals may become positive for moderate albuminuria (in Asia) [21]. Targeted screening of moderate albuminuria, previously called "microalbuminuria", has been suggested for high-risk groups in addition to diabetes patients, such as those with hypertension [22, 23].

A moderately increased albuminuria corresponds to a persistent albumin excretion rate (AER) 3-30 mg albumin/mmol creatinine, not reached with a conventional protein/albumin strip test, and a severe albuminuria an AER of 30 mg albumin/mmol creatinine or more [24]. Quantitative measurements of proteinuria are discussed in **Chapter 5.3.** in more detail.

Proteinuria is not always related to a renal disease. Causes of proteinuria are listed in **Table 5-4.**

RECOMMENDATION: Sensitive albuminuria screening for incipient chronic nephropathy is not recommended at an epidemiological level because of costs of follow-up investigations. A targeted screening of high-risk patient populations (e.g., patients with diabetes and cardiovascular diseases) is recommended. (1, B)

Table 5-4. Causes of proteinuria
Modified from reference [25].

Main groups	Classification	Examples
<i>Intermittent</i>	Functional	Fever proteinuria Exercise proteinuria Congestive heart failure Epileptic seizures
	Orthostatic	Occurs in upright position only
	Factitious	Urine manipulation (Munchausen's syndrome)
<i>Persistent</i>	<i>Pre-renal</i>	Immunoglobulin heavy and light chain excretion (= Bence-Jones proteinuria) Myoglobinuria (in rhabdomyolysis) Haemoglobinuria (haemolysis)

<i>Renal</i> , divided into	
Glomerular	Albuminuria in glomerular nephropathies
Tubular	Low-molecular-mass proteinuria caused by nephrotoxic drugs, tubulo-interstitial nephritis
Mixed (glomerular and tubular)	Ischaemia Excretion of high molecular protein (IgG) in advanced renal disease
<i>Post-renal</i>	Urinary tract infection Postrenal bleeding Prostatic or bladder disease Vaginal discharge

5.2.1.4. Measurements of urine concentration on test strips

Relative density (official Nomenclature term: Relative volumic mass; old term: Specific gravity)

Results from all chemical measurements and particle examinations from single-voided specimens need to be related to the state of water excretion (volume rate, diuresis) to allow proper clinical interpretations [19] (**Chapter 2.2.1**). The relative density obtained with the chemical test strip is a rough estimate of urine concentration [26], see **Chapter 5.2.2**.

Medical indications for proper quantitative measurements of urine concentration are described in **Chapter 5.4.1**, and their measurement procedures in **Chapter 5.4.2**.

Creatinine

Creatinine measurement has been traditionally used to estimate excretion rates by relating urine concentrations of proteins [27], hormones [28] or other analytes to that of water in single-voided specimens. New applications have been introduced for test strips to sensitively measure albumin-to-creatinine ratios from patient urines, see **Chapter 5.2.2**.

RECOMMENDATION: Urine concentration is recommended to be reported together with all chemical and particle examinations from single-voided urine specimens. (1, B)

5.2.1.5. Tests related to diabetes and other metabolic conditions

Glucose

Examinations of urine glucose concentrations have largely been replaced by measurements of blood glucose concentration [29, 30]. Measurements of glycosuria are used for specific clinical or scientific purposes only.

Urine glucose measurements were traditionally advocated to check for inappropriate use of blood examinations, or for patients unwilling to use blood sampling in addition to laboratory monitoring of haemoglobin HbA1c concentrations. However, it is NOT a sensitive screening tool for diabetes [31]. Occasionally, finding of marked glycosuria may reveal patients with uncompensated diabetes mellitus in acutely ill patients (to be confirmed from blood glucose measurements), or in pregnant women. Glycosuria is the mechanism of action of inhibitors of sodium-glucose cotransporter protein 2 (gliflozins) used to treat type 2 diabetes.

Ketone bodies

Ketone bodies (acetoacetate, beta-hydroxy butyrate and acetone) are excreted into urine in diabetic acidosis, during strenuous exercise, fasting, during enteric inflammations or periods of vomiting. The chemical reaction used is sensitive to acetoacetate and acetone, but not beta-hydroxybutyrate. Ketone bodies serve to classify or treat specified patient populations, such as patients admitted as emergencies (especially paediatric patients), juvenile-onset and specific subtypes of diabetic patients, or patients with toxemia of pregnancy. Ketosis may be created also by ingesting popular ketogenic diets. Plasma hydroxybutyrate measurements are important for the follow-up of comatose ketoacidosis patients to improve the adjustments of clinical treatment. Slight ketosis is detected even after overnight fasting, indicating an acceptable clinical sensitivity.

pH

Urinary pH varies between 5 and 9. Concentrated morning urine is usually acidic, with a pH around 6. Urines from children are often alkaline. Urea metabolising bacteria transform urea to ammonia and may increase the pH of urine to become alkaline. Survival of leukocytes [32] may be reduced in dilute and alkaline urines, typically in children UTI [33]. Casts are also lost in alkaline urine [34]. Measurements of urine pH are needed for the diagnosis of acid-base disturbances, or in monitoring of specific diseases, such as renal tubular acidosis or recurrent renal stone disease. Elimination of specific drugs (e.g., cytotoxic drugs) may be enhanced by medical acidification or alkalinisation of urine.

Measurements of urine pH have been suggested to help in avoiding nitrofurantoin treatment of UTI in patients with urine pH 8 or higher, because *Proteae* group bacteria (e.g., *Proteus mirabilis*, and other *Proteus* spp.) increase urine pH by breaking urea. An increased resistance of 33% to nitrofurantoin by *Proteae* group is detected in urine specimens with pH 8-9, as compared to that of 20% in specimens with pH 5-7. In a retrospective study of emergency department, concerning 67127 urine cultures, only 3% (369/12275) of positive specimens in bacterial culture had both bacteria resistant to nitrofurantoin and pH 8-9, downshifting the importance of urine pH measurements in selection of antimicrobial treatment [35].

Bile pigments

Measurements of urinary urobilinogen and bilirubin concentrations have lost their clinical significance in the detection of liver disease after application of modern blood tests with better

diagnostic performance [36]. Routine measurements of bile pigments in urine are considered obsolete tests, as concluded in a national guideline as well [19].

Ascorbic acid

Because many patients ingest vitamin C in large quantities (> 1 g/day), measurement of ascorbic acid concentration in urine helps in identifying those patients prone to false negative test strip results. In a Korean study, vitamin C was detected in 18% of urine samples. False negative results were observed in 42 % samples with glycosuria, in 11 % of those with haemoglobinuria, and in 8 % of those with leukocyte esterase after ingestion of vitamin C [37]. Another, more direct approach is to develop test strips insensitive to interference by ascorbate.

RECOMMENDATION: Plasma hydroxybutyrate measurements are recommended for the follow-up of comatose ketoacidosis patients instead of urine strip tests. (1, B)

5.2.2. Measurement procedures with multiproperty test strips

5.2.2.1. Detection principles

The technology and principles employed in traditional test strips have been widely studied since 1960's. The limitations of strip technology have been summarised in textbooks [1, 38], and are quoted in the manufacturers' documents. Summary of these analytical principles, as modified from the mentioned textbooks, is compiled in the **Table 5-5**. Any new drug may, however, represent a new potential source of interference.

Table 5-5. Detection principles and their limitations on multiple strips

Modified from references [1] and [38].

Analyte	Measurement principle	False negative results	False positive results
Leukocytes (WBC)	Indoxyl esterase activity (granulocytes and macrophages; not present in lymphocytes)	Vitamin C (intake Grams/day), protein > 5 g/L, Glucose > 20 g/L, mucous specimen, cephalosporins, nitrofurantoin; mercuric salts, trypsin inhibitor, oxalate, 1% boric acid	Oxidizing detergents, formaldehyde (0.4 g/L), sodium azide, coloured urine (beet ingestion, bilirubinuria)
Bacteria (Nitrate reductase positive) ^a	Nitrite detected with Griess' test (azo dye)	No vegetables in diet, short bladder incubation time, vitamin C, Gram+ bacteria, <i>Pseudomonas aeruginosa</i>	Coloured urine, in vitro growth
Erythrocytes (RBC)	Pseudoperoxidase activity by the haem moiety of haemoglobin	High nitrite concentration, delayed examination, high density of urine, formaldehyde (0.5 g/L)	Microbial peroxidases, oxidizing detergents, hydrochloric acid
Albumin (protein), conventional	Non-specific binding to indicator dye	Globulins, immunoglobulin light chains hardly detected; coloured urine	Alkaline urine (pH 9), quaternary ammonium detergents, chlorhexidine, polyvinylpyrrolidone (blood substitute)
Glucose	Glucose oxidase and peroxidase	Vitamin C, urinary tract infection	Oxidizing detergents, hydrochloric acid
Ketone bodies (acetoacetate; acetone)	Nitroprusside reaction (Legal's test)	Improper storage, beta-hydroxybutyrate not detected	Free sulphhydryl groups (e.g. captopril), coloured urines, L-dopa

pH	Two indicator dyes giving a pH range 5-9	Formaldehyde lowers pH	
Relative density (specific gravity)	Ionic solutes of urine react with poly-electrolytes on the strip	Falsely low: glucose, urea, alkaline urine	Falsely high: protein > 1 g/L, ketoacids
Creatinine	Oxidative reaction, copper chelate, or dinitro benzoate reaction ^b	EDTA	
Urobilinogen	Azo reaction with a diazonium salt; Ehrlich's aldehyde reaction	Formaldehyde (2 g/L), exposure to light	Sulphonamide and other drugs, coloured urine; porphobilinogen (Ehrlich)
Bilirubin	Azo reaction with a diazonium salt	Vitamin C, high nitrite concentration, exposure to light	Coloured urine, chlorpromazine metabolites
Ascorbic acid	Reduction reaction with an indole dye	Not known	Similar reducing agents
ADDITIONAL ANALYTES			
Albumin, sensitive	Immunochemical or dye-binding procedure ^c	Not known for dye-binding procedures; immunochemistry may suffer from hook effect in high concentrations, or non-reactivity to modified albumins	Haemoglobin or myoglobin above 50 mg/L [39]; quaternary ammonium disinfectants, chlorhexidine [40]

^a Bacteria are detected on the basis of nitrate reductase present in most Gram-negative uropathogenic rods, such as *E.coli* (Griess's test), reducing dietary nitrates into nitrite.

^b Example measurements on strip are described in the text.

^c Examples for the dye-binding principle of albumin measurement on the strip are given in the text.

Leukocytes (WBC, Esterase)

The analytical sensitivity of the esterase strip is about 80-90% at the detection limit of 20×10^6 WBC/L against visual or automated counting of fresh uncentrifuged specimens [41]. The agreement between test strip and particle counting depends on the statistical imprecision of reference counts, analytical imprecision of reflectance readings, level of lysis of the granulocytes on the strip, and preservation of urine specimens before counting. At 100×10^6 WBC/L, a sensitivity of 95% should be reached. Specificity at a detection limit of 20×10^6 WBC/L is about 80-90%, also for statistical reasons. Lysed cells are classified as negative in particle counting, but show enzymatic activity on the strip pad, reducing the observed specificity. Subtilisin of known activity may be used as a quality control solution.

NOTE. Sensitivity to detect pyuria (leukocytes) is not the same as a sensitivity to detect an infection with either leukocyte or nitrite field of the strip. See **Chapter 5.2.1.1**.

Bacteria (Nitrite)

Nitrite examination is based on activity of nitrate reductase that is present in most Gram-negative uropathogenic rods, such as *E.coli* (Griess's examination). Nitrate reductase is, however, lacking from *Pseudomonas aeruginosa* and Gram-positive uropathogens such as *Enterococcus spp.* and *Staphylococcus spp.*, and will therefore not be detected whatever their urinary concentration. The positive detection of bacteria requires, in addition, ingestion of nitrate by the patient (vegetables), its excretion into urine and a sufficient incubation time in bladder for reduction to nitrite. The analytical sensitivity of the method is reported to vary between 20-80% against the culture, depending on the patient population and the cut-off limit for positive culture (with the highest performance against 10^5 CFU/mL, or 10^8 CFB/L) [42, 43, 13]. The diagnostic specificity of this field for bacteria is high (>90%).

Erythrocytes

The presence of red blood cells (RBC), haemoglobin or myoglobin in urine is seen either in dotted (cells) or homogenous appearance of colour on the reagent pad. Unfortunately, this pseudoperoxidase activity degrades rapidly even when the specimen is refrigerated, and is remarkably sensitive to various preservatives. The analytical sensitivity of the test strip is about 80% at 10×10^6 RBC/L against particle counting [41]. Specificity of RBC detection with a strip test is reduced when compared with particle counts because RBC lyse easily in urine, and occasional urine specimens contain haemoglobin from in vivo haemolysis or myoglobin in rhabdomyolysis. Also, statistical imprecision of both low counts and low reflectance signals affects the agreement.

Protein

Total urinary protein is a mixture of high molecular weight (e.g., albumin, transferrin, intact immunoglobulins, α_2 -macroglobulin) and low molecular weight proteins (e.g., α_1 -microglobulin, retinol-binding protein, immunoglobulin light chains) sieved from plasma, proteins secreted by the kidney (uromucoid or Tamm-Horsfall protein) and those derived from the urinary tract. The traditional test strip field is 90-95% sensitive to clinical albuminuria at a concentration of about 200 mg/L protein or 100 mg/L albumin [44]. It is less sensitive for mucoproteins and low molecular weight protein, and almost insensitive for immunoglobulin light chains. The quantitative comparison methods, e.g., pyrogallol red or benzethonium chloride precipitation, measure better the various globulins than the strip (see **Chapter 5.3.2**), which affects the analytical sensitivity and specificity of a strip measurement against these comparison methods, in addition to imprecisions of procedures.

Albumin, sensitive procedure on the strip

For early detection of glomerular damage, sensitive immunochemical procedures [or cheaper dye-binding procedures [45, 39] have been introduced. Later, tetrachloro-tetraiodo-fluorescein [46], or tetrabromo-phenol blue [40] have been adopted to measure albumin concentration. Sensitive strips should reach a limit of quantitation at 10 mg/L albumin (or albumin-to-creatinine ratio 3 mg/mmol) to qualify moderate albuminuria screening in detecting incipient nephropathy.

Relative density (old term: specific gravity; official term: relative volumic mass)

The conventional measurement on a multiple strip is dependent on the ion exchange reaction with polyelectrolytes on the strip pad that has a tendency to provide falsely high and low densities of urine even after correction of pH [47]. In particular, diluted samples may remain unnoticed despite that those samples should be detected to reveal false negative results. Refractometric measurements used by automated test strip analysers in the laboratory are less prone to that error. Relating urine WBC counts to the relative density values of urine specimens of infants with a cut-off of 1.015 improves accuracy of laboratory assessment, in particular with dilute specimens [18], although impact on clinical decisions of antimicrobial treatment for infants is less pronounced [48]. A measurement on the strip pad is not recommended for intensive care patients and only with limitations for in-patients [49]. For quantitative measurements of urine concentration, see **Chapter 5.4.2**.

Creatinine

Dye-binding procedures for determining the creatinine concentration in urine on a test strip include complexing with Cu^{2+} ions added with an oxidizable dye [39], a chelate reaction [46], or dye-binding with dinitrobenzoic acid [40].

Glucose

Enzymatic measurements are usually based on glucose oxidase reaction that is almost quantitative. The analytical performance of ordinal scale glucose measurement usually satisfies the clinical need.

Ketone bodies

The nitroprusside reaction (Legal's test) does not detect the most important ketone body, beta-hydroxybutyrate, but it can be used for screening ketosis states due to various causes. Unspecific reactions, lack of sufficient reference material, and inaccuracy of detection limit obscures clinical interpretation of this examination.

pH

pH of urine is measured with a pair of pH-sensitive indicator dyes. The accuracy within 0.5-1 pH unit is usually obtained in the External Quality Assessment schemes.

Ascorbate

Ascorbic acid (vitamin C) interferes with the measurement of several test strip analytes. Its specific measurement may improve detection of false negative results in patient specimens.

RECOMMENDATION: From specimens of intensive care and in-patient groups with needs of improved accuracy, urine concentration is recommended to be measured by using refractometry or osmolality. (2, B)

5.2.2.2. Instruments used for multiple test strip examinations

Instruments (rather than the naked eye) are recommended for reading a multiple test strip, whether in the laboratory or at point-of-care, because observer-related major errors occur

frequently in practice and are not traceable afterwards. All laboratory examinations including those performed at point-of-care sites should meet the required quality as described in the ISO 15189:2022 [50].

The selection of different instruments is determined by the local diagnostic processes. Centralised laboratories providing a 24-hour service tend to automate the analysis of large numbers of specimens, while point-of-care sites show an increasing interest to improve the quality of their single patient investigations. Automated urinalysis aims to improve the precision and accuracy of results at higher level than that achieved by traditional semi-automated methods. Automated systems shall be verified against quantitative reference procedures, using their own quantitative reflectances in measurement comparisons [51, 41]. Smaller devices (from regional laboratories or point-of-care sites) may be verified against the index instrument at the central laboratory using ordinal scale cross-tables if quantitative signals are not accessible to the laboratory.

Turnaround time, cost containment and safety of the working environment are important issues in routine workflow in all diagnostic environments. Low-resource environments with limited access to centralised testing are particularly interested in studying the possibilities of point-of-care technology for health screening programmes [52]. Advanced mobile phones may also become tools for instrumental reading of laboratory tests in the future.

RECOMMENDATION: Urine strip tests are recommended to be read with instruments both in laboratories and points-of-care, using qualified procedures, to avoid human errors in interpretation of results. (1, A)

5.2.2.3. Qualified procedures for test strip reading

The following tables **Table 5-6** and **Table 5-7** are provided for practical help in auditing the various steps of test strip reading. The given lines intend to help in developing qualified routine operating procedures.

Table 5-6. Visual reading of test strips (auditing list)

Item	Standard	Method of checking
Identification of specimen	Label the specimen	Compare label with the computerised or manual working list if analysing several specimens at once
Homogenous specimen	Mix immediately before dipping	Even colour
Temperature of the specimen	+20°C ± 5°C	Allow to stand for 15-30 min before analysis to cool down after voiding, or warm up
Quality of strips	Date still acceptable	Expiration date checked
Environment	Sufficient light	Artificial light is an adequate substitute for daylight to allow easy reading;
	Calm space for working	Allow no other activity during the procedure
Dipping	Follow manufacturer's guidance for routine practice	Observation by trainer
Timing	Use a timer showing time in seconds at reading	Not possible afterwards
Reading	Compare with the colours on the packing vial	Train before actual patient analysis
Internal quality control	Control solutions measured daily if analysis is done daily	Follow-up charts maintained
External quality control	Participation expected, organised with local supporting laboratory that typically contacts an EQA service provider nationally available	Reports available
Storage of strips	No physical problems associated with storage	Outlook of the strips (bent, wet etc), closed vials, temperature of the storage shelf
Reporting	Use the predefined format and units; Fill in the patient record or working list immediately	Train before actual patient analysis

Table 5-7. Reflectometric reading (auditing list)

Item	Standard	Method of checking
Identification of specimen	Label the specimen	Compare label with the working list on screen if analysing several specimens at once; Confirm data transfer according to local protocol
Homogenous specimen	Mix immediately before dipping	Even colour
Temperature of the specimen	$++20^{\circ}\text{C} \pm 5^{\circ}\text{C}$	Allow to stand for 15-30 min before analysis to warm up, or cool down after voiding
Quality of strips	Date still acceptable	Expiration date checked
Protocol for instrumental measurement	Protocol written locally after training for both instrument and data transfer	Written protocols available
Internal quality control	Control solutions measured daily, following the principles described in Chapter 5.2.3.3	Follow-up charts maintained
External quality control	Participation expected, organised with national or foreign EQAS provider or within smaller groups	Reports available
Maintenance	Instrument manual followed	Documentation of service and repairs
Calibration of the instrument and methods, changes of reagents	Analytical performance specifications are given in Tables 5-8 to 5-12 of Chapter 5.2.3. IVDR regulations by the European Council followed	Documentation of validation by the manufacturer (IVDR), as verified by the end-user laboratory; changes of strip lots recorded

5.2.3. Performance specifications for test strips

The quality of test strip measurements is included in the current ISO 15189:2022 standard [50]. The analytical performance specifications (APS) for ordinal scale strip tests are suggested in this chapter. For optimal results, performance of each instrument must be verified and ambiguities clarified with the manufacturer. When operating procedures have been developed, instructions must be carefully followed in the analytical process for optimal results.

When verifying the performance of a test strip analyser, quantitative signals (original reflectances) are preferred over the categorised ordinal scale arbitrary concentrations, to be able to visualise the observed imprecisions of reflectance readings [53, 51, 41].

5.2.3.1. Trueness in ordinal quantity scale

APS for ordinal scale measurements have not been discussed widely. Criteria are suggested for multiproperty (multiple) urine test strips based on upper health-related reference limits, analytical performance and statistical tests applicable to ordinal scale. The detection limit was created by multiplying the approximate healthy upper reference limits of concentrations in morning urine by a factor of 2, to avoid transient positivity at the grey zone due to intra-individual (biological) variation. The trueness of ordinal scale measurement may be expressed by using a detection limit (LoD) and a confirmation limit (LoC) from the comparison measurement. The ratio between concentrations LoC / LoD is about five based on the experience on the accuracy of reflectance measurements. They delineate a grey zone [54]. Below the detection limit, a strip examination should remain negative, while above the confirmation limit, it should be positive. At the grey zone, a gradual transition from negative to positive results should occur.

The following detection limits (LoD) and confirmation limits (LoC) are proposed for the usual test strip fields (**Table 5-8**).

Table 5-8. Suggested detection and confirmation limits for multiple test strips

Property (analyte)	Comparison method	Detection limit (LoD)	Confirmation limit (LoC)
Leukocytes (x 10 ⁶ /L)	Chamber counting ^a	20	100
Erythrocytes (x 10 ⁶ /L)	Chamber counting ^a	10	50
Albumin (protein) (g/L)	Dye binding	0.1 (alb), 0.2 (prot)	0.5 (alb), 1 (prot)
Nitrite (mg/L)	Weighing out dry sodium nitrite, applicable comparison method	0.5	2.5

Glucose (mmol/L)	Quantitative method (glucose dehydrogenase or hexokinase method)	3	15
Ketones (acetoacetate; mmol/L)	Weighing out Li acetoacetate	1	5
pH	pH meter (potentiometry)	± 1 unit ^b	N/A ^b
Relative density	Refractometry	± 0.005 ^b	N/A ^b
Creatinine (mmol/L)	Enzymatic (kinetic recommended)	4	N/A ^b
Urobilinogen (μ mol/L)	Not commonly available	20 ^c	100 ^c
Bilirubin (μ mol/L)	Bilirubin solution	10 ^c	50 ^c

^a Chamber counting of fresh (less than 2 hours) uncentrifuged specimens.

^b N/A = detection and confirmation limits not applicable; an arbitrary class width is given.

^c Urobilinogen and Bilirubin are considered obsolete tests in detection of liver disease, as compared to blood tests. For urobilinogen, no commonly available comparison methods exist. Manufacturers should document their validation.

To allow sensitive detection of albuminuria (microalbuminuria range), the following performance specifications are given to sensitive rapid albumin measurements (**Table 5-9**). Albumin concentrations (mg/L) are not expressed with substance-based unit (mol/L) to be comparable with total protein concentrations used in **Table 5-8**.

Table 5-9. Detection and confirmation limits for sensitive albumin examinations

Property	Comparison method	Detection limit (LoD)	Confirmation limit (LoC)
Albumin (sensitive; mg/L)	Immunochemical (quantitative)	10	50
Albumin (sensitive): Creatinine ratio (mg/mmol)	Albumin as above, ratio to quantitative creatinine method	3	15

5.2.3.2. Analytical performance specifications for trueness of test strips

The ordinal scale performance can be described as sensitivities and specificities, i.e., as maximal allowable fractions of analytically false positive (FP) or false negative (FN) measurements against best practical comparison methods. If not otherwise clinically justified, the trueness of a test strip field is judged as shown in **Table 5-10**. Optimal trueness of measurements is suggested to be a FP rate < 10% at LoD and a FN < 5% at LoC, when compared with an applicable quantitative procedure. The tighter optimum of FN reflects the fact that detection of existing pathology is clinically more critical than reinvestigation of FP cases. In many situations or with a less optimal comparison method, a minimum performance may be acceptable.

Table 5-10. Analytical performance specifications for trueness of test strip examinations

Performance	$FP_D = b / (a+b)$	$FN_G = c / (c+d)$	$FN_C = e / (e+f)$
Optimum	< 10%	< 30 %	< 5%
Minimum	< 20%	< 50%	< 10%

Example: Leukocyte detection by esterase activity

Acute urinary tract infections are associated with urinary leukocyte counts ≥ 100 -200 WBC $\times 10^6/L$, while at the level of < 10 WBC $\times 10^6/L$, no association exists [55, 56]. What is the performance of a strip procedure with leukocyte esterase to detect pyuria? Test strip results are compared with chamber counts of WBC from freshly voided (< 2 hours) urines with the following example data (**Table 5-11**):

Table 5-11. Example data for estimation of trueness of test strip examinations

Comparison method (WBC $\times 10^6/L$)	Negative < 20	Grey zone 20 - 99	Positive > 100	Total
Test strip result				
Negative	200 (a)	25 (c)	5 (e)	230
Positive (1+ or more)	80 (b)	100 (d)	40 (f)	220
TOTAL	280	125	45	450

Limits:

LoD

LoC

The following fractions describe the trueness of measurements:

- 1) The fraction of false positives at the detection limit (LoD) = $FP_D = b / (a+b)$
(in the example: $80 / 280 = 0.29$ or 29%)

- 2) The fraction of false negatives at the grey zone area = $FN_G = c / (c+d)$
(in the example: $25 / 125 = 0.20$ or 20%)
- 3) The fraction of false negatives at the confirmation limit (LoC) = $FN_C = e / (e+f)$
(in the example: $5 / 45 = 0.11$ or 11%)

In this example, a theoretical strip field has a poor performance in FP_D , but an optimum performance at FN_G , and a minimum performance of FN_C (due to random variation, or possible problems with lysis of leukocytes on the reagent pad etc.). In this case, the “too high” sensitivity may, however, not be true, but reflect delayed counting and disruption of leukocytes in diluted urines, in addition to random variation of the measurement. With many comparisons, such as in leukocyte and erythrocyte detection, the different principles of measurement procedure (enzyme activity vs. chamber counting) must be understood for correct interpretation. The same applies to comparisons of bacterial culture with chemical strips or particle counting.

5.2.3.3. Concordance analysis

Agreement of ordinal scale data should be visualised with cross-tabulation. In statistical analysis, the agreement expected by chance must be subtracted. One possible tool is to calculate Cohen's kappa coefficient [57, 58]. It is an easily understandable way to show agreement between two or more ordinal scale categories, such as test strip results obtained from two different measurement procedures. Weighted kappa should be calculated, when assessing agreement of cross-tables with four or more ordinal scale results to those measured with a quantitative comparison procedure.

If a formal significance testing is needed, the p value from McNemar's test can be calculated. Modules to calculate simple and weighted κ (kappa) coefficients are found in many existing statistical software packages. The description below is intended to be a simplified explanation for laboratory professionals.

$$\kappa \text{ (kappa)} = (P(o) - P(e)) / (1 - P(e)) = 1 - (Q(o) / Q(e))$$

where $P(o)$ = observed probability of agreement,
 $P(e)$ = expected probability of agreement by chance,
 $Q(o)$ = observed disagreement = $1 - P(o)$, and
 $Q(e)$ = expected disagreement by chance = $1 - P(e)$.

In a 2x2 table, an analytical sensitivity of 90% and specificity of 90% result in a κ (kappa) = 0.8. $\kappa = 0.8$ means that the non-random agreement = $P(o) - P(e)$ was obtained with the examined method in 80% out of all expected disagreement by chance = $Q(e) = 1 - P(e)$. A sensitivity of 80% and specificity of 80% result in $\kappa = 0.6$. A zero value means no deviation from a random distribution (equivalent to sensitivity of 50% and specificity of 50%). Kappa coefficient varies between -1 (complete disagreement) and +1 (complete agreement).

For multiple (4-5) categories, the agreement should be calculated based on weighted Kappa coefficients. Since the sum of expected disagreement exceeds 100% because of the squared weighting factors, the goal must be tighter (**Table 5-12**).

Table 5-12. Targets of ordinal scale agreement using Kappa coefficients

Type of recommended coefficient	Optimum	Minimum
κ coefficient (simple), 2-3 categories	> 0.8	> 0.6
κ coefficient (weighted), 4-5 categories	> 0.9	> 0.7

5.2.3.4. Precision and internal quality control

The low positive range (1+) is more important than the high positive range (3+) in rapid examinations screening for positivity. It is recommended that internal quality control is established by using continuous reflectance values from reflectometers organised as Levey-Jennings quality control charts. These allow verification of reproducibility and routine follow-up of measurements [46, 41].

Dilutions of control solutions (with buffer or pooled human negative urine) help in following performance at low concentrations if a stable low positive control solution is not available. However, since the pad on a test strip has an impact on measured reflectance, a commercial low positive control solution to the corresponding reflectometer is preferred, because dilution of a highly positive control solution with aqueous buffers may create unexpected uncertainty.

RECOMMENDATION: Performance of test strip measurements is recommended to be verified against quantitative measurement procedures, and monitored internally by using continuous reflectance values from reflectometers, and control solutions close to the limit of positivity of each measurement. (1, B)

5.3. Proteinuria measurements

The principal classification of proteinuria is described in **Chapter 5.2.1.3**.

For many urine components, a quantitative result is needed in the diagnosis and follow-up of patients. This used to involve timed 24-hour or other collections of urine with calculated excretion rates of the analytes. As a practical alternative, a reference measurement to adjust for water excretion (creatinine) is recommended, and calculation of a measurand-to-creatinine ratio as a routine measurement of protein excretion. See **Chapter 3.1.5** for detailed discussion.

5.3.1. Diagnostic significance of proteinuria

In 2017, about 700 million people have been estimated to suffer from chronic kidney disease (CKD) globally, corresponding to an age-standardised prevalence of about 8.7%, with a range from 5% in Western Europe to 12% in Eastern Europe and Oceania, associated with about 1.2 million deaths annually [59]. Early detection of kidney diseases and their differentiation challenge laboratory diagnostics and interdisciplinary care because [60]:

- Kidney diseases are usually asymptomatic initially, and become diagnosed late
- Patients with kidney disease have an increased morbidity and mortality already in the early stages of their disease
- Kidney diseases diagnosed late have an increased rate of progression
- High costs of treatment of end-stage renal disease (ESRD) may be avoided, or delayed with early intervention

KDIGO Work Group for Chronic Kidney Disease (CKD) suggests detection of CKD with measurements (1) of plasma (serum) concentrations of creatinine or another glomerular marker to estimate glomerular filtration rate (GFR), and (2) of urinary albumin/protein with the following priority [24]:

1. urine albumin-to-creatinine ratio (ACR),
2. urine protein-to-creatinine ratio (PCR),
3. test strip urinalysis for total protein with automated reading, or
4. test strip urinalysis for total protein with manual reading.

5.3.1.1. Total protein, albumin and other glomerular proteins

A recent review summarises clinical uses of glomerular filtration rate (GFR) estimates and albuminuria measurements in the evaluation of **acute and chronic kidney diseases** [61].

Measurement of total protein excretion was traditionally used to detect a kidney disease. Total protein measurement fails to provide both accurate and highly sensitive screening for CKD [62]. It is, however, useful in screening for proteinuria in situations beyond albumin excretion. Glomerular nephropathies are characterised by increased excretion of albumin, transferrin, and in the advanced stage with unselective leakage, additionally by high molecular mass proteins such as immunoglobulin G (IgG) [63].

Detection of an early glomerular disease, such as incipient nephropathy needs a measurement of albuminuria that is more sensitive than the traditional total protein or strip test measurement. Albumin excretion rate is elevated years before the reduction in glomerular filtration rate (GFR), stressing the importance of its predictive value [64].

With respect to *cardiovascular disease* (CVD), albuminuria is known to be a strong predictor of cardiovascular damage in type 2 diabetes mellitus [65]. The detection of albuminuria is a risk factor in non-diabetic hypertensive nephrosclerosis [66], and vascular disease [67]. Thus, measurements of albuminuria are important (1) in exploring possible kidney damage in all hypertensive patients, and (2) in cardiovascular risk stratification of patients with chronic kidney disease (CKD) [68].

KDIGO classification of albuminuria is as follows [24]:

Normal or mildly increased albuminuria (A1): <30 mg/24 hours
(equals <3 mg/mmol creatinine; or <30 mg/g creatinine)

Moderately increased albuminuria (A2): 30-300 mg/24 hours
(equals 3-30 mg/mmol creatinine; or 30-300 mg/g creatinine)

Severely increased albuminuria (A3): >300 mg/24 hours
(equals >30 mg/mmol creatinine, or >300 mg/g creatinine)

5.3.1.2. Diagnostic significance of measurements tubular proteins in addition to glomerular proteins

Incidence of tubular diseases in end-stage renal disease (ESRD)

The incidence of new end-stage renal diseases needing kidney replacement therapy (KRT) was 145 patients/million inhabitants/year in 2021 in Europe (range 53 to 283/million inhabitants/year in different countries) [69]. Out of these, 5% to 20% may have been caused by tubulo-interstitial nephropathies. Uncertainty relates to the 30% of KRT cases where the primary kidney disease remained unknown, and to combined damages of different renal compartments. Renal tubulopathy may result from nephrotoxic medication (nonsteroidal anti-inflammatory drugs, aminoglycosides, or cytostatic drugs), acute renal failure, pyelonephritis, or specific tubulopathies, e.g., due to myeloma or epidemic nephropathy (Hanta virus infection). Tubulopathy finally appears in all advanced kidney diseases.

Detection of renal diseases with proteinuria markers

Quantitative measurements of both glomerular and tubular marker proteins are needed for sensitive detection of all renal disorders [70]. An increased excretion of albumin and IgG in urine, as seen in diabetes mellitus, nephrosclerosis, or glomerulonephritis, reflects a defect in the permselectivity of glomerular basement membrane. Low molecular mass proteins, such as α 1-microglobulin, β 2-microglobulin, retinol-binding protein, and immunoglobulin light chains, are excreted into the end urine, when the absorption capacity of the tubular epithelium is reduced due to overload or tubular damage as a sign of tubular dysfunction. This occurs in inflammation of

tubulo-interstitial space, i.e., interstitial nephritis and acute pyelonephritis, in vascular damage, or in excretion of immunoglobulin light chains, i.e., Bence Jones proteinuria of myeloma. - Occasionally, toxic damage to the kidneys caused by administration of analgesics, cytostatic drugs or aminoglycosides, or metabolic inhibition, e.g., inhibition of tubular prenylation by statins, may cause increased excretion of tubular markers into urine [71].

Pathophysiological mechanisms explain combined excretion of both high and low molecular mass marker proteins into urine, but different major proteins allow differentiation of renal and post-renal diseases by means of specific measurements from a single urine specimen [72, 73, 60] (**Table 5-13**, and graphically in **Figure 5-1**).

Table 5-13. Pathophysiological events behind different types of proteinuria

Type of proteinuria	Pathophysiological events
Normal (healthy state)	Minor amounts of high molecular mass plasma proteins (e.g., albumin, IgG) leak through the glomerular basement membrane, most of them being reabsorbed in proximal tubuli. Despite remarkable leaks of low molecular mass proteins (α_1 -microglobulin and others) through glomerular basement membrane, most of them are being reabsorbed in the tubuli.
Prerenal proteinuria	Increased secretion of immunoglobulins and their fragments by myeloma cells results in abundant secretion of immunoglobulin (Ig) light chains in urine (Bence-Jones proteinuria). A secondary albuminuria and α_1 -microglobulinuria derives from saturation and damage of renal tubulointerstitium caused by overflow of various Ig fragments.
Glomerular nephropathy	Major excretion of high molecular mass proteins (albumin, IgG) is caused by defective glomerular permselectivity.
Glomerular-tubular nephropathy	A combination of both glomerular and tubular nephropathy may be observed in some types of kidney diseases. A moderate secondary α_1 -microglobulinuria usually results from saturation and damage of renal tubulointerstitium caused by overflow of albumin and IgG.
Tubulointerstitial nephropathy	Major excretion of low molecular mass proteins (α_1 -microglobulin and others) due to reduced reabsorption caused by tubular saturation and damage. In addition, excretion of tubular damage proteins (KIM-1 and others) is observed. Reabsorption of high molecular mass proteins is also reduced, but excretion of albumin and IgG remains lower than that seen in glomerulopathies.
Postrenal proteinuria	All plasma proteins leak into urine through a damaged mucosal membrane of the lower urinary tract, e.g., in UTI. Concentration ratios of largest plasma proteins (α_2 -macroglobulin to albumin, and IgG to albumin), correspond to those seen in plasma.

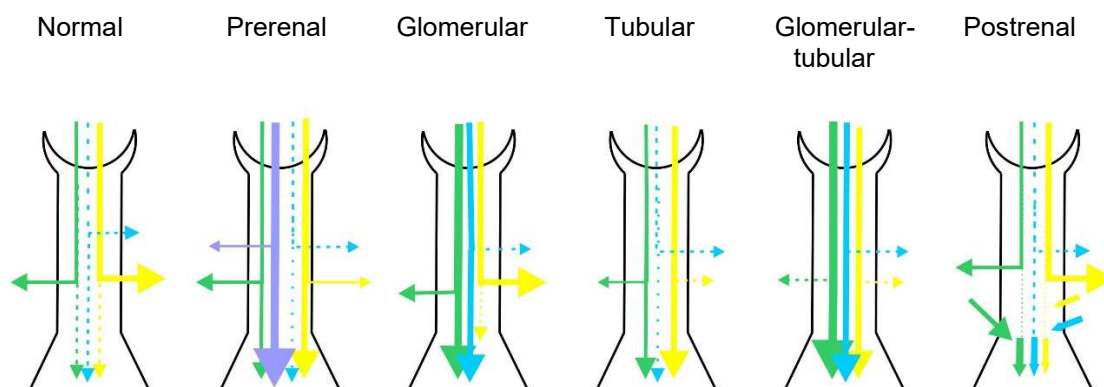






Figure 5-1. Graphical presentation of proteinuria types.

Schematic excretion of example proteins in the pathophysiological categories of **Table 5-13**: Normal, prerenal, glomerular, tubular, mixed (glomerular + tubular), and postrenal proteinuria.

The arrows depict the following example proteins:

-  Albumin
-  α_1 -microglobulin
-  Immunoglobulin light chains (Bence-Jones protein)
-  IgG

Correlation of urinary protein pattern with detailed diagnosis in renal biopsy may vary in patients with complex renal diseases, but the high negative predictive value was repeated in a study of more than 500 biopsy-proven renal patients [74]. In a study of 65 renal patients, sensitivity of urine particles was 41% – 50% against renal biopsy, while all patients were detected by specific urinary protein measurements [75]. In addition to proteinuria, red blood cell (RBC), white blood cell (WBC) and bacteria counts in urine are needed to detect or rule out haematuria or UTI.

In routine laboratory service, the tubular dysfunction marker α_1 -microglobulin is available for automated instrumental platforms with computerised interpretations [76, 77, 78, 73] (**Figure 5-2**). Elevated excretion of renal marker proteins is possible even when the total protein concentration in urine is normal [79]. With the proposed algorithm, the information obtained from a urine sample has increased substantially, allowing detection and differentiation of proteinuria, and providing suggestions for the clinical evaluation of patients [80].

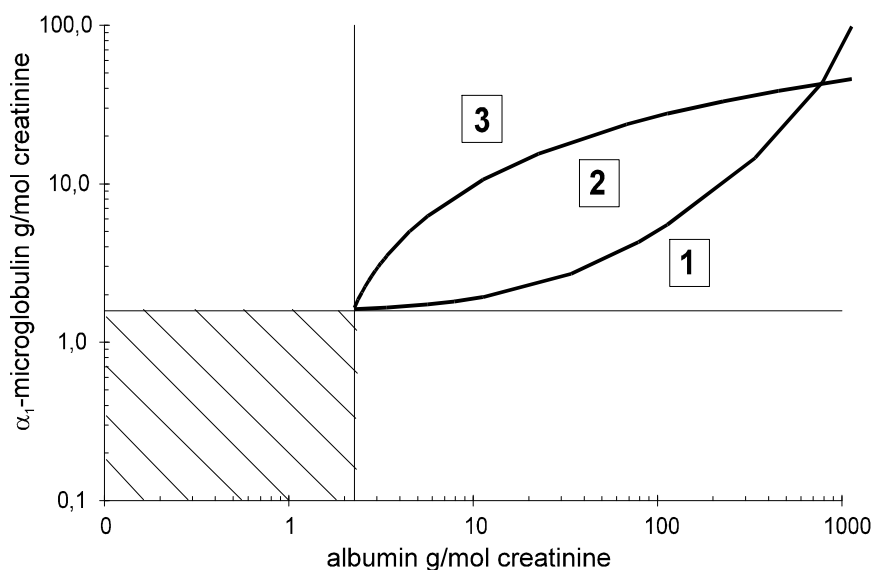


Figure 5-2. Differentiation of proteinurias

Specific measurements of albumin and α_1 -microglobulin-to-creatinine ratios can differentiate between (1) primary glomerulopathies, (2) secondary glomerulopathies, and (3) tubulo-interstitial nephropathies. The shaded area represents the health-associated concentration ratios.

Differentiation of proteinuria is recommended for specific patient groups in the initial diagnostics of kidney disease, while estimations of GFR (eGFR) are of primary importance in the follow-up. Differentiation should include measurements of different “guide proteins” representing defined kidney compartments (glomeruli, tubulo-interstitium), or postrenal bleeding, as well as measurement of creatinine in urine [79, 73] (Table 5-14).

Table 5-14. Individual guide proteins for the differentiation of proteinuria

Guide protein	M _r	Type of proteinuria: physiology, diagnostic significance
α_1 -Microglobulin	33 kDa	tubular proteinuria: restricted tubular reabsorption, tubulointerstitial damage (nephritis, nephropathy)
Albumin	67 kDa	selective or unselective (+ IgG) glomerular proteinuria: increased glomerular filtration pressure, glomerular hyperfiltration, glomerulopathy
Immunoglobulin G (IgG)	150 kDa	unselective glomerular proteinuria: filtration defect; IgG / albumin quotient > 0.03, glomerulopathy
α_2 -Macroglobulin	725 kDa	postrenal proteinuria: bleeding / exudation; α_2 -macroglobulin / albumin quotient > 0.02

RECOMMENDATION: Sensitive detection of kidney disease in high-risk groups requires measurements of both urine albumin, and a tubular marker in urine, such as α 1-microglobulin, in the diagnostics of kidney disease. Measurement of urine total protein remains important in validation of specific protein measurements. Estimation of GFR (eGFR) is of primary importance in the follow-up of the detected kidney disease. (1, B)

5.3.1.3. Prognostic assessment of chronic kidney diseases

Established prognostication markers of CKD are eGFR and albuminuria of patients. Because of repeated gaps in prediction of progress to end-stage renal disease (ESRD), cardiovascular disease, and mortality after accounting for eGFR and albuminuria, markers of tubular injury and dysfunction are being investigated to elucidate their prognostic role in CKD, and predicting adverse events in acute kidney injuries (AKI). The end-stage renal fibrosis occurs, anyway, in the tubulointerstitial space [81, 82]. Tubular damage has been assessed both by means of dysfunction markers, such as α 1-microglobulin, β 2-microglobulin [83], or retinol-binding protein (RBP) [84, 85, 86], and by means of injury markers, such as kidney injury molecule-1 (KIM-1), or neutrophil gelatinase-associated lipocalin (NGAL) in urine [87].

Elevated urinary α 1-microglobulin excretion correlates with interstitial fibrosis and tubular atrophy in kidney biopsy after transplantation, representing chronic kidney damage [88]. Increased excretion of tubular markers also predicts adverse effects after cardiac surgery [89]. Tubular markers KIM-1 and NGAL in urine reflect progression of diabetic nephropathy, but not independently of eGFR or albuminuria [87]. In IgA nephropathy, urinary KIM-1 was an independent prognostic factor from eGFR to predict ESRD, while α 1-microglobulin excretion correlated with proteinuria [90]. In minimal change nephrotic syndrome, low level of tubular proteinuria predicts a good prognosis [91].

An independent role in prognostication of CKD was not found for urinary tubular markers KIM-1, NGAL, N-acetyl beta-D-glucosaminidase and liver fatty acid binding protein (L-FABP) in a prospective Chronic Renal Insufficiency Cohort (CRIC) with 2512 established CKD patients [92]. Out of tubular injury markers KIM-1, NGAL, and NAG assessed in a meta-analysis of 29366 participants, only urinary NGAL had a prognostic value for end-stage renal disease among CKD patients (Relative Risk 1.40) [93]. It is still possible that tubular markers have a prognostic importance in specific diseases or clinical situations causing CKD, in patients with incipient renal insufficiency, or in CKD cases without albuminuria [94].

5.3.2. Quantitative measurement procedures of proteinuria

5.3.2.1. Detailed measurement procedures

Total protein

Principles of measurement

Benzethonium chloride [95] and trichloroacetic acid precipitation [96], dye binding methods with Brome-phenol blue [97], Coomassie brilliant blue [98], Ponceau red [99] and pyrogallol-red [100], nephelometry and turbidimetry have been applied for measurement of total protein in urine. All these methods can be automated except the biuret examination [101]. Determination of total protein is a compromise because no procedure detects all the proteins in urine.

Calibration

Calibration of total protein concentration can be performed by using a human protein calibrator intended for concentrations found in human urine, traced back to the National Institute of Standards and Technology (NIST), Standard Reference Material® (SRM) 927f for protein quantitation [102].

Interpretation: Upper reference limits (URL) in health

Because of dependency on measurement procedure, several URL are cited. As a practical consensus of URL, 150 mg/day is recommended [103].

Measurements of urinary total protein are traditionally used as a cheap method to screen or follow-up a kidney disease. Increased excretion of immunoglobulin light chains in urine (Bence Jones proteinuria) is also detected with measurements of urinary total protein. As a plausibility test, measured sum of excretions of specific proteins, i.e., those of albumin and α 1-microglobulin may be compared with excretion of total protein to reveal a possible protein gap that should be confirmed by specific measurements of free immunoglobulin light chains (in serum) and typing of the monoclonal components with immunofixation.

Albumin

Principles of measurement

Nephelometry, turbidimetry, radioimmunoassay (RIA), enzyme-linked immune-sorbent assay (ELISA), or other immunometric procedures are applied. Chromatographic procedures have been used for research purposes only.

Calibration

Because total urinary protein is an ill-defined measurand that cannot be standardized satisfactorily, a broad consensus has developed over the years that total urinary protein should be replaced by urinary albumin. A separate NIST Standard Reference Material® 3666 for urinary albumin and creatinine is available for this purpose [104].

Interpretation

Measurements from single-voided urine specimens are recommended, adjusting the measurand concentrations to that of urine creatinine (see **Chapter 2.2.1**). KDIGO classification of albuminuria [24] is quoted in **Chapter 5.3.1.1**. The cut-off limit of moderately increased albumin excretion in a

single-voided, and that in a 24-hour collection correlate approximately to the cut-off in the formal timed albumin excretion rate as follows:

Albumin excretion rate of $> 20 \mu\text{g}/\text{min}$ (formal unit) corresponds to an albumin-to-creatinine ratio $> 3 \text{ mg}/\text{mmol}$ ($30 \text{ mg}/\text{g}$ in conventional units), or an albumin mass $> 30 \text{ mg}$ in 24-hour collection.

Albumin-to-creatinine ratios decrease slightly with age [105]. Albumin-to-creatinine ratio is also slightly higher in women than in men due to lower creatinine excretion in women. The average biological intra-individual coefficient of variation of albumin excretion from day to day is approximately 20-30%, and appears larger in diabetic nephropathy and other renal patients [106, 107]. Diagnostic decisions should not be based on a single measurement due to this variability, especially in borderlines of diagnostic categories.

α_1 -microglobulin (also called protein HC)

Principles of measurement

Nephelometry, turbidimetry, RIA and ELISA with polyclonal antibodies are commonly used.

Calibration

Measurement of α_1 -microglobulin or protein HC concentration has not been standardized yet. An international calibrator is highly desirable.

Interpretation

The within-subject coefficient of variation of healthy individuals is 20% on average between days [106]. α_1 -microglobulin (30-33 kDa) is produced in the liver and lymphocytes. This glycoprotein appears in serum in free (50%), albumin bound ($<10\%$) and IgA-bound forms (40%). Only the free form is filtered and reabsorbed in the proximal tubule $> 99\%$. Increased concentrations in the urine are found in tubulo-interstitial dysfunction or in nephropathies (**Figure 5-1**).

5.3.2.2. Health-associated upper reference limits of urine proteins

The health-associated upper reference limits (URL) shown for excreted urine proteins quote the references [106] and [108] (**Table 5-15**). These point estimates have wide confidence intervals due to skewed distributions of values.

Table 5-15. Upper 95% health-related reference limits (URL) for protein-creatinine ratios in urine

Protein	Type of specimen	Upper 95% reference limit (mg/mmol creatinine; SI unit)	Upper 95% reference limit (mg/g creatinine; conventional unit)
Total protein	Second morning	8 ^a	70 ^a
Albumin	First morning	3.0	27
IgG	First morning	0.7	6
α1-microglobulin	First morning	0.5	4

^a Turbidometric trichloroacetic acid precipitation method.

As a practical estimate, a 95% URL for protein-to-creatinine ratio is 10 mg/mmol or 100 mg/g creatinine independently of measurement principle, as calculated from the consensus limit of 150 mg protein/day (**Chapter 5.3.2.1**).

Variability in the physiological excretion of renal marker proteins between day and night are important to know, when assessing orthostatic or exercise-related proteinuria. Differences in the upper health-related 95% reference limits between nightly and daily albumin-to-creatinine ratios are shown in the **Table 5-16**. Similar estimates of nightly and daily excretion of α1-microglobulin and IgG for females and males are also published [109].

Table 5-16. Albumin-to-creatinine ratio in collections of night and daytime urine
Upper health-related 95% reference limits with 90% confidence intervals (CI).

Night urine	females	1.3 mg/mmol	(0.9 – 2.4 mg/mmol, 90% CI)
	males	0.6 mg/mmol	(0.5 – 1.8 mg/mmol; 90% CI)
Day urine	females	2.7 mg/mmol	(1.6 – 9.0 mg/mmol, 90 % CI)
	males	1.2 mg/mmol	(0.8 – 2.1 mg/mmol, 90% CI)

5.3.3. Performance specifications of quantitative proteinuria measurements

For urine chemistry, a proposal for analytical performance specification should be adjusted to reflect changes in pathological states that appear exponential as compared to the low, or almost

negative concentrations seen in health. Since the same measurement is often used for both monitoring and diagnostic testing, the quality criteria should satisfy both needs.

5.3.3.1. Analytical performance specifications (APS)

Analytical performance specifications should consider within-subject biological variation of urine constituents, and clinical needs. A diagnostic classification of albuminuria has limits of 30 and 300 mg/L albumin (corresponding arbitrarily to 3 and 30 mg/mmol albumin-to-creatinine ratio). Clinical need is suggested to be at least a differentiation between 30 and 100 mg/L albumin (70/30 = +230 % difference) [110]. In monitoring of patients, the total diagnostic uncertainty of two laboratory results should allow detection of a *two- to threefold* change (+100% to +200%).

The provisional clinically acceptable APS for quantitative urine albumin measurements at moderate albuminuria range is shown in **Table 5-17**.

Table 5-17. Analytical performance specification for albumin in urine

	Optimum	Minimum
Albumin, analytical uncertainty at 20-100 mg/L	< 20 %	< 40 %

5.4 Quantitative measurements of volume rate (diuresis)

5.4.1 Diagnostic significance of volume rate measurements

Concentration of urine is important to consider in diseases of both kidneys and lower urinary tract, since measured concentrations of both formed elements and dissolved chemical constituents in urine depend on diuresis (volume rate of water). Mass excretion rates of diagnostics measurands have classically been adjusted over a defined collection period, but currently more practically, by using measurand-to-reference ratios from single-voided specimens (**Chapter 2.2.1**).

Strip tests for urine density were described in **Chapter 5.2.2**. When assessing urine concentration in specific diagnostics of water and electrolyte disorders, hypothalamic or kidney function, quantitative measurements are required.

5.4.1.1 Osmolality

Renal concentrating capacity is a key function of renal tubuli and interstitium, guided by arginine-vasopressin hormone [111]. The recommended quantity related to volume rate (diuresis) is urine osmolality, representing the combined solutes in urine. Urine osmolality is dependent on diet and ingestion of salts.

Osmolality is particularly important for basic diagnostics of water and electrolyte disorders [112], and diabetes insipidus [113]. Osmolality should be measured as well, when other measurands in urine need to be related to excretion of water, but the other analytes of volume rate are less accurate. Because a separate instrument is required for osmolality measurements, measurand-to-osmolality ratios have not become a routine. In specific cases, e.g., under parenteral nutrition, an improved accuracy of urine concentration is, however, obtained by osmolality measurements (see also **Chapter 5.2.2.1**).

5.4.1.2 Relative density (official term: Relative volumic mass)

(old term: Specific gravity)

Relative density is officially named by the IFCC-IUPAC Committee for Nomenclature of Properties and Units, C-NPU, as relative volumic mass, NPU03694, with the following description: Pt–Urine; relative volumic mass, ratio of patient urine at 20°C to that of water, 20°C; procedure defined units, e.g. any proprietary unit not traceable to an international certified reference material. The C-NPU has made their codes publicly available [114, 115, 116]. Because of its rare use in clinical laboratories yet, the conventional term “relative density” is still repeated in this guideline. The old term specific gravity is no more recommended. Since the reference density (reference volumic mass) is the density of water at +20°C, no practical difference between density and relative density of urine exists in clinical medicine.

Urine relative density is closely related to osmolality [117]. The correlation between relative density (relative volumic mass) and osmolality decreases, however, in disease because relative density depends on the concentration of electrolytes, glucose, phosphate, carbonate and occasionally excreted iodine-containing radiocontrast media (after radiological investigations),

while osmolality is dependent on urea, ammonia and electrolytes [49, 118]. Relative density may work better than muscle mass-dependent creatinine in adjusting excretion of occupational toxic substances among healthy individuals [119].

5.4.1.3. Creatinine

Creatinine is secreted tubularly up to a maximum of about 10%. Tubular secretion increases in parallel with renal function impairment in a compensatory manner. Serum or plasma creatinine is measured as falsely high if its tubular secretion is inhibited, e.g., by drugs (including trimethoprim, cimetidine, fenofibrate, ritonavir, hydroxycarbamide).

Correction of diuresis using urinary creatinine concentration to calculate measurand-to-creatinine ratios has gained general acceptance despite its theoretical problems [24]. Creatinine measurement is easily performed and only minimally affected by protein-containing diet. Creatinine excretion suffers from inaccuracies related to body weight, age, gender, and tubular secretion in uraemia [120]. Chronic diseases, such as hypo- and hyperthyroidism, may also affect it. The accuracy of measurand-to-creatinine ratios is, however, clinically sufficient to be used as part of routine quantitative measurements from single-voided urine specimens from clinical patient groups to large epidemiological studies [24, 121], instead of timed collections of overnight or 24-hour urine specimens (see **Chapter 2.2.1**).

5.4.1.4. Conductivity

Conductivity is a new analyte that was brought to clinical laboratories with novel instruments. It is related to osmolality since both are dependent on concentration of salts in urine. Conductivity seems to correlate to osmolality even better than creatinine [49, 122]. It serves as an estimate of urine osmolality together with the concentrations of urine particles.

5.4.2. Measurement procedures of volume rate (or urine concentration)

5.4.2.1. Creatinine

Principles of measurement

Methods based on the Jaffe reaction are recommended to be replaced with more specific enzymatic methods to improve standardisation of results [123]. Gas chromatography-mass spectrometry (GC-MS) or liquid chromatography followed by mass spectrometry (LC-MS) is used in the reference measurement procedures [124].

Calibration

Calibration of creatinine measurement is recommended to be performed using the specific urine calibrator for urine albumin and creatinine Standard Reference Material® 3666 [104].

Interpretation

Creatinine in 24-hour urine, 95% central health-related reference intervals [125]:

Adults (n=241):	7 - 20 mmol (SI Unit), with a median of 12 mmol or 0.8 - 2.2 g (conventional unit), with a median of 1.3 g
Men (n=121):	7 - 21 mmol, with a median of 15 mmol
Women (n=116):	7 - 14 mmol, with a median of 10 mmol

Creatinine excretion rate depends on muscle mass. Creatinine is almost entirely filtered by the glomeruli and only traces are secreted by the tubules. The fraction of tubular secretion increases, however, with reduced glomerular filtration rate. High protein meals and intense physical exercise lead to increased urinary creatinine concentrations.

5.4.2.2 Osmolality*Principles of measurement*

Osmometry follows directly the definition of osmolality: it is based on either a decrease in freezing point or an increase in the evaporation point of solutions.

Interpretation

Osmolality in 24-hour urine collection, freezing point procedure, 95% central health-related reference interval [125].

Adults (n= 241):	163 – 990, with a median of 388 mOsm/kg H ₂ O
Men (n= 121):	165 – 1011, with a median of 463 mOsm/kg H ₂ O
Women (n= 117):	146 – 743, with a median of 327 mOsm/kg H ₂ O

Urea, ammonia and monovalent ions are mostly responsible for urine osmolality. With the maximum antidiuresis the urine reaches an osmolality of about 1200 mOsm/kg H₂O. Maximal diuresis may result in an osmolality as low as 50 mOsm/kg H₂O [117]. The concentrated morning urine after an overnight restriction of fluid intake reaches an osmolality of at least 700 mOsm/kg H₂O in healthy individuals. In chronic renal failure, the urine remains isotonic within the range of 300-350 mOsm/kg H₂O.

5.4.2.3 Relative density (official term: Relative volumic mass)*Calibration*

Relative density measurement can be calibrated in practice by measuring densities of pooled human urine, i.e., by weighing out accurately known volumes of pooled human urine. In this way, refractometers and related instruments can be adjusted using a calibrated balance.

Principles of measurement

Measuring principles include urinometry, refractometry, oscillometry and test strips. These have been reviewed extensively [117]. It is to be noted that there are marked differences in the accuracy of these methods [126].

Interpretation

Relative density is primarily a function of glucose, phosphate, and carbonate.

For human urine, the values are within the interval of 1.003-1.035. Morning urine of healthy individuals has a relative density of 1.020 or more after overnight restriction of fluid intake.

Isotonic range in chronic renal failure corresponds to relative densities 1.010 - 1.012 [117].

5.4.2.4. Conductivity

Conductometry of urine (measured as a current flow between two electrodes) has become easily available with urine flow cytometres [127, 128]. Since the number of charges in urine (the ionic strength) is related to urine concentration, the conductivity is also related with water excretion. A benefit of urine conductivity is that it is insensitive to the contribution of uncharged particles and the presence of X-ray contrast media into urine concentration. Diet-dependent intake affects the excretion of salt from healthy individuals as well as from patients.

RECOMMENDATION: Physiological and biochemical limits of each measurand for urine concentration (volume rate) need to be considered when interpreting them clinically. (1, B)

5.5. Diagnostics of renal stone formers

5.5.1. Diagnostic strategy

The primary diagnostics of renal stone disease should be based on X-ray diffraction or infrared spectroscopy of the stones [129]. The different stone types include the following:

- calcium oxalate, calcium phosphate
- uric acid, ammonium urate
- struvite (= magnesium ammonium phosphate), and infection stones
- cysteine, xanthine and 2,8-dihydroxyadenine
- drug stones
- stones of unknown composition

In emergency cases, the following urinary findings are essential: detection of haematuria, possible UTI and excreted urinary particles. Imaging of the patient provides initial diagnostics of the stone, and possible location and size for initial treatment.

Only high-risk stone formers require specific metabolic evaluation. All children with kidney stones belong to the high-risk group. Compliance and motivation of the patient or her/his guardian needs to be discussed for optimal results in treatment efforts. The European Association of Urology (EAU) Guideline on Urolithiasis contains both detailed diagnostics and treatment advice to different patient groups suffering from renal stones [130]. A parallel Canadian urological guideline also exists [131].

RECOMMENDATION: The EFLM European Urinalysis Guideline endorses the diagnostic strategy for renal stone formers given by the European Association of Urology on Urolithiasis. (1, B)

5.5.2. Details of measurements from specimens of renal stone formers

Initial measurements of serum (or plasma) concentrations of intact parathormone, calcium, urate, inorganic phosphate, and creatinine or cystatin C (to estimate GFR) are of value, as well as examination of acid-base homeostasis, to allow a general assessment and separate diagnostics of hyperparathyroidism, renal tubular acidosis or other diseases.

The important measurands in 24-hour urine collections from patients with specific metabolic evaluation are suggested to include at least excreted daily volume, relative density, pH, creatinine, calcium, oxalate, urate / uric acid, citrate, magnesium, inorganic phosphate, ammonium, and cystine (or amino acid analysis), and possibly sodium and potassium, as specifically indicated [130].

Two consecutive 24-hour collections reduce intra-individual biological variation of results if practically amenable [132, 131]. In non-toilet trained children, age-specific measurand-to-creatinine ratios provide estimates of daily excretion rates in difficulties with timed collections [130]. The EAU guideline also provides therapeutic decision limits based on concentrations of urinary risk factors to kidney stones. A selective targeted approach based on the found risk factor is probably more fruitful than a non-selective approach [133].

Out of the listed measurands, analysis of ammonia is difficult to outsource to specialised laboratories. A direct ammonium measurement would improve assessment of acid excretion from kidneys, and diagnostics of various forms of renal tubular acidosis [134, 135].

Detailed instructions for *specimen collection and preservation* are described in **Annex I, Chapter I.1** and **Chapter I.2**, respectively. Acidification of 24-hour collections is recommended to be carried out after the collection in laboratories only, to avoid chemical hazards at patients' homes [136].

Dietary background must be known and understood when interpreting quantitative excretions of metabolites in urine. Therapeutic approaches are dependent on the availability of therapeutic possibilities and motivation of the patient [137, 138].

RECOMMENDATION: Preservation of measurands related to renal stones is no more recommended for 24-hour urine collections by patients at home. Additions of preservatives may be needed after receiving the specimen at the laboratory, depending on local preanalytical processes. (1, A)

Microscopic analysis of urinary crystals is valuable in specific cases of renal stone formers, as discussed in the chapter on Particle analysis (**Chapter 6**).

5.6. New markers for non-infectious diseases of kidneys

5.6.1. Significance of new kidney disease markers

Prognostic markers of chronic kidney disease were already discussed in **Chapter 5.3.1.3**. For diagnostics of acute kidney injury (AKI) or prognostic assessment to a chronic disease, numerous new "cellular and humoral" components have been described in urine and serum. Determination of these markers should help to detect kidney disease early, specifically and with little effort [139, 140, 141, 142]. Prognostic evaluation and potential treatment success should also be recognisable from the dynamics of relevant markers in the clinical follow-up [143, 144].

5.6.1.1. Investigated biomarkers

New markers have been proposed within genomics [145, 146], transcriptomics [147, 148, 149], proteomics [144, 150, 151, 152], metabolomics, micro-RNAs, and free/modified DNA [148].

Some new polypeptide or protein markers have also been reported. None of these markers is established for clinical use yet. The compiled table divides these suggested biomarkers into functional and structural markers, with elevated excretion usually reflecting kidney injury (**Table 5-18**). In contrast to the other proposed markers of kidney damage, urinary uromodulin may be a functional renoprotective marker in diverse clinical situations, preventing AKI after cardiac surgery or progress of CKD of different etiologies [153].

Table 5-18. Urine protein or peptide biomarkers suggested for acute or progressive chronic kidney disease

Clinical associations and other remarks are given in brackets.

Marker	References
Functional markers	
α 1-Microglobulin, other microproteins (tubular damage, reduced tubular reabsorption)	[140]
CKD273 classifier (selective group of marker peptides for kidney damage)	[154]
Uromodulin (increased risk for AKI or CKD with decreased excretion)	[153]
Structural markers	
Microvillous membrane proteins/ exosomes (proximal tubule); renal tissue proteins/ epitopes of distal tubules or collecting ducts (tubular damage, increased elimination)	[155, 156]

Marker	References
Soluble CD80 in glomerulus (minimal change GN, relapses)	[141, 157]
DKK3 = Dickkopf-related protein 3 (tubulointerstitium; increase in fibrosis)	158, 159]
IL-18 = interleukin 18, including cytokines / chemokines (kidney inflammation, infiltrates)	[148]
L-FABP = liver-type fatty acid-binding protein-1 (tubular damage)	[143]
KIM-1 = kidney injury molecule-1 (tubular injury)	[139]
NGAL, neutrophil-gelatinase-associated lipocalin, plasma and urine (tubular injury)	[160]
NEP, neprilysin (diabetic nephropathy)	[161]
TIMP-2 * IGFBP-7 = product of tissue inhibitor of metalloproteinase-2 x insulin-like growth factor binding protein-7 (tubular injury)	[162, 163]
TNF-alpha and IL-9 (interstitial inflammation)	[164]
Mitochondrial DNA (metabolic, oxidative cell damage)	[165]
EGF (epidermal growth factor) decreased concentration (tubule damage, tubular atrophy)	[154]
Soluble CD163 (sCD163) increased concentration (AKI, acute GN, LE nephritis, ANCA associated GN)	[142, 144]
Active ANCA GN; elevated	[165]

Abbreviations: AKI= acute kidney injury; ANCA = anti-neutrophil cytoplasmic antibody; GN = glomerulonephritis.

5.6.1.2. Detection of acute kidney injury during operations, intensive care, and drug treatment

Detection of AKI during major operations or intensive care periods, or following a drug treatment has a special importance because of its marked impact in patient prognosis. This chapter reviews some existing clinical studies.

Urine Neutrophil Gelatinase–Associated Lipocalin, NGAL, is a predictive biomarker for AKI after paediatric cardiac surgery. It may permit earlier intervention and improved outcome from AKI. Urine NGAL-to-creatinine ratio improves prediction of AKI severity, but offers no advantage in the diagnosis of AKI [166]. In a meta-analysis of patients submitted to cardiac surgery, the pooled sensitivity of NGAL for the diagnosis of AKI was 0.68, and the specificity was 0.79 [167]. It should be noted that also leukocytes contain NGAL, which is why urinary tract infections should be considered when interpreting elevated NGAL concentrations in urine [168].

Urinary NGAL and Liver-type Fatty Acid Binding Protein, L-FABP, have been shown to detect injuries of the renal tubular system in a cross-sectional study of several clinical conditions. L-FABP showed a better diagnostic performance and a lower interference by leukocyturia and hematuria than NGAL [169].

Urinary Kidney injury molecule-1, KIM-1, and Cystatin C and NGAL can predict platinum-induced AKI in earlier stages than serum creatinine. KIM-1 was the most sensitive biomarker for early detection of AKI in patients treated for their bronchopulmonary dysplasia [139].

A recent marker in urine for predicting progression to end-stage renal disease might be the Dickkopf-related protein 3, DKK3, shown in renal tubulointerstitium [158]. Further studies are still needed to clarify its clinical value as well.

Levels of Urine Insulin-like Growth Factor-Binding Protein 7, IGFBP7, measured at admission and in the follow-up of patients in intensive care unit, ICU, can be used as a biomarker for the early diagnosis of septic AKI development before being affected by sepsis (with an AUC = 0.79) [170].

A combination (product) of two urinary biomarkers, Tissue Inhibitor of Metalloproteinases-2, TIMP-2, and IGFBP7, calculated as $[TIMP-2] * [IGFBP7]$, has been used to identify patients at high risk to AKI in intensive care units (ICU). Numerous clinical studies have evaluated the utility of several biomarkers, e.g., NGAL, L-FABP, interleukin-18, KIM-1, and cystatin C, in the early diagnosis and risk stratification of AKI. Among these biomarkers, $[TIMP-2] * [IGFBP7]$ has been shown to be superior in early detection of AKI, before the decrease of renal function is evident. Several clinical studies are evaluating its application, interpretation and measurements in different clinical settings [171, 163].

Only limited number of systematic reviews or meta-analyses on clinical studies exist so far, and only on some of the listed markers. The new urine biomarkers NGAL, KIM-1, L-FABP, and $[TIMP-2] * [IGFBP2]$ have not reached the diagnostic performance criteria (sensitivity, specificity) for routine clinical use [172, 173, 174, 175, 176]. Further studies are needed to establish their medical benefits.

Diagnostic tests for AKI in the ICU may offer a potential to improve patient care, but cost-effectiveness remains highly uncertain. Further research should focus also on the mechanisms by which a new test might change current care processes in the ICU and the subsequent cost and quality-associated life years (QALY) implications, to justify adoption in clinical practice [177].

5.6.2. Application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)

In recent years, the differentiation of polypeptides in urine has opened up new diagnostic possibilities. By means of capillary electrophoresis and subsequent mass spectrometry, e.g., MALDI-TOF MS, more than 2000 different polypeptides have been differentiated in urine. Typical patterns characterise various kidney diseases. In addition to IgA nephropathy, an early diagnosis of diabetic nephropathy has also been described. One of the focuses is a profile of 273 peptides (so-called CKD273 proteome classifier) that varies depending on the underlying disease [178].

Certain protein or peptide patterns, or protein fragments within an overall profile (so-called "multimarker patterns") are associated with the progression of kidney disease, or should also provide indications for more favourable disease courses. "Proteomics" from urine samples merge smoothly with aspects of "metabolomics", "genomics" and other "omics". Analysis of proteomics in urine has not yet been established for routine use.

5.7. RECOMMENDATIONS FOR CHEMISTRY

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
24	Multiple (multiproperty) test strips are still recommended as screening tools for routine patient populations because of their cost-efficiency. Conventional strip tests are NOT sensitive enough for diagnostics of patients with high-risk to kidney disease (patients with diabetes or cardiovascular diseases), or complicated UTI patients.	1, A	5.2.1
25	No laboratory tests are recommended for otherwise healthy non-pregnant female patients with sporadic symptoms of uncomplicated lower UTI.	1, A	5.2.1.1
26	Rapid tests to detect UTI should include tests for detection of both leukocytes and bacteria.	1, A	5.2.1.1
27	Rapid tests are recommended to be requested from elderly patients after a clinical intention to treat only because of a high prevalence of asymptomatic bacteriuria.	1, A	5.2.1.1
28	Concentration of urine is valuable in interpretation of urine specimens of paediatric patients, to alert of dilute specimens.	2, B	5.2.1.1
29	Sensitive albuminuria screening for incipient chronic nephropathy is not recommended at an epidemiological level because of costs of follow-up investigations. A targeted screening of high-risk patient populations (e.g., patients with diabetes and cardiovascular diseases) is recommended.	1, B	5.2.1.3
30	Urine concentration is recommended to be reported together with all chemical and particle examinations from single-voided urine specimens.	1, B	5.2.1.4
31	Plasma hydroxybutyrate measurements are recommended for the follow-up of comatose ketoacidosis patients instead of urine strip tests.	1, B	5.2.1.5
32	From specimens of intensive care and in-patient groups with needs of improved accuracy, urine concentration is suggested to be measured by using refractometry or osmolality.	2, B	5.2.2.1
33	Urine strip tests are recommended to be read with instruments both in laboratories and points-of-care, using qualified procedures to avoid human errors in interpretation of results.	1, A	5.2.2.2

34	Performance of test strip measurements is recommended to be verified against quantitative measurement procedures and monitored internally by using continuous reflectance values from reflectometers, and control solutions close to the limit of positivity of each measurement.	1, B	5.2.3
35	Sensitive detection of kidney disease in high-risk groups requires measurements of both urine albumin, and a tubular marker in urine, such as α 1-microglobulin, in the diagnostics of kidney disease. Measurement of urine total protein remains important in validation of specific protein measurements. Estimation of GFR (eGFR) is of primary importance in the follow-up of the detected kidney disease.	1, B	5.3.1.2
36	Physiological and biochemical limits of each measurand for urine concentration (volume rate) need to be considered when interpreting them clinically.	1, B	5.4.2
37	The EFLM European Urinalysis Guideline endorses the diagnostic strategy for renal stone formers given by the European Association of Urology on Urolithiasis.	1, B	5.5.1
38	Preservation of measurands related to renal stones is no more recommended for 24-hour urine collections by patients at home. Additions of preservatives may be needed after receiving the specimen at the laboratory, depending on local preanalytical processes.	1, A	5.5.2

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction.

5.8. References, Chapter 5

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The EFLM European Urinalysis Guideline

Update 2023

Chapter 6. Particles

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List of abbreviations, Chapter 6

<i>APS</i>	Analytical performance specification
<i>CAAPS</i>	Clinically acceptable analytical performance specification
<i>CFB</i>	Colony-forming bacteria
<i>CFU</i>	Colony-forming unit
<i>CLSI</i>	Clinical Laboratory Standards Institute
<i>CV</i>	Coefficient of variation; often used to express imprecision of results
<i>CV_A</i>	(Coefficient of) analytical variation
<i>CV_D</i>	(Coefficient of) diagnostic variation
<i>CV_I</i>	(Coefficient of) intra-individual biological variation
<i>CV_{PRE}</i>	(Coefficient of) preanalytical (technical) variation
<i>DHA</i>	Dihydroxyadenine (crystals)
<i>EFLM</i>	European Federation of Clinical Chemistry and Laboratory Medicine
<i>ICSH</i>	International Committee for Standardization in Hematology
<i>ISO</i>	International Organisation for Standardization
<i>IVDR</i>	In vitro diagnostic medical device regulation
<i>JCGM</i>	Joint Committee for Guides in Metrology
<i>R(CV)</i>	Relative coefficient of variation; ratio between observed-to-theoretical variation
<i>RBC</i>	Red blood cells
<i>RTC</i>	Renal tubular (epithelial) cells
<i>s(n)</i>	Standard deviation (of n observations)
<i>SEC</i>	Squamous epithelial cells
<i>SI</i>	International system of units
<i>TEC</i>	Transitional epithelial cells
<i>UFC</i>	Urine flow cytometry
<i>UTI</i>	Urinary tract infection
<i>VIM</i>	International vocabulary of metrological terms
<i>WBC</i>	White blood cells

6.1. Clinically significant particles in urine

6.1.1. Urinary particles with diagnostic significance

Urine particles are traditionally used to detect urinary tract infections, i.e. pyuria and bacteriuria [1], and haematuria [2]. Microscopy of urine particles is specifically used to detect or follow-up kidney diseases [3, 4]. The development of automated particle analysers has brought a new level of accuracy to urine particle analysis [5]. In addition to specifying particles indicating a renal disease, urine particle analysis provides rapid diagnostics of UTI and haematuria, and is affordable in different health care environments.

Morphological features of urine particles are described in the **Annex II, Table II-1** by using phase contrast microscopy strongly recommended by these guidelines [6]. Additional differentiation by supravital staining methods, such as Sternheimer staining [7], is also shown in the **Annex II, Table II-2**.

6.1.1.1. Pyuria and urinary microbes

Leukocytes (WBC, white blood cells)

The most frequent leukocytes found in urine are polymorphonuclear neutrophilic granulocytes. Granulocytes are most frequently detected in the urine of patients with urinary tract infections together with bacteria [1, 8]. They are excreted into urine also with other formed elements, in other inflammatory states, such as active proliferative glomerular diseases, acute interstitial nephritis, in which they are the most frequent element, and in urological disorders [9]. Leukocytes degenerate or lyse easily in low-density urine, in inflammatory specimens, or after delayed examination [10].

Microbes

Bacteria may be seen on visual bright-field microscopy. They are particularly visible with phase-contrast optics. Rods are typically identifiable, but cocci may be confused with salt precipitates if they are not motile. Automated particle analysers have been improved in their ability to detect bacteria allowing the ruling out of bacteriuria for general patient populations (see **Chapter 6.3.3.1**). Challenges to detect bacteria remain for uropathogens below 10^4 colony-forming units (CFU)/mL - equivalent to 10^7 colony-forming bacteria (CFB)/L in culture - suggesting specific analytical workflows for specimens investigated for significant bacteriuria at $10^2 - 10^3$ CFU/mL level, corresponding to 10^5 - 10^6 CFB/L [11].

A Gram stain of urinary samples as screening technique for UTI is labour-intensive and requires experience. Therefore, it is no longer recommended for routine detection of urinary bacteria [12]. See **Chapter 7.3.1.1** for description of some specific bacteriological needs.

Other microorganisms that may be found in urine:

- Fungi. On most occasions, they are due to vaginal contamination in specimen collection, although they may represent true kidney infection in chronically debilitated or immunosuppressed patients.
- Protozoa. *Trichomonas vaginalis* is found in urine as a consequence of genital contamination.

- Helminths. The diagnosis of parasitic infestation by *Schistosoma haematobium* relies on the observation of the eggs in the urine. Occasionally, eggs of *Enterobius vermicularis* may be seen in paediatric urine specimens [9].

Macrophages (histiocytes)

Macrophages (mononuclear phagocytes, histiocytes) appear fairly often in the urine of patients with urinary tract infection without established clinical significance. It is suggested that they reflect inflammatory activity of renal disease, as detected with specific immunostaining [13, 14]. In heavy proteinuria, they may be loaded with lipids together with renal tubular cells, both lipid-laden cells called “oval fat bodies”.

Lymphocytes

The appearance of lymphocytes in urine is associated with chronic inflammatory conditions, viral diseases, and renal transplant rejection [15].

Eosinophils

In the past, determination of eosinophil granulocytes was suggested for the diagnosis of AKI or interstitial nephritis. They are no more considered specific for these pathological conditions [16, 17]. Reporting eosinophils in urine is therefore not clinically useful.

6.1.1.2. Haematuria**Erythrocytes (RBC, red blood cells)**

The appearance of red blood cells in urine generally reflects origin of bleeding: dysmorphic erythrocytes suggest glomerular disease, whereas red blood cells with normal morphology usually arise from the lower urinary tract [2, 18, 19]. A subgroup of abnormally shaped red blood cells, **acanthocytes** or G1 cells (= ring-shaped cells with blebs), has been described [20, 21, 22]. Phase-contrast microscopy clearly visualizes the acanthocytes that are important in establishing glomerular haematuria [20, 23, 24].

Haematuria remains a major sign of disease in urinary tract or kidneys. It may also reflect a general bleeding tendency. Haematuria due to physiological reasons (strenuous exercise) or vaginal contamination (menstruation) should be avoided with careful patient preparation. The clinical value of RBC morphology is related to patients with isolated haematuria, because dysmorphism guides the subsequent diagnostics towards urological or nephrological disease [25, 26].

6.1.1.3. Epithelial cells

Released epithelial cells in the urine may help to localize urinary tract diseases according to their origin.

Squamous epithelial cells (SEC)

Squamous cells derive from the urethra and vagina. During pregnancy, their exfoliation is increased. The presence of squamous epithelial cells has traditionally been associated to unsuccessful urine mid-stream collection, predicting polymicrobial growth in culture [27]. Most recent assessments have shown that the correlation of squamous cells with polymicrobial growth

is not strong enough to support their use for either ruling in or ruling out contaminated samples [28, 29, 30, 31].

Transitional epithelial (Urothelial) cells (TEC)

The urinary tract is mostly covered by a multi-layered epithelium with a variable number of cellular layers, called transitional epithelium that goes from the calyces of the renal pelvis to the bladder in the female, and to the proximal urethra in the male. Transitional epithelium, also called uroepithelium or urothelium, may be divided into superficial and deep cells, with intermediate forms [32]. Deep urothelial cells are usually associated with ureteric stones, urothelial carcinoma, or hydronephrosis.

Urinary cytology in detection of urothelial cancer

The examination of voided urine specimens for exfoliated cancer cells has a high sensitivity in high-grade tumours, but a low sensitivity in low-grade tumours. The sensitivity in carcinoma in situ detection may be less than 50%. Cytology is useful as an adjunct to cystoscopy, but it is not designed to detect low-grade tumours. A negative cytology does not exclude the presence of a urothelial cancer [33].

Atypical forms of urothelial cells are an incidental finding with phase-contrast or rapid supravital techniques in routine urinalysis [34, 35]. Automated urinalysis instruments may also help in identifying markedly atypical urothelial cells [36, 37, 38]. General laboratories examining urine cells at an advanced level may report a **suspicion** of atypical or malignant cells [39] as agreed within the local cytopathology laboratory.

Generally applicable tumour cell markers for clinical diagnostics are still under development [40]. Diagnosis and follow-up of patients with urothelial cancers should be undertaken by experienced cytopathologists from specifically collected specimens (usually, a second morning urine after a 2-hour incubation in bladder, using specific fixatives). Laboratories devoted to cancer cells look at the surface of urinary cells and check for certain expression profiles or certain clusters of molecular differentiation markers [41].

6.1.1.4. Detection of kidney disease with urinary particles

Sensitivity and specificity to detect a kidney disease by means of urine particles depends on the type and clinical phase of each disease. Urine particles have been compared with new biomarkers in detection of acute kidney injury (AKI) [42]. The sensitivity of urine particles in detecting kidney disease is generally lower but the specificity is higher than that of protein markers [43, 4]. Patients with chronic proliferative glomerulonephritis have a higher prevalence of urinary particles than those with non-proliferative glomerulonephritides [44]. The worsening of AKI in hospitalised patients may be predicted by the presence of renal tubular cells or casts in urine with a similar overall performance to that of modern biomarkers, such as urinary neutrophil gelatinase-associated lipocalin (NGAL) [45].

Renal tubular epithelial cells (RTC)

Different types of tubular cells line the segments of renal tubuli. As a consequence, several types of detached tubular epithelial cells can be found in urine in renal damage. Renal tubular epithelial

cells are found in patients with glomerulonephritides, in nephrotic syndromes [44], and in some metabolic storage diseases, such as Fabry's disease [46]. In patients with severe proteinuria, they may appear as "oval fat bodies" if excessively loaded with lipids [9]. They are also found in the urine of patients with acute tubular necrosis, acute interstitial nephritis, and acute rejection of renal allograft [47]. Renal tubular epithelial cells have been shown to aid in the discrimination between upper and lower urinary tract infections [48].

Casts

Casts are formed in distal tubules and collecting ducts from aggregation and gel-transformation of the fibrils of uromodulin, also called Tamm-Horsfall glycoprotein [49]. This material is produced by the cells of the ascending limb of Henle's loop and forms the hyaline matrix of casts. A cast is formed as a precipitate when concentrations of excreted soluble uromodulin fibrils, plasma proteins, or small molecular weight components exceed the saturation point of the colloidal solution [50]. They are elongated elements with a cylindrical shape with variable bending, wrinkling, and irregular edges. Partially formed shapes, called "cylindroids" are created in identical conditions. Casts usually reflect the presence of renal disease, but hyaline casts may also reflect physiological conditions [50].

Within casts, plasma proteins, lipids, different types of cells, microorganisms (bacteria or yeasts), pigments (haemoglobin, myoglobin, bilirubin) and crystals may be found. The inclusions inside the casts describe different pathogenetic subtypes as described below.

Hyaline casts. They are found in both renal parenchymal diseases and also in normal subjects, such as in concentrated morning urine, during dehydration, or after strenuous exercise of healthy individuals.

Granular casts. They suggest the presence of a renal disease or stasis in urine flow.

Waxy casts. They are found in patients with chronic renal insufficiency or failure.

Fatty casts. They are typical in patients with heavy proteinuria associated with lipoprotein excretion into urine. See *Lipids* below.

Pseudocasts (artefacts). These may represent hair, synthetic fibres or toilet tissue, or technical artefacts during preparation of the sediment under a coverslip. Pseudocasts are not reported.

Cellular casts. According to the cells contained, cellular casts are classified as:

- *Erythrocyte casts*, always indicating bleeding from the renal parenchyma
- *Leukocyte casts*, usually containing granulocytes, indicating acute pyelonephritis, interstitial nephritis, or proliferative glomerulonephritis
- *Renal tubular epithelial cell, RTC casts*, suggesting acute tubular necrosis, acute interstitial nephritis, acute cellular rejection of grafted kidney, or glomerular disorders

Haemoglobin and myoglobin casts. Frequently, haemoglobin casts derive from erythrocyte casts. Therefore, they also indicate renal parenchymal bleeding. However, haemoglobin casts may also be due to haemoglobinuria caused by intravascular haemolysis. Myoglobin casts may be seen in the urine of patients with renal failure caused by rhabdomyolysis with myoglobinuria.

Bilirubin casts. Urinary bilirubin was used in the differentiation of icteric patients when serum measurements were lacking. Currently, conjugated bilirubin is measured from blood.

Bacterial and yeast casts. These indicate an upper urinary tract infection.

Lipids (Fat).

Lipids are found in urine when plasma lipoproteins leak through the damaged basement membranes of glomeruli. As lipoprotein particles are larger than protein molecules, lipiduria is typical in patients with heavy proteinuria. Lipids are most often identified as refractile droplets, but they are detected essentially better by using polarised light (seen as “Maltese crosses”). Lipids also appear as cholesterol crystals or lipid-containing casts (fatty casts).

6.1.1.5. Other particles in urine with occasional clinical significance

Crystals

In most instances, crystals in urine represent transient supersaturation caused, for instance, by food rich in urate or oxalate, or by *in vitro* changes due to refrigerated temperature or change in pH of urine during storage. Detailed investigation for crystals in all specimens is unwarranted.

Detection of crystals has clinical value in recurrent renal stone formers needing urological treatment [51, 52] (see also **Chapter 5.5**). They may also be significant for some patients with acute renal failure. In such cases, crystalluria is a marker of a major disorder and is diagnostically important. Typical examples include acute uric acid nephropathy, or ethylene glycol poisoning, which is associated with calcium oxalate monohydrate crystalluria. All the above circumstances are suggested by the finding of either massive or atypical crystalluria, including crystalline casts. When there is a high clinical suspicion, a specific request should be sent to the laboratory for investigation of crystals from a concentrated urine specimen with relevant clinical information.

Urine crystals are usually described based on their shapes [9]. A review with pH dependency of common and rare crystals has also been published [53]. The tridimensional morphology of crystals is best seen with bright-field as opposed to phase-contrast optics [6].

Common crystals

Common crystals with occasional significance in some patients include uric acid, calcium oxalate dihydrate, calcium oxalate monohydrate, calcium phosphate, and triple phosphate = magnesium ammonium phosphate crystals. Amorphous precipitates in urine usually contain urates or phosphates.

Rare crystals

Cystine. Cystinuria can be detected with a prevalence from 1:2500 in Libyan Jews to 1:100'000 in Sweden [54]. Cystinuria may be confirmed by urine amino acid analysis.

2,8-Dihydroxyadenine (DHA). Rare 2,8-dihydroxyadenine crystals occur in a genetic deficiency of adenine phosphoribosyltransferase (APRT) enzyme. Their morphology resembles that of other xanthine (e.g., uric acid) crystals. The disease is sometimes diagnosed after repeated renal transplantations only [55]. At least one European genetic isolate has been published from Iceland [56].

Xanthine. Another very rare xanthine crystalluria occurs in deficiency of xanthine oxidase [57].

Tyrosine and Leucine.

Tyrosine and leucine crystals are associated with severe liver disease, and may indicate inborn errors of metabolism, such as tyrosinemia or maple syrup urine disease. Measurements of urinary (and plasma) concentrations of amino acids, organic acids, and relevant genetic tests are recommended for confirmation of inborn errors.

Cholesterol. These crystals are associated with heavy proteinuria without specific clinical significance.

Crystals of drugs. Therapeutic drugs possibly crystallising in urine include sulphadiazine (appearing as “sheaves of wheat”), triamterene, acyclovir (birefringent and needle-shaped crystals), indinavir (plate or star-like crystals) [58], ciprofloxacin [59]; amoxycillin [60] and phenyltoloxamine [61], and vitamin C [62].

RECOMMENDATION: Urine particle analysis has a role in the diagnostics of urinary tract infections, haematuria, and kidney diseases. (SoR 1, LoE A) ^a

RECOMMENDATION: Urine crystals are NOT recommended to be looked for, nor to be reported for all specimens. In specific situations, urinary crystals may indicate an inherited or metabolic disease, or a drug precipitated in the kidneys, causing stone formation or renal failure. Most commonly, crystals or amorphous precipitate interfere with identification of other particles in urine. (SoR 1, LoE A) ^a

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

6.1.2. Levels of differentiation

Differentiation of the above-mentioned particles by microscopy can be divided into **basic and advanced levels (Table 6-1)**. The basic level for routine urine microscopy is a positive, specific identification of the usual formed elements, grouping kidney disease-related elements into screening groups of RBC, non-squamous, or small epithelial cells, and casts (left column). The advanced level of urine microscopy is intended to provide detailed features of renal damage (right column, requested in nephrological needs). The basic level is considered to be satisfactory in screening or emergency needs in most health care environments, while the advanced level needs in-depth training in visual microscopy.

In both cases, a quantitative count is recommended to be reported for urine cells and casts, whereas microbes (bacteria, yeasts) or crystals are difficult to quantitate in visual microscopy, and are amenable to ordinal scale categories only (**Table 6-1**). See **Chapter 6.2.4** for details of routine quantitative counting (*Level 2*) procedures.

When using automated instruments, the defined basic level allows reproducible differentiation of urine particles and standardised patient reports by multiple users. That is why clinical laboratories should discuss and decide the practice of differentiation of urine particles with their clinicians, also considering the performance of the used automated instruments. An agreed level of differentiation also allows for a systematic framework for training of laboratory personnel and a harmonised interpretation of delivered results.

RECOMMENDATION: Laboratories are recommended to discuss and clearly describe their basic or advanced differentiation of urinary particles with their clinicians, in order to harmonise clinical interpretation of their results. (1, B)

Table 6-1. Levels of particle differentiation in clinical urinalysis

Basic level	Advanced level in addition
Red blood cells (RBC)	Detailed subclasses: dysmorphic RBCs (G1-cells), isomorphic RBCs
White blood cells (WBC)	Differentiation of WBCs
Epithelial cells	
Squamous epithelial cells	Differentiation of <i>non-squamous</i> epithelial cells
<i>Non-squamous (small) epithelial cells</i>	Renal tubular epithelial cells
	Transitional epithelial cells (superficial and deep)
	Intestinal epithelial cells (usually not clinically significant, occurring after bladder surgery)
	Atypical cells (by experienced cytopathologist)
Casts	
Hyaline casts	Differentiation of <i>non-hyaline</i> casts
<i>Non-hyaline (pathological) casts</i>	Erythrocyte, granulocyte casts
	Renal tubular cell casts
	Hyaline, granular, waxy, fatty casts
	Bacteria and yeast-containing casts
	Haemoglobin, myoglobin and bilirubin casts
Bacteria ^a	Bacteria ^a
Yeast ^{a,b}	Yeast ^b
(Protozoa) ^b	Trichomonas
(Helminths)	

	<i>Schistosoma haematobium</i> (in appropriate geographical locations)
Spermatozoa ^b	Spermatozoa ^b
Lipids	Lipids, in addition to droplets: Oval fat bodies (lipid-laden cells), cholesterol crystals
Crystals ^{a,b}	Crystals: urate, oxalate (mono- and dihydrate), and phosphate Additional rare crystals: drugs, cystine, leucine, tyrosine, 2,8-dihydroxyadenine, xanthine
Artefacts (if present) and mucus	Artefact details to be differentiated from casts or other rare particles (such as hair, paper and textile fibres, starch, glass, and plastics)

^a Particle concentrations are to be reported quantitatively. Ordinal scale is sufficient for microbial counts or crystals in visual microscopy, e.g., negative (-), positive: few (+), moderate (++), or abundant (+++). Quantitative counts for bacteria are possible with automated instruments, to be reported in patient results as agreed locally, to avoid confusion with colony counts from urine bacterial cultures.

^b Yeast cells are important to be differentiated from RBCs. Trichomonas and Helminths are important if frequent in local specimens.

6.2. Measurement procedures of particle counting

6.2.1. Levels of accuracy of particle counting procedures

General terms describing accuracy levels of measurement procedures (methods) are provided in the international vocabulary of metrology, VIM [63], see **Chapter 4**. A primary reference measurement procedure for urine microscopy does not exist. The different levels of accuracy in urine particle counting may be described as follows:

Level 3: Advanced comparison method for routine quantitative counting

Level 2: Quantitative visual or automated counting (standardised routine procedures)

Level 1: Ordinal scale methods (non-standardized particle counting)

Standardisation of the method used is essential to improve accuracy and limit of detection. In urine particle analysis, special attention should be paid to different sources of error and training of personnel [64, 65]. In the assessment of urine particles, the centrifugation step with removal of supernatant is a common procedure to detect rare particles, but also a major source of error. Standardisation also includes an accurate urine volume where the particles were originally found.

6.2.2. Unit of reporting urine particle concentrations

Counts of urinary particles shall be related to the original volume of urine to reach concentrations that are comparable between different procedures, e.g., between a candidate and the reference procedure, or between routine procedures of various clinical laboratories [66]. The standardised SI Unit for reporting particle concentrations is defined as number of particles in a volume of litre (capital “L” is preferred over “l”) that is accepted to be in use with SI [67]. The IUPAC (International Union of Pure and Applied Chemistry) and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) has a Committee on Nomenclature, Properties and Units (NPU) [68, 69, 70, 71].

The recommended NPU units of concentration of particles in body fluids are shown with examples of RBC and WBC in **Table 6-2**, using exponentials with litre volumes. The concentration of leukocytes in urine, such as WBC 15/ μL , is written $15 \times 10^6/\text{L}$, or $15 \times \text{E}6/\text{L}$. Non-standard units, such as particles/high-power field (HPF), or particles/low-power field (in microscopy) are recommended to be (1) converted to litre units based on standardised factors, and (2) harmonised at national level to avoid confusions in clinical interpretation.

Table 6-2. IUPAC-IFCC Nomenclature, Properties and Units (NPU) definitions for number concentrations of erythrocytes and leukocytes in body fluids

Body fluid and cell type	Standardised unit for reporting	NPU code (ratio scale)
^a Blood—Erythrocytes	? x 10 ¹² /liter	NPU01960
^a Blood—Leukocytes	? x 10 ⁹ /liter	NPU02593
Urine—Erythrocytes	? x 10 ⁶ /liter	NPU03842
Urine—Leukocytes	? x 10 ⁶ /liter	NPU10505
Cerebrospinal fluid—Erythrocytes	? x 10 ⁶ /liter	NPU01962
Cerebrospinal fluid—Leukocytes	? x 10 ⁶ /liter	NPU02594

^a Note that the format of NPU code includes a long hyphen, when searching the NPU database.

RECOMMENDATION: The standard unit for urine particle concentrations is particles/litre (L), the SI unit. Unit of routine clinical reports is recommended to be harmonised at national level, to avoid clinical confusions. (1, C)

6.2.3. Advanced comparison method for urine particle counting (Level 3)

The advanced comparison procedure for urine particle counting has been described [72]. Some principles of that document are repeated below.

Identification

Particle identification needs an optical method to discern formed elements from their background and a differentiation method to allocate these elements into correct classes (**Table 6-1**). Bright-field microscopy of unstained preparations is inadequate for detection of bacteria, red blood cells, and hyaline casts, and therefore not applicable for advanced differentiation. For this reason, phase-contrast microscopy is necessary in the detection and discrimination of elements [6]. An optional supravital staining may be additionally used to differentiate nucleated cells.

Detection of microbes by Gram staining is used in the microbiology laboratories for specific needs only (see **Chapter 7.3.1.1**). Urine particle analysis either, focussing on rapid differentiation of basic and kidney-related particles. Identification of specific types of cells may require sophisticated procedures such as use of immunochemical markers of specific proteins, or *in situ* markers of specific genes. These are beyond the need in routine diagnostics.

Counting with the reference visual microscopy

Counting of native urine is required to avoid the error created by centrifugation. Then, a sufficient volume is needed to detect rare particles related with renal damage. Particle concentrations close to the low positive range can vary remarkably due to pre-analytical variation, including diuresis, collection, and preservation of specimen.

The reference procedure contains a requirement of statistically sufficient total counts derived from Poisson distribution: a total of 200 cells for WBC and RBC at high concentrations, and at least 50 cells for rare particles. Details of Poisson statistics for urine particle counting are provided as supplemental material in a recent verification study [73].

RECOMMENDATION: Phase-contrast optics is strongly recommended in the detection and discrimination of urine particles both in routine and reference microscopy. (1, A)

6.2.4. Routine identification and quantitation of urine particles (Level 2)

Routine reports of urine particle counts and differentiation should follow locally agreed standardised procedures and reporting formats, to support clinically needed accuracy and reproducible interpretation of results in clinical units. The laboratories should select one of the routine visual microscopy procedures as a major operating frame for their specimen workflow either alone, or as a confirmatory tool for results of their automated devices (**Chapter 6.3.3**).

6.2.4.1. Standardized urine sediment under a coverslip

A standardised volume of urine must be centrifuged, a precise volume of supernatant removed, and the sediment resuspended into an accurate final volume, to define an accurate concentration factor. With or without staining, an aliquot of resuspended urine sediment is investigated under a defined size coverslip that results in a defined height of the fluid layer. Then, a known volume of original specimen is counted when the size of the view field is known (from the ocular viewfield number). These steps are needed to obtain quantitative urine particle concentrations in counting urine particles under a coverslip on a microscopic slide, with a possibility to convert the results into particles/L units. Detailed auditing list for standardised urine sediment is given in **Table 6-3**. Without these steps, the urine particle counts remain inaccurate corresponding to *Level 1* only.

Table 6-3. Details of standardized urinary sediment examination

Item	Standard	Method of checking
Delay	Use of preservatives, described in Annex I.2, Chapter I-2	Documented times of collection
Original volume of urine	5 to 12 mL (1 mL for paediatric specimens)	Line marked on the tube
Centrifugation	400 <i>g</i> for 5 min, preferably at +5°C ± 3°C if delays occur	Check with the supplier of the centrifuge
Removal of supernatant	Suction to a defined final concentration factor, e.g., concentration x20	Calibrate the final volume by weighing pooled urine (buffer solutions have a different surface tension)
Method of staining and microscopy	Phase-contrast microscopy, or staining + bright-field microscopy; polarized optics when needed; low (x100) and high-power (x400) magnification	Consult local supplier
Volume of original urine investigated under microscopic field	Define and calculate	Microscopic slide with a metric scale
List of reported components	Define the report format	These guidelines
Units of reporting	Particles/L (SI unit), or as nationally harmonised	Calculate the equivalence
Reproducible process	Written operating procedures	Training of personnel, blind peer reviews
Internal quality control	Training courses and peer reviews organised locally	Two independent investigations for the same specimen
External quality control	Participation in an EQA scheme	Documents of results available
Calibration	Traceability of measured quantities	Evaluation against uncentrifuged specimens

The concentrated, standardised sediment is the traditional visual procedure of *examination for kidney-related urine particles*, because detection of renal particles (casts and renal tubular epithelial cells) suffers from their low concentrations in urine. When using uncentrifuged specimens, the investigator may miss rare elements if a small volume is investigated. This is overcome by concentrating the specimen at a low-speed of 400 x *g* for 5 min. To confirm even distribution of particles under a coverslip and to see all existing particles, a low-power

magnification (usually x100) is used before counting at a high-power magnification (usually x400). Converting to particles/L unit is done as nationally agreed. The standardised operating procedure may be adapted to review of flagged specimens, or all urine specimens depending on the workflow and daily amount of specimens. Particle concentration under a coverslip remains inaccurate despite standardisation efforts due to the centrifugation step, and to the small and somewhat arbitrary volume of original urine counted.

6.2.4.2. Urine sediment counted in a chamber after centrifugation

Counting concentrated, centrifuged sediments in a chamber was advocated by some investigators to improve accuracy of counts [74, 75], because counting a concentrated sample in a precise chamber volume, such as 3.2 μL of Fuchs-Rosenthal chamber, is more accurate than counting viewfields under a coverslip. A standardised procedure of both centrifugation and chamber counting needs careful training to reach the assumed benefits [76]. Otherwise, chamber counting of concentrated samples leads to higher mean counts than those obtained from uncentrifuged samples, and an imprecision that is similar to the coverslip procedure after more tedious work.

6.2.4.3. Chamber counting of uncentrifuged specimens

A quantitative count for urine particles is more reliably obtained by direct counting of uncentrifuged specimens in a chamber than after centrifugation. The centrifugation procedure is prone to uncertainty of particle counts, because centrifugal forces, removal of non-sedimented particles with the supernatant, and resuspension of the sediment create variable 20-80 % losses of RBC and WBC [64], and even fragmentation of casts. The identification of acute patients with suspected UTI particularly needs an accurate count of leukocytes [77]. Bacteria do not concentrate during centrifugation at 400 x g, but they are clearly visible by using phase-contrast optics in a chamber. The chamber counting of uncentrifuged specimens is easiest for counting WBC, RBC, and bacteria in urine, but different epithelial cells and casts can be identified with high probability after appropriate training.

As a part of the automated workstation process, conversion of results from visual chamber counting to the same metric units as used in automated counting is recommended when applied to primary review of specimens flagged by an automated device due to its accuracy and speed [73]. In addition, centrifugation, supravital staining, or counting of several chamber volumes of a flagged specimen is occasionally needed to confirm detection or identification of low-count particles, as decided in the local workflow.

Routine counting is usually performed in a 1- μL chamber volume, such as in a Bürker chamber or an equivalent commercial disposable chamber, with a height of 100 μm , and grids both for 0.10 μL (A) and 0.00625 μL (B) squares, when a 1 A square is divided into 16 B squares. Other chambers, such as Fuchs-Rosenthal or Goryaev chambers with a 3.2- μL volume and a height of 200 μm , may be used even in routine to improve precision of counts.

RECOMMENDATION: Laboratories should verify one of the (Level 2) procedures of visual microscopy for their routine analysis to ensure accuracy of their results. (1, B)

6.2.4.4. Procedure of counting dysmorphic erythrocytes in urine

Dysmorphic erythrocytes (RBC) suggest glomerular bleeding (kidney disease), while isomorphic erythrocytes (RBCs with regular size or shape) indicate bleeding originating from the tubuli or a lower site in the urinary tract, a general bleeding tendency, or contamination from vaginal bleeding (**Chapter 6.1.1.2**).

Specimen: Mid-stream urine (MSU) collection from the second morning urine within 2 hours from the previous voiding is the preferred specimen. A minimum drinking is recommended in the morning to increase urine density. A random single-voided MSU collection is the second best option. Analysis should be performed on a fresh collected specimen, preferably within 2 hours after collection if the used preservative is not separately verified.

Background measurements: Investigation of urine without centrifugation is preferred to avoid losses of RBC in the specimen. If needed, percentage of dysmorphic RBC remains unchanged during centrifugation. As a background assessment, basic counts of the other particles in the specimen and a test strip analysis are needed.

Validity check:

Specimens with RBC $< 20 \times 10^6/\text{L}$ in uncentrifuged urine are not diagnostic, since physiological haematuria may be dysmorphic [2]. Increased concentrations of WBC ($> 30 \times 10^6/\text{L}$), or presence of bacteria or yeast, crystals or amorphous precipitate may obscure RBC differentiation. A new specimen should be requested after treating the infection, or should be transported into the laboratory fresh after voiding in case of precipitates. Test strip results support assessment of RBC results, in particular density of urine, pH, RBC (pseudoperoxidase reaction), or presence of albuminuria (also related to kidney disease).

Counting procedure

Direct chamber counting without centrifugation is preferred, using phase contrast optics to discern different shapes. No staining is recommended to avoid extra background. During centrifugation, RBC may be lost with the supernatant – dysmorphic RBC more easily than isomorphic RBC – because their internal density may be equal to that of the urine matrix. (Laboratories may wish to centrifuge their urine samples to reach higher concentrations for counting; in that case they need to confirm RBC yields after centrifugation against the original RBC counts of their specimens.)

Counting of a minimum of 100 RBC is required to classify the morphology of the majority of RBC with a 10% uncertainty: with 50% of the RBCs in the specimen being dysmorphic, the 95% binomial confidence interval is 40-60%.

The binomial standard deviation is $s(n) = \sqrt{n \cdot p \cdot q}$, where n = number of counted RBC, p = probability of dysmorphic RBC, and $q = 1 - p$, probability of isomorphic RBC. Coefficient of variation $CV = s/n$.

Write down the total volume (1-10 μL) counted to compare the result with the total RBC concentration. Note also additional particles from the specimen if influencing interpretation of results.

Detection and classification of dysmorphic RBC needs practicing, using peer review to reduce inter-observer variability. Dysmorphic shapes include most specifically acanthocytes = G1 cells: doughnut-shaped or ring-formed RBC with remarkable protrusions or blebs [20, 9] that should be reported as a separate subcategory. A total of nine abnormal shapes of RBCs in urine have been described [20]. Ring-shaped cells with a hole in the middle, or “target cells” with a dark centre piece, but without external blebs (called together codocytes) are fairly easy to identify in addition to acanthocytes. Also, broken or distorted RBC fragments (schizocytes) may be learned to identify. Ghost cells and echinocytes are definitely not dysmorphic cells. The other shapes tend to create variability due to mild abnormality or reversibility with osmotic changes while standing, thus reducing specificity of findings.

Report format: In the report, indicate the total RBC concentration ($\times 10^6/L$, recommended unit or a nationally agreed unit), and differentiation as Dysmorphic RBC (including acanthocytes as a subgroup), % out of total RBC, and Acanthocytic RBC, % out of total RBC, as well as presence and concentration of RBC casts if detected.

Interpretation: Presence of dysmorphic haematuria is suggested by the presence of dysmorphic RBC $\geq 40\%$, or presence of acanthocytes $\geq 2\%$, or presence of RBC casts. The probability of dysmorphic haematuria is increased with the presence of dysmorphic RBC $\geq 80\%$ or presence of acanthocytes $\geq 5\%$. See **Table 6-4** for an arbitrary performance at different cut-off limits. The incidence of glomerular haematuria varies initially in different kidney diseases and in their follow-up, among different patient groups, and due to differences in laboratory examination. Thus, published estimates of diagnostic performance (sensitivity and specificity) have a wide uncertainty [18, 23, 78]. The investigation seems to be valuable for paediatric patients with isolated haematuria [79], and for urological consultations to exclude nephrological diseases [26]. Diagnostic performance should be confirmed with local clinicians and patient groups after confirmation of local examination procedure.

Table 6-4. Diagnostic limits for dysmorphic erythrocytes in urine

Category	Cut-off limit	Sensitivity %	Specificity %	Probability of glomerular disease
Dysmorphic RBC	40%	40-80	50	possible
	80%	20-60	70	probable
Acanthocytes	2%	40-80	80	possible
	5%	20-60	90	probable

6.3. Automated particle analysis

Automation has made urinalysis more standardized, quicker, and less observer dependent. The purpose is to provide *Level 2* quantitative counts on clinically significant urine particles. Automated instruments have improved precision because of increased number of particles in counting as compared to visual microscopy [80].

Simplification of particle differentiation improves the efficiency of the laboratory process if the nonspecific categories or ambiguous findings can be flagged and confirmed by proper visual microscopy based on local clinical needs [5]. With technical evolution, new measurands have become available [81].

6.3.1. Flow cytometry

Automated urinary flow cytometry (UFC) analysers use flow cytometry along with staining to count and classify urine particles. The first UFC analysers, introduced in the 1990s, used argon laser (at 488 nm) and could quantify RBCs, WBCs, squamous epithelial cells, and partially casts and bacteria [82, 83, 84, 85].

Since 2005, classical argon lasers in UFC have been replaced by semi-conductor lasers (operating at 630 nm) with longer lifetime and hence better economy [86]. Also, a dedicated channel for bacteria specific staining was made available, thereby allowing sensitive bacteria detection. The latest generation of UFC employs fluorescence technology by using a new blue semi-conductor laser at 488 nm.

Before particles are sent through a laser beam, they are stained by specific fluorochromes for nucleic acids and for surface structures. Hydrodynamic focussing is then used to improve detection and quantification performance. The recognition, counting, and classification of urinary particles is based on signals of forward and side scatter, side fluorescent and depolarized side scattered light. Analytical and diagnostic performance evaluations of later generations of UFCs have been published since the primary studies, including counting of different types of casts and small epithelial cells [87, 48]. Despite continuous efforts, performance of automated UFC is not sufficient to replace visual microscopy in detection of dysmorphic RBC [88, 89, 90].

6.3.2. Automated imaging technologies

6.3.2.1. Flow cell morphology

Automated imaging analysers are equipped with a microscopic camera along with a software system to classify the different urinary particles. The first principle of digital urine microscopy takes images of urine particles in a flow cell ("Digital Flow Morphology"). A strobe lamp and video camera capture images of the particles, continued with automated recognition software. The original instrument was published 40 years ago [91]. A later generation of the instrument uses charged coupled device cameras [92].

Identification software classifies and quantifies cells and particles in native, uncentrifuged urine using a single, laminar flow of the specimen through the lens of a charged-coupled device

(CCD) camera. Hundreds of digital camera captures are evaluated by identification software, and each particle is classified based on characteristics, such as shape, contrast, and texture. After classification by the instrument, the operator has the ability to reclassify or correct the obtained images in the correct categories if needed. Some studies also exist on counting with a similar analyser FUS-2000 against visual counts of RBCs, WBCs, and epithelial cells [93, 94].

6.3.2.2. Digital cuvette counting

In the digital cuvette instruments, whole field digital images are taken from prepared monolayers in a specific cuvette. Magnifications and images of particles on computer screen resemble those observed by visual microscopy, allowing reclassifications if needed [95]. The whole field images allow the user to see numerous particles at the same time, which facilitates classification of urinary particles, and form combined clinical profiles. Automated particle classification is performed by a neural network based artificial intelligence, and confirmed on screen by the operator if needed.

Bright-field optics have been supplemented with phase-contrast optics in the most recent versions of these automated image analysers. Phase-contrast optics enable a better identification of particles with a low refractive index, most importantly hyaline casts, RBC that have lost their haemoglobin called “ghost RBC”, and bacteria. Phase-contrast also allows visualisation of intracellular details, improving evaluation of RBC morphology [96, 73].

6.3.3. Applications of automated particle counting for specific clinical purposes

New technologies are now capable of carrying out more than the basic level of urine particle analysis, being adapted in large laboratories. Specific clinical needs have focused on the detection of findings related to UTI (bacteriuria and pyuria), or to kidney disease with automated instruments.

6.3.3.1. Bacteriuria detection

Detection of bacteriuria (with a sensitivity higher than 90 – 95 %) is made possible by automated counting techniques, allowing the ruling out of urine samples that probably remain negative in bacterial culture [97, 98, 99, 100, 101]. The specificity to detect uropathogens may remain low (about 40-50 %) with current instruments if the sensitivity is kept high (> 95%) and significant growth includes lower colony counts of $10^3 - 10^4$ CFU/mL (colony-forming units/mL) in culture, corresponding to $10^6 - 10^7$ CFB/L (colony-forming bacteria/L). Automated particle counting is most appealing to mid-stream urine and other routine collections that constitute the majority of urine specimens sent for bacterial culture.

A sensitivity of 99 % with a specificity of 80 % has been achieved with UFC against bacterial culture at $\geq 10^8$ CFB/L ($\geq 10^5$ CFU/mL) in a mixed patient population [102]. A sensitivity of 99 % with a specificity of 51 % against $\geq 10^8$ CFB/L ($\geq 10^5$ CFU/mL) in bacterial culture, or a sensitivity of 97% with a specificity of 47 % at $\geq 10^7$ CFB/L ($\geq 10^4$ CFU/mL) has been shown among elderly patients at the emergency department [103]. Initial detection of Gram-negative bacteria has been suggested by using the UF-5000, still needing further development and studies [104, 105].

A sensitivity of 98% and specificity of 48% has been described for $\geq 10^8$ CFB/L ($\geq 10^5$ CFU/mL) identified species in bacterial culture, or a sensitivity of 87% and specificity of 54% against $\geq 10^7$ CFB/L ($\geq 10^4$ CFU/mL) in culture - including mixed growth - by using automated digital counting in cuvette with phase-contrast optics and samples from a mixed patient population [31].

Novel technologies improve rapid diagnosis of UTI at the emergency department, and may help in organising workflow in large clinical laboratories if the process can be designed to improve efficiency of analytics and to create economic benefits. Performance specifications for rapid UTI diagnostics against urine bacterial culture are suggested in **Chapter 7.8.3**.

6.3.3.2. Kidney diseases

Kidney damage is detected by identifying different types of pathological casts, renal tubular epithelial cells or dysmorphic (often small) erythrocytes in urine. Detection of kidney damage is developing along with improvements in detection and classification of kidney-related particles, i.e., casts and RTC, by the automated instruments (see **Chapter 6.3.1** and **6.3.2** for details). Sensitivity to detect and classify dysmorphic RBC is particularly not sufficient by automated instruments at the moment.

6.4. Reference and diagnostic limits of urine particles

6.4.1. Health-associated upper reference limits of urine particles

Health-associated reference intervals depend heavily on pre-analytical procedures as well as analytical standardisation and delay of examination. Some visual microscopy-based 98% or 95% upper reference limits (URL) have been published earlier [64, 106, 107, 108]. Adjustment to diuresis was not reported in those studies. Since the detection and counting of WBC and RBC in urine has become reliable with several automated instruments, the experimentally produced URL estimates for WBC and RBC in urine are analytically reliable [109]. Preanalytical standardisation is of key importance when preparing reference individuals for mid-stream urine collection in the morning [85, 110]. Specimen collection may create a problem in newborns and older children, resulting in higher counts than those from older individuals without a disease in the kidneys or the urinary tract [111].

RECOMMENDATION: A rough estimate for health-associated 95% Upper Reference Limit both for leukocytes and erythrocytes is 10 (to 20) $\times 10^6/L$ from a mid-stream urine collection of uncentrifuged morning urine. The uncertainty contains both preanalytical and analytical factors. (1, A)

An estimate to the URL of *squamous epithelial cells (SEC)* has been published by using standard approaches [109]. Counting of *bacteria* is also reproducible because of high numbers, but the results are method-dependent due to different principles and specificity of detection. A challenge of imprecision of counting is obvious when particle concentrations in health are markedly below $10 \times 10^6/L$, such as those for *casts*, or *small epithelial cells (RTC and TEC)* in urine.

Both in visual microscopy (with 1-3 μL volume of original urine counted) and in automated counting (with 2-10 μL volume), uncertainty of the low counts needs extra efforts to get an estimate for URL below the limit of quantitation (LoQ). If the URL remains statistically uncertain in standard (visual or automated) procedure, URL should be expressed by using the expression “below LoQ” (see **Chapter 6.5.1.1**).

The actual 95% URL at low counts may be obtained by increasing counting volume with repeated measurements until at least a total of 50 particles has been counted in each of the specimens within the 90th to 100th percentiles (the highest concentrations) of the particle type measured. The obtained median concentration of the specimens represents then the 95% URL [72].

6.4.2. Diagnostic cut-off limits between health and disease

Distributions of particle concentrations in urine both in health and in diseases are needed to define discriminatory cut-offs for diagnostics of diseases. For infants, cut-off concentrations of WBC in urine were investigated in the diagnostics of acute UTI, as obtained from catheterised specimens [77]. A WBC count $> 10 \times 10^6/L$ had a sensitivity of 91% with a specificity of 97% in detecting bacteriuria at 5×10^4 CFU/mL or higher in symptomatic, acutely ill infants. In a regional

study, a median of about $200 \text{ WBC} \times 10^6/\text{L}$ was reported to be associated with a positive bacterial culture $\geq 10^3 \text{ CFU/mL}$ considering also symptoms for UTI [97]. The diagnostic grey zone in leukocyturia is approximately a 10-fold range from health-associated to diseases concentrations (**Table 6-5**).

Isolated microscopic haematuria has been found in 4-13% of population, mostly due to UTI or calculi of the urinary tract, and often at concentrations below $30 \times 10^6/\text{L}$ in uncentrifuged urine [25]. Stratification of 15779 patients with haematuria was studied using the American Urology Association guideline with a cut-off of 3 RBC/HPF in sediment microscopy (about $30 \text{ RBC} \times 10^6/\text{L}$, assuming that 1 HPF equals $0.1 \mu\text{L}$ volume of centrifuged particles), resulting in a total risk of 5.4% for urothelial cancer [112]. A multi-factorial risk stratification subdivided the patients to a risk of 0.4% in the low, 1.0% in the medium, and 6.3% in the high risk group for this cancer. One of the risk factors for the high risk was $\text{RBC} > 25/\text{HPF}$ (about $200\text{-}250 \times 10^6/\text{L}$) or gross haematuria [112]. A diagnostic differentiation of RBC may be approximated with a 10-fold concentration range between 20 to $200 \text{ RBC} \times 10^6/\text{L}$ in isolated haematuria in the association with urothelial cancer.

In established kidney disease, haematuria was shown to be present in 98% of patients with proliferative glomerulopathies (GN) and 67% of those with non-proliferative GN at about $10 \times 10^6/\text{L}$ or more ($> 1 \text{ RBC}/\text{HPF}$), with a median of about $400 \times 10^6/\text{L}$ ($38 \text{ RBC}/\text{HPF}$) in proliferative GN and a median of about $50 \times 10^6/\text{L}$ ($5 \text{ RBC}/\text{HPF}$) in non-proliferative GN [44]. Renal tubular cells, granular casts, and RBC casts were present at concentrations $\geq 1 \times 10^6/\text{L}$ ($> 1/20 \text{ HPF}$) in 83%, 52% and 85% in proliferative GN, and 65%, 50% and 40% in non-proliferative GN, respectively. For kidney-related urine particles, i.e, casts and RTC, evidence of diagnostic or prognostic significance between low or high positive concentrations is lacking. A concentration of about 5 x health-associated URL is suggested to indicate significant presence of kidney-related particles in urine, with uncertainties both in preanalytical and analytical phases (**Table 6-5**).

Presence of squamous epithelial cells (SEC) in urine may be associated with improper mid-stream collections, and that of TEC with any disease of the urinary tract with no data on significant concentrations in routine particle counting. A 5-fold concentration is suggested to represent significant presence of these, similar to kidney-related particles. No quantitative cut-offs of significant concentration can be given to crystals, or other microbes than bacteria if present in routine particle analysis.

Table 6-5. Examples of clinical cut-off concentrations (x 10⁶/L) of urine particles

Particle type	URL ^a	LoC ^a	Notes
Leukocytes, WBC	10-20	100-200	Preanalytical ^b
Erythrocytes, RBC	20	200	Preanalytical, Analytical ^c
Squamous epithelial cells, SEC	10	50	Preanalytical, Diagnostic ^d
Transitional epithelial cells, TEC	2	10	Diagnostic
Casts	1-2	5-10	Preanalytical, Analytical, Diagnostic
Renal tubular epithelial cells, RTC	1-2	5-10	Preanalytical, Analytical, Diagnostic

^a Abbreviations used: URL, 95% upper reference limit in health; LoC, limit of confirmation, estimated significant or reproducible presence of a particle (5 x URL).

^b *Preanalytical uncertainty:*

Increase: Concentration of WBC increases in asymptomatic bacteriuria, that of RBC during a menstrual period or strenuous exercise. Concentrations of these and SEC also increase in inadequate mid-stream collections.

Decrease: Concentrations of WBC and RBC decrease after extended storage in dilute urine.

Concentrations of kidney-related particles (casts and RTC) may decrease while transferring the specimen from the primary collection container to secondary tubes by vacuum aspiration.

^c *Analytical uncertainty:* Losses of particles may result from removing supernatant after centrifugation, or by heavy resuspension of the specimen before analysis. Kidney-related particles are prone to deficient detection and excessive imprecision at the low concentrations representing URL.

^d *Diagnostic uncertainty:* Evidence for diagnostic or prognostic significance between low or high concentrations of kidney-related particles. Diagnostic significance of quantitative SEC or TEC concentrations is lacking.

6.5. Verification of particle counting procedures

6.5.1. Performance evaluation of instrumental particle counting

The advanced comparison procedure in urine particle counting for the manufacturer's validation, and for the verification of primary (index) instrument at the end-user's laboratory is a *Level 3* procedure (see **Chapter 6.2.3**). In addition to comparing quantitative counts with scatter plots, the correct detection and differentiation of particles is important, expressed as sensitivity and specificity against the reference procedure. Comparisons using receiver-operating characteristic curves may be informative.

For a standard evaluation of analytical performance, such as imprecision, linearity, and limits of blank, detection, and quantitation by automated urine particle instruments, international guidelines should be consulted as needed, as available from the Clinical Laboratory Standards Institute (CLSI) [113, 114, 115], International Committee for Standardization in Hematology (ICSH) [66], Joint Committee for Guides in Metrology (JCGM) [116], and similar organisations.

Method comparison should be performed by linear regression analysis using non-parametric Passing-Bablok procedure [117], and Spearman's ordinal scale coefficient of correlation. Difference plots according to Bland and Altman [118] are applicable for urine particles as well. Logarithmic transformation helps in assessing exponential changes.

If comparing in ordinal scale categories, inter-rater agreement with kappa statistics can be applied for comparisons (see **Chapter 5.2.3** for examples of Performance specifications for urinary test strips). Advice to estimate carry-over is available from the ICSH guideline for verification of instruments counting body fluids [66].

6.5.1.1. Imprecision of counting

Low particle concentrations (less than $200 \times 10^6/\text{L}$) in clinical urine specimens need additional consideration of statistical imprecision based on the Poisson distribution, with standard deviation $s(n) = \sqrt{n}$, where n = total number of counted particles [72]. Correspondingly, the minimum coefficient of variation, $CV = s(n)/n = \sqrt{n}/n$. In addition, technology of instruments and variable morphology of particles increase the imprecision of counts.

The analytical CV of imprecision is obtained by 20 replicate countings of low positive specimens (in a range of 1-15 particles $\times 10^6/\text{L}$) according to the standard protocol [115]. After confirmation of the zero level (Limit of blank, *LoB*) by measuring supernatant solution of centrifuged urine, the limit of detection (*LoD*) is obtained by replicate counting after dilution of stable particles, such as those in quality control specimens, into the prepared supernatant urine, to obtain $LoD = LoB + 2s$ (two standard deviations of the observed imprecision) [96]. Due to the Poisson distribution of low counts, the limit of quantitation (*LoQ*) obtained with natural particles is more critical. It may be estimated with patient specimens positive for the assessed particle. A *LoQ* is a concentration where the observed CV is at 30%, to obtain $3 \times LoQ$ that is above *LoB* (different from zero). It may appear that the estimated 95% upper reference limit (*URL*) is below the *LoQ* when obtained from repeated counting (**Figure 6-1**). If that case, *LoQ* should be given in clinical reports instead of the exact *URL*, e.g. $95\% URL < n \times 10^6/\text{L}$, where $n = LoQ$.

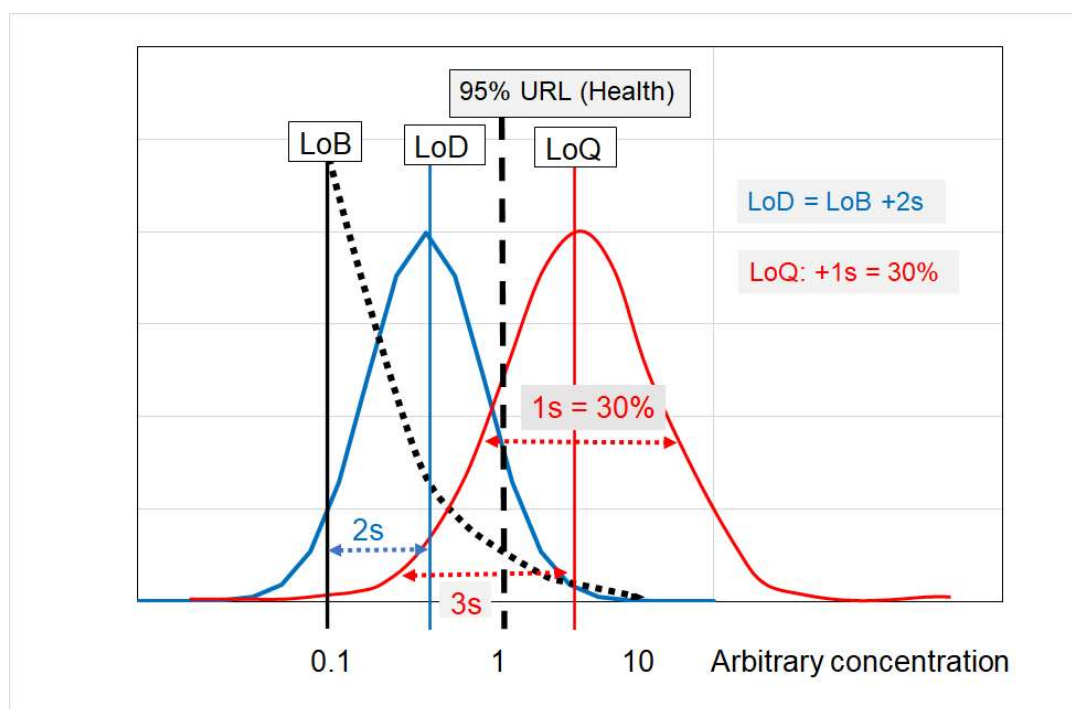


Figure 6-1. Schematic order of analytical limits in urine particle counting

A typical distribution of health-associated concentrations of urine particles is shown with a dashed line, with a 95% upper reference limit (URL). The baseline = Limit of Blank (*LoB*) needs to be confirmed in the method. Limit of Detection (*LoD*) is 2 standard deviations above *LoB*. Limit of Quantitation (*LoQ*) is at the concentration where the CV of analytical imprecision is 30%.

Since the imprecision is dependent directly on particle concentration, it is suggested to estimate the *LoQ* by duplicate counting of a range of positive specimens (e.g., 1-100 particles xE6/L depending on the type of particles in question), out of which an estimate to the actual imprecision is obtained from defined subgroups of concentrations by using Dahlberg's equation [119]:

$$s = \sqrt{[\sum(x_{i1} - x_{i2})^2 / (2n)]},$$

The imprecision of counting, CV_{observed} should also be related to the mathematical Poisson imprecision CV_{Poisson} . The *relative imprecision* $R(CV)$ is equal to $CV_{\text{observed}} / CV_{\text{Poisson}}$. The increment of $R(CV)$ above 1 is caused both by technology and biology of urinary particles, and may be up to 1.5-2 with available instruments, while CV_{Poisson} may be decreased by increasing counting volumes only [73].

In addition to quantitative results, the following qualitative aspects are recommended to be reviewed in the evaluation of urine particle analysers (**Table 6-6**).

Table 6-6. Qualitative features in the assessment of a urine particle analyser

Sufficient number of pathological patient specimens both qualitatively and quantitatively
Ease of use and robustness
Ability to self-check and recognise faulty performance, flagging
Instrument throughput
Data transmission with laboratory computers, preferably bi-directional
Cost/benefit assessment including all costs (reagents, manpower, maintenance, and indirect costs)
Impact on patient care (impact on outcomes if changing specimen or patient processes)

6.5.1.2. Regulations

Any medical device intended for *in vitro* diagnostics should be compatible with the EU Regulation 2017/746 on *in vitro* diagnostic medical devices [120].

RECOMMENDATION: Automated particle analysers need to be verified before being implemented into routine, based on the published performance specifications (against Level 3 procedure), as repeated in these guidelines. Performances in detecting urinary tract infections or kidney diseases need a special attention. (1, A)

6.5.2. Suggested analytical performance specifications

6.5.2.1. Quantitative counting

Imprecision

The imprecision of particle counts theoretically follows Poisson distribution (**Chapter 6.5.1.1**). A recommended *optimum specification* for the relative imprecision is $R(CV) \leq 1.5$, and for a *desirable specification* is $R(CV) \leq 2$.

$$R(CV) = CV_{\text{observed}} / CV_{\text{theoretical}}$$

where $R(CV)$ = relative imprecision, CV_{observed} = observed imprecision, and $CV_{\text{theoretical}}$ = statistical Poisson imprecision of counts

Limits of detection, at least

$5 \times 10^6/\text{L}$	for WBC, RBC and squamous epithelial cells (SEC)
$1\text{--}3 \times 10^6/\text{L}$	for casts and other epithelial cells (RTC and TEC)
> 90 %	sensitivity to detect uropathogenic bacteria in defined patient population, against colony counts in culture at 10^4 CFU/mL (10^7 CFB/L) or 10^3 CFU/mL (10^6 CFB/L) if applicable)

Trueness, correlation to advanced comparison counting

Spearman's correlation coefficient $r_s > 0.9$ (for WBC and RBC), $r_s > 0.8$ for other particles.

Allowable analytical variation

The specification for performance of urine particle counting (including bias and imprecision) has not yet been harmonised. It may be derived clinically from differentiation between health and disease-related concentrations. Clinically acceptable analytical performance specification (CAAPS) for particle counting, expressed as maximum allowable analytical variation (CV_A), or measurement uncertainty, is derived from the equation of reference change value, RCV , considering also intra-individual biological variation (CV_I) of the counts [121, 122].

Analytical performance specifications derived from clinically significant difference, CD , are based on the following two equations:

(1) $CD = z * \sqrt{2} * CV_D$, converted into $CV_D = CD / (z * \sqrt{2})$

where z is the Gaussian statistic, using $z = 3$ to reach a 85% sensitivity of detection, CV_D = coefficient of diagnostic variation, and $\sqrt{2}$ models two identical distributions in the compared measurements.

(2) $CV_D^2 = CV_I^2 + CV_{PRE}^2 + CV_A^2$

where CV_I = intra-individual biological variation, CV_{PRE} = preanalytical technical variation, and CV_A = maximum allowable analytical variation, or measurement uncertainty.

Analytical performance specification (APS)

Maximum allowable analytical variation (calculated as a CAAPS) is recommended to be 30% (optimum) or 50% (desirable), to detect 3 to 10-fold differences related to pyuria or haematuria, assuming an intra-individual biological variation (CV) within 30-200% (Table 6-7) [122].

Table 6-7. Analytical performance specifications from clinical differences in concentrations of urine particles

Estimated difference from the lower limit	Example difference in counts (LL -> HL)	Decision interval (UL-LL)/ LL, %	Maximum allowable variation for diagnostics, $z=3$	Biological intra-individual variation, estimate, %	Preanalytical technical variation, estimate, %	CAAPS based on decision limit, %
3 x LL	10 -> 30 x10 ⁶ /L	200 %	47 %	30 %	20 %	30 %
5 x LL	2 -> 10 x10 ⁶ /L	400 %	94 %	60 %	50 %	53 %
10 x LL	10 -> 100 x10 ⁶ /L	900 %	212 %	200 %	50 %	50 %

CAAPS = clinically acceptable analytical performance specification; LL = lower limit, UL = upper limit.

6.5.2.2. Visual microscopy and ordinal scale specifications of low-count particles

The standardised visual microscopy (*Level 2*) should be reported with quantitative counts that can be compared to ISLH reference procedure (*Level 3*). Both automated instruments and visual microscopy suffer imprecision of low counts and rare particles. Centrifugation improves detection of rare particles, but reduces accuracy due to losses during centrifugation. Laboratories are recommended to select relevant procedures from those described in **Chapters 6.2.4** for their routine visual microscopy. They should also verify that their procedure satisfies clinical needs in urine particle detection and quantitation, applying details from **Chapter 6.2.3** as necessary.

In evaluation studies, a positive selection of specimens with rare particles should be attempted, to maximise fractions of specimens positive for each particle to be compared, and to avoid comparison of specimens with negative counts. If the assessed patient material does not allow comparison of precise counts, an ordinal scale cross-table helps to assess agreement, i.e., sensitivity and specificity against the comparative procedure. Ordinal scale statistics still require a sufficient number of positive cases to allow balanced distribution of results along ordinal scale categories. A logarithmic grouping of particle counts is recommended.

Specification of ordinal scale categories

An example of ordinal scale statistics is shown in **Chapter 5.2.3**. If using Cohen's kappa coefficient to eliminate random agreement, the following specification is recommended: A weighted kappa ≥ 0.9 as an optimum, and ≥ 0.7 (as a minimum performance with 4 or more ordinal categories).

Qualitative assessment, detection and differentiation

Identification and differentiation of clinically significant urine particles should be internally reviewed (peer review between staff members) and externally evaluated (EQA schemes), in addition to initial verification of the routine procedure. Each site should document training of its laboratory technicians.

RECOMMENDATION: It is recommended to adopt relevant statistical procedures when presenting verification data for urine particles. (1, B)

6.5.3. Microscopic review after automated particle analysis

The combination of automated routine urine particle analysis and microscopic re-analysis is employed to screen for otherwise undetectable or doubtful urine samples [123]. Due to the differences in analytical performance, each analyser should have its own review flags [124, 125, 126], based on cross-checks between automated urinary test strip and visual microscopy results, or on unreliable particle counts. Those criteria must be validated and confirmed to meet local clinical and laboratory needs.

RECOMMENDATION: Based on the verification, appropriate review rules need to be defined and implemented to support reliability of all results. (1, B)

6.6. RECOMMENDATIONS FOR PARTICLE ANALYSIS

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
39	Urine particle analysis has a role in the diagnostics of urinary tract infections, haematuria, and kidney diseases.	1, A	6.1.1
40	Urine crystals are not recommended to be looked for, nor be reported, for all specimens. In specific situations, urinary crystals may indicate an inherited or metabolic disease, or a drug precipitated in the kidneys, causing stone formation or renal failure. Most commonly, crystals or amorphous precipitate interfere with identification of other particles in urine.	1, A	6.1.1
41	Laboratories are recommended to clearly discuss and describe their basic and advanced differentiation of urinary particles with their clinicians, in order to harmonise clinical interpretation of their results.	1, B	6.1.2
42	The standard unit for urine particle concentrations is particles/litre (L), the SI unit. Unit of routine clinical reports is recommended to be harmonised at national level, to avoid clinical confusions.	1, C	6.2.2
43	Phase-contrast optics is recommended in the detection and discrimination of urine particles both in routine and reference microscopy.	1, A	6.2.3
44	Laboratories should verify one of the (<i>Level 2</i>) procedures of visual microscopy for their routine analysis to ensure accuracy of their results.	1, B	6.2.4
45	A rough estimate for health-associated 95% Upper Reference Limit both for leukocytes and erythrocytes is 10 (to 20) x10 ⁶ /L from a mid-stream urine collection of uncentrifuged morning urine. The uncertainty contains both preanalytical and analytical factors.	1, A	6.4.1
46	Automated particle analysers need to be verified before being implemented into routine, based on the published performance specifications (against <i>Level 3</i> procedure), as repeated in these guidelines. Performances in detecting urinary tract infections or kidney diseases need special attention.	1, A	6.5.1
47	It is recommended to adopt relevant statistical procedures when presenting verification data for urine particles.	1, B	6.5.2
48	Based on the verification, appropriate review rules need to be defined and implemented to support reliability of all results.	1, B	6.5.3

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction.

6.7. References, Chapter 6

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The EFLM European Urinalysis Guideline

Update 2023

Chapter 7. Bacteriology

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List of abbreviations, Chapter 7

ACSS	Acute cystitis symptoms score
AST	Antimicrobial susceptibility testing
ATCC	American type culture collection
BIPM	Bureau International des Poids et Mesures
CFB	Colony-forming bacteria
CFU	Colony-forming unit
CV	Coefficient of variation (relative standard deviation)
dAST	Direct AST
EAU	European Association of Urology
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
EQA	External Quality Assessment
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ICSH	International Committee for Standardization in Hematology
ID	Identification (of species)
IDSA	Infectious Diseases Society of America
ISO	International Organisation for Standardization
IVD	In vitro diagnostic medical device
IVDR	In vitro diagnostic medical device regulation
JCGM	Joint Committee for Guides in Metrology
MALDI-TOF	Matrix-assisted laser desorption ionisation time-of-flight (mass spectrometry)
MDR	Medical device regulation
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
MSU	Mid-stream urine
SI	International system of unit
SPA	Suprapubic aspiration (specimen)
TAT	Turn-around time
TLA	Total laboratory automation
UTI	Urinary tract infection
VIM	International vocabulary of metrological terms

7.1. Medical indications for bacteriology investigation of urine

The aims of urine bacterial culture are

- to identify aetiological agents of urinary tract infection, i.e., relevant pathogens, but also mixed flora (> 2 species) as a sign of contamination,
- to estimate the concentration of bacteria,
- to offer susceptibility testing for antimicrobial treatment, and
- to look for a relapse or re-infection in patients not responding to antimicrobial treatment.

In clinical practice, it is not necessary to perform all examinations for every patient suspected of having urinary tract infection (UTI) (see **Chapter 1.2, Figure 1-2**). A simple division of the patients into common cases suspected of lower uncomplicated UTI, and other more demanding cases will improve the efficiency of clinical laboratory practice.

7.1.1. Indications for rapid urine examinations in diagnostics of urinary tract infections

Clinical questionnaires, such as ACSS (Acute Cystitis Symptoms Score), may be used to support in diagnosing uncomplicated lower UTI in non-pregnant women, as validated already for several languages [1, 2], see **Chapter 1.2.1**. Rapid examinations are recommended in situations described in **Table 7-1**. In the context of UTI diagnostics, test strips and particle analysis are both rapid or emergency tests compared to bacterial cultures. Usually, rapid tests mean point-of-care tests with robust methods and devices (**Chapter 4**).

Table 7-1. Suggested indications for use of rapid tests in UTI diagnostics

(1) Classical frequency/ dysuria syndrome in young, low-risk women if clinically needed
(2) Emergency medical services, as a first rapid diagnostic examination
(3) Screening for selected asymptomatic individuals (Chapter 1.2.2)
(4) Selecting specimens for extended investigation in the laboratory (Chapters 1.2 and 7.1)

In sporadic uncomplicated lower urinary tract infection from otherwise healthy non-pregnant women (item 1), no laboratory examinations are usually necessary when the symptoms are clear-cut [1, 3, 4, 5, 6, 7, 8]. If symptoms remain unclear, rapid methods to detect bacteriuria and leukocyturia help in the differential diagnosis of patients with medical emergencies (**Figure 1-2**). Before classifying otherwise healthy women into this group, anatomic abnormalities in the urinary tract and pregnancy should be considered (**Table 7-2**). The topic of recurrent UTI is covered in **Chapter 7.1.2**.

Usually, asymptomatic bacteriuria represents colonisation with or without leukocyturia, and should not be sought nor treated, to avoid enrichment of multi-resistant bacterial strains, particularly in patients with indwelling catheters [4, 9, 10]. Screening of selected clinical populations, such as pregnant women and patients before urological operations that penetrate

the mucosal membrane is, however, warranted (**Chapter 1.2.2**), as specified by international guidelines [4, 5, 6, 9].

RECOMMENDATION: Commensal urogenital microbiota are not recommended to be sought nor treated from asymptomatic individuals (Asymptomatic Bacteriuria) (SoR 1, LoE A) ^a

RECOMMENDATION: Suspicions of sporadic uncomplicated lower urinary tract infections in otherwise healthy women are recommended to be screened for the presence of infection by using a validated questionnaire, to reduce routine workflow in bacteriology laboratory. Rapid tests for leukocytes and bacteria are recommended into diagnostics of unclear and other cases (1, A) ^a

^a Laboratory modification of the grades is described in the Introduction of this guideline. Strengths of Recommendations (SoR) are rated as: 1= strong, 2 = weak recommendation. Levels of Evidence (LoE) are rated as: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts.

7.1.2. Indications for urine bacterial culture and identification of species

Strategies to reduce the number of non-significant bacterial cultures are highly encouraged, to improve the quality of those cultures that are clearly indicated. An advisory flowchart for test requisition in suspicions of UTI is shown in **Chapter 1.2, Figure 1-2**. Urine specimens from other symptomatic patients than non-pregnant otherwise healthy women suffering from sporadic uncomplicated lower UTI should be sent to the bacteriology laboratory for quantitative culture and susceptibility testing (**Chapter 1.2**). A representative list of these patients with UTI symptoms is in **Table 7-2**. Special cases and specimens needing special urine cultures are pointed out in **Chapter 1.2.1.2** and **Figure 1-2**.

Table 7-2. Medical indications for urine culture

(1) Suspicion of acute pyelonephritis or febrile urinary tract infection
(2) Suspicion of hospital-acquired urinary tract infections (possibility of reduced antibiotic sensitivity)
(3) Suspicion of urinary tract infection in patients with a predisposing disease, such as diabetes [11], anomaly of the urinary tract, recurrent stone disease, or immunocompromised state
(4) Patients failing first line antimicrobial chemotherapy
(5) Clinical suspicion of urinary tract infection in febrile patients with indwelling catheters
(6) Clinical suspicion of urinary tract infection in men (symptomatic) [12]
(7) Clinical suspicion of urinary tract infection in pregnant women (symptomatic)
(8) Suspicion of urinary tract infection in children and adolescents (symptomatic)
(9) Recurrent UTI

Detailed backgrounds of item (3) and (6) are in the quoted references [11] and [12], respectively.

Consider also *national guidelines* for diagnostics and treatment of urinary tract infections, or other reviews on management of urine cultures, as shown with the listed examples:

- European Association of Urology Guideline [9]
- Public Health England Quick reference guide [8]
- Spanish guidelines for diagnosis and treatment of UTI [4]
- American Urology Association Guidelines for rUTI [13]
- IDSA Guideline for catheter-associated UTI in adults [14]
- Belgian BILULU consensus guideline [15]
- German multidisciplinary clinical guideline on ambulatory UTI of adults [10, 16]
- Updated EAU/ESPU guidelines on urinary tract infections in children [17]
- Reviews on urine culture management [5, 18]

RECOMMENDATION: Urine specimens from most routine patients suspected for UTI are recommended to be sent to quantitative urine culture and possible antimicrobial susceptibility testing. Sensitive screening procedures are encouraged to reduce the number of specimens from the routine workflow. Special cultures of specimens from special patient groups are recommended to be organised as nationally or locally defined. (1, A)

7.1.3. Indications for urine bacterial culture after completed treatment

When the patient become asymptomatic after treatment for acute cystitis, no control urine culture is needed [4, 9, 19]. There is insufficient evidence to guide management after acute cystitis treatment in pregnancy. The Committee on Clinical Consensus-Obstetrics of the American College of Obstetricians and Gynecologists (ACOG) has written a recommendation allowing clinicians to consider either repeating the urine culture 1-2 weeks after treatment for acute cystitis for pregnant individuals, or requesting a urine culture only if symptoms recur [20]. No recommendation is given to control cultures for pregnant women after antimicrobial treatment.

RECOMMENDATION: No control cultures are needed from patients with lower UTI if becoming asymptomatic after an antimicrobial treatment (1, A).

7.2. Microbes of the urinary tract

Specific bacteria, e.g., those causing tuberculosis, leptospirosis, salmonellosis, or sexually transmitted diseases, such as *N. gonorrhoeae*, or *C. trachomatis*, and fungal infections need special examination methods not discussed in detail in these guidelines.

7.2.1. Urinary microbes in health and disease

7.2.1.1 Urobiome in healthy individuals

The presence of organisms in urine *per se* is not diagnostic of an infection, since the urogenital tract of asymptomatic individuals contains numerous and diverse microbiota when studied with extensive culturomics and gene sequencing [21, 22, 23, 24, 25]. Indeed, despite that urine was historically considered sterile in healthy individuals, many recent studies with genomic technologies and expanded urine cultures describe a variable resident bacterial community in the bladder of healthy individuals.

The term urobiome refers in this guideline to microbiome of the urinary tract (group of microbial genomes in a specific environment) that encompasses viable urinary microbiota. It is variable between individuals and changes over time and in different physiological conditions [25, 26, 27].

The microbiome obtained in the urinary bladder (collected with methods that avoid contamination by other anatomically close microbiota) is estimated to encompass 10^2 to 10^5 CFU/mL (10^5 to 10^8 CFB/L). Its size is smaller than those of other human microbiomes, consisting both cultivable and non-cultivable bacteria. In both genders, Firmicutes is the major phylum identified, followed by Actinobacteria, Bacteroidetes, and Proteobacteria (8% in male, 3% in female for this last phylum). Many genera are frequently identified: in healthy women *Lactobacillus*, *Gardnerella*, *Atopobium*, *Prevotella*, *Staphylococcus* are found, whereas in males *Corynebacterium* and *Streptococcus* are more prevalent. *Escherichia* and *Enterococcus* genera are also described as members of urinary microbiota in healthy individuals [28].

The composition of bladder microbiota differs from that of periurethral and genital tract and that of gut microbiota, but shares a wide range of species with both of them. Given a great similarity between strains isolated from vaginal and bladder microbiota, some authors even propose existence of a single urogenital microbiota in both niches. Most authors prefer to consider an interconnection [27, 29, 30].

The urinary microbiota could play a major role in maintenance of homeostasis and preventing UTI. The diversity and the proportion of bacterial species identified in the urobiome are modified in many urinary diseases or disorders, including urgency incontinence. The relationship between specific urotypes and specific urinary symptoms is still poorly understood [31, 32]. Interactions within microbiota probably play a critical role affecting the capacity of potential pathogens to successfully establish and sustain colonization to outcompete the other microorganisms [33].

7.2.1.2. Uropathogens and urinary tract infection

Detection of primary pathogens in urine does not necessarily mean a diagnosis of infection. UTI symptoms depend on the combination of virulent invasion of uropathogens, inadequate host defences and other predisposing factors. Some lineages of *Escherichia coli* (UPECs, uropathogenic *E. coli*) and *Staphylococcus saprophyticus* are more commonly associated with urinary tract infections than other species because of their virulence gene repertoire. They are therefore regarded as primary pathogens [34, 35, 36, 37, 38] (see **Table 7-3**).

Even primary pathogens can be cultured in urine of women without any symptoms. As an example, *Escherichia coli* was detected by extended quantitative cultures and 16S RNA gene sequencing in the urine collected via transurethral catheter in some continent adult women without UTI symptoms [26]. In routine laboratory practice, primary pathogens can be cultured even at significant colony counts from urine of individuals without symptoms of UTI, defined clinically as *asymptomatic bacteriuria* (see **Chapter 1.2.2**) [6]. Thus, urobiome and/or host-related factors influence the development of UTIs by these primary pathogens.

Consequently, several clinical factors affect the specificity of detected bacteriuria in the diagnosis of UTI. These include presence of local or general symptoms, bladder incubation time, way of collection - including catheters, anatomical or functional abnormalities of the urinary tract, and patient-related factors, such as age and sex, pregnancy, kidney disease, concomitant immunocompromising diseases, or glycosuria in diabetes.

7.2.1.3. Contamination of urine specimens during collection

A major variable that cannot be accurately controlled is the technique of mid-stream (MS) urine collection. Despite patient instructions, a fraction of specimens contain commensal urogenital contaminants in high enough quantities to make interpretation difficult. For the recommended efforts, see **Chapter 3.2**. Diagnostic rules therefore depend on whether bacterial growth is pure or polymicrobial. This underscores the importance of clinical and preanalytical detail for each laboratory specimen, as well as infection-related test results such as leukocyturia. Effective patient management requires inclusion of these concomitant data in the interpretation of results of urine bacterial cultures.

7.2.2. Classification based on uropathogenicity

Uropathogens were classified into 16 categories based on four degrees of pathogenicity (I – IV) and four frequencies in different clinical populations [3, 7, 15, 35, 39, 40, 41, 42, 43, 44]. The examples shown in **Table 7-3** for each category must be adjusted locally to cover most relevant clinical uropathogens.

Table 7-3. The pathogenicity and frequency of example microorganisms in urine

		Frequency (percent of isolates)			
Pathogenicity in the urinary tract		uUTI ^a	cUTI	HA-UTI	CA-UTI
I. Primary pathogens	<i>E. coli</i>	70-75	55-65	45	30
	<i>S. saprophyticus</i> ^b	3-6	-	-	-
II. Secondary pathogens	<i>Enterobacter</i> spp.	2	4	6	3
	<i>Enterococcus</i> spp. ^e	4-5	6-11	10	10
	<i>Klebsiella</i> spp.	5-6	8-9	12	5
	<i>Proteus</i> spp.	2-4	2-5	6	11
	<i>P. aeruginosa</i>	1-3	2-7	9	11
	<i>S. aureus</i>	1-2	2-3	3	4
	<i>Citrobacter</i> spp.	2	3	1.5	5
	<i>M. morganii</i>	<1	5	<1	4
	<i>Serratia</i> spp.	<1	7	<1	<1
	<i>Aerococcus</i> spp. ^e	1	1	-	-
	<i>Actinotignum schaalii</i> ^e	<0.1	<0.1	-	-
	<i>C. urealyticum</i>	-	-	-	-
III. Doubtful pathogens	<i>Streptococcus agalactiae</i> ^c	3-4	2-3	<1	<1
	Yeast ^d	1	3-7	2	7
	<i>Acinetobacter</i> spp.	<1	2	2	2
IV. Contaminants	Coagulase negative staphylococci, CNS ^d (except <i>S. saprophyticus</i>)				
	<i>Corynebacterium</i> spp. (except <i>C. urealyticum</i>)				
	<i>Gardnerella vaginalis</i>				
	<i>Lactobacillus</i> spp.				

^a Abbreviations used: uUTI, uncomplicated urinary tract infection; cUTI, complicated urinary tract infection; HA-UTI, healthcare-associated urinary tract infection; CA-UTI, catheter-associated urinary tract infection; -, data not available.

^b More important in sexually active young women [45]

^c *Streptococcus agalactiae* (Group B streptococci) [46]. GBS are pathogenic to the babies of pregnant women at childbirth and a few weeks before, and should always be reported [44].

^d Yeast [47] and CNS [48] are members of urobiome. Probability that they cause a true infection must be evaluated case-by-case to avoid unnecessary antimicrobial treatment.

^e Classification as class II pathogen only in case of monomicrobial culture, otherwise considered as a class III pathogen with an AST carried out based on local decision.

Pathogenicity was classified as follows:

I. *Primary pathogenic species*: Species that can cause urinary tract infection in individuals with normal urinary tract.

II. *Secondary pathogenic species*: Species that rarely cause primary infection in patients with normal urinary tract.

III. *Doubtful pathogenic species*: These microorganisms sometimes colonise urinary tract, and occasionally cause mostly hospital-acquired urinary tract infections.

IV. *Contaminants*: Microorganisms that are found in urine culture due to contamination of the specimen with skin, urethral or genital microbiota. These may be considered to cause UTI only after assessing the details of the specimen and the specific clinical request. A control with a new specimen is encouraged.

Specimen data and clinical background have an impact on pathogenic role of listed pathogenic groups. When the specimen is NOT obtained by SPA or puncture of renal pelvis, consider the following:

- quality of the actual way of specimen collection
- results from urine particle analysis or microscopy
- count and types of species grown in culture
- host conditions (pregnancy, immunosuppression, another predisposition to UTI)

Suggested changes in classification

Class II is now enriched with *Aerococcus* spp (*A. urinae*, *A. sanguinocola*) and *Actinotignum schaalii* that can be considered as secondary pathogens when isolated in monomicrobial culture. These have been underreported and underestimated. Being previously considered as contaminating microbes and overlooked in routine diagnostics, accumulating evidence shows that these bacteria are a rare but real cause of UTI (see **Chapter 7.2.3**). Their role when detected with an other uropathogen remains to be explored.

Corynebacterium urealyticum also belongs to class II uropathogens. Due to its urease enzyme, it is associated with alkaline incrustated cystitis and pyelitis, particularly in patients with underlying urologic disease, such as renal transplant patients [49].

Coagulase-negative staphylococci (CNS) were moved to contaminants, being members of urobiome [48].

RECOMMENDATION: *Classification of uropathogens has been slightly updated. In addition to uropathogenicity, predisposing host conditions, quality of specimen collection, results from particle analysis (leukocytes and bacteria), and quantity and types of species grown in culture have an effect on the diagnostic value of detected bacteriuria. (1, A)*

7.2.3. Emerging pathogens

Improvement of traditional culture techniques, introduction of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) (see **Chapter 7.6.2**) and molecular techniques, and finally development of laboratory automation (see **Chapter 7.4.3**) have considerably improved the efficacy and accuracy of microbial detection and identification from urine specimens. Indeed, implementation of MALDI-TOF MS, prolonged incubation up to 48h, use of anaerobic or 5 to 10 % CO₂ atmosphere have enlarged the number of identifiable bacteria from urine samples. Examples of organisms with fastidious growth requirements include *Aerococcus* spp, *Actinotignum schaalii*, and *Alloscardovia omnicolens* [50, 51, 52, 53, 54, 55].

The role of *Aerococcus* spp. (especially *A. urinae*) and *Actinotignum schaalii* in urinary tract infections is nowadays well established, being considered as class II secondary pathogens (see **Table 7-3**). These species are more commonly isolated in elderly patients with underlying urological diseases, e.g., urgency urinary incontinence, over-active bladder, prostate or bladder cancer, or benign prostatic hyperplasia [56, 57, 58]. However, while *A. urinae* has been isolated from both female and male elderly patients [55], *A. schaalii* is more frequently cultured from male patients and can also be isolated from young children [52].

Implementation of automated systems in microbiology laboratory has increased the recovery of microorganisms, including fastidious ones (such as Gram-positive bacteria) thanks to closed systems allowing stable incubation atmospheres and high-quality plate images (see **Chapter 7.4.3**) [59, 60]. However, the clinical relevance of some of these emerging species, e.g., *Actinomyces* spp, *Lactobacillus* spp, *Gardnerella vaginalis*, and *A. omnicolens* needs to be confirmed as they have also been described as members of the bacterial communities colonising the urinary tract [61, 62, 63] and are often found in low numbers (10² to 10³ CFU/mL; corresponding to 10⁵ to 10⁶ CFB/L) [59]. Midstream urine specimens are also prone to contaminants of commensal species during collection, in addition to members of bladder urobiome, see **Chapter 7.2.1** for detailed discussion.

RECOMMENDATION: New species *Aerococcus* spp, *Actinotignum schaalii* and *Corynebacterium urealyticum* are proposed into the list of class II uropathogens if detected in monomicrobial culture. (2, B)

7.3. Bacterial detection by non-culture methods

There is a need for high performance rapid methods for the detection of bacteria in urine. This applies for the routine laboratory handling of large numbers of specimens, for emergency diagnostics, and for detection of asymptomatic bacteriuria in selected patient groups, such as pregnant women. Development of analytically sensitive and specific rapid procedures for detection of bacteria is encouraged for both adults and children (see **Chapter 6.3.3.1**).

Sensitivity view: A high performance high-throughput screening procedure with low false negative rate would identify true negative specimens (most often, with WBC and bacteria detection) and allow significant reduction in unnecessary urine cultures. Besides sensitivity, health care savings are dependent on the obtained specificity or false-positive rate. The validation of method performance for detection of bacteria at low counts, i.e., less than 10^5 CFU/mL (less than 10^8 CFB/L) becomes very important.

Specificity view: Patients at emergency rooms need a rapid examination with high specificity to suggest presence of uropathogenic bacteria, in particular when the focus of infection is not obvious. At higher diagnostic cut-off of UTI-related urine particles (high concentrations of WBC and bacteria), a rapid test supports immediate treatment decision while cases with borderline counts of particles need to wait results from urine bacterial cultures.

7.3.1. Microscopy methods in bacteriology

7.3.1.1. Gram staining procedure (*Level 2*)

Gram staining of urine is traditional, but it has rather low sensitivity ($\geq 10^4$ bacteria/mL) and low discriminatory power as only Gram positive versus Gram negative, and cocci versus rods can be detected. It is no more a mandatory procedure for urine specimens because it is tedious, time-consuming and strongly dependent of interfering factors (see below) [41].

Gram staining is, however, important to be available for special requests or patient groups, e.g., young children, severe infections, or atypical clinical forms [64, 65, 66, 67, 68]. Gram staining may occasionally be used for presumptive etiologic diagnosis - leading for example to addition of extra culture media, to guide empirical antimicrobial treatment [69], or to detect polymicrobial contamination of a specimen [70].

Preliminary results on Gram staining of urine bacteria with flow cytometric particle counting are reviewed in **Chapter 6.3.3.1**.

When Gram stain is performed on fresh uncentrifuged urine, the sensitivity of microscopy is 10^5 bacteria/mL (10^4 bacteria/mL when centrifuged) [41, 70, 71]. When compared to culture results, major errors of Gram stain results are related to inappropriate staining processing, examination of a limited number of fields or characteristics of some organisms, e.g., Gram positive species that stain Gram negative naturally or because of antimicrobial therapy. This can be improved by training and maintenance of proficiency in microbiology [70].

Discordant results with culture (false Gram stain results) may also be due to fastidious or non-viable micro-organisms (like anaerobic bacteria) that failed to grow under the culture conditions used, or due to presence of antibiotics in the sample [41, 70]. Thus, to be accurate and

helpful, Gram stain requires a careful follow-up of the technical procedures [41] and interpretation criteria. The sensitivity of Gram staining may vary from 82% to 98%, and the specificity from 66% to 95% compared to $\geq 10^4$ CFU/mL in culture [72].

7.3.2. Screening procedures for detecting bacteria in urine

Multiple test strips are discussed in detail in **Chapter 5.2**, including diagnostic performance (**Chapter 5.2.1**). Analytical performance of strip test analytes with false negative and false positive results are discussed in **Chapter 5.2.2**, including **Table 5-5**.

Urine particle analysis (of both living and non-revivable bacteria) by visual microscopy or automated instruments is discussed in detail in **Chapter 6**, including performance of WBC and bacteria counting by automated instruments against bacterial culture (**Chapter 6.3.3.1**). Detection of leukocyturia and bacteriuria may be used in several ways for diagnostics of UTI:

- Diagnostic specificity > 90% against clinical UTI, to be used to support decisions on emergency patients, although the sensitivity may remain less optimal
- Analytical sensitivity > 95% against culture at $\geq 10^5$ CFU/mL (10^8 CFB/L), or >80% at $\geq 10^3$ CFU/mL (10^6 CFB/L), to be used to rule out unnecessary specimens from cultures at a specificity of at least 50% (see also **Chapter 7.8.3**)
- Presence of increased WBC concentrations in urine specimens to focus workflow of routine cultures in bacteriology laboratories (see **Chapter 7.5.2**)

Bacterial cultures have been modified for emergency diagnostics by **automated rapid culturing devices** using specific technologies and media [73, 74]. Despite clinical need, these instruments have not been widely applied into routine.

7.3.3. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been implemented in many clinical microbiology laboratories for more than a decade now [75]. This technique has changed the way to identify bacteria, but also yeast and even some fungi.

The detection of species-specific MALDI-TOF spectra from essentially ribosomal polypeptides provides a robust identification of bacteria and fungi. For this, microorganisms or their respective protein extractions are placed together with an organic matrix solution (e.g., alpha-cyano-4-hydroxycinnamic acid) on a metal target plate. After inserting the target plate into the MALDI device, a laser beam transfers energy to the bacteria-matrix mixture. The energy causes disruption of the bacteria, and subsequently release and ionization of highly prevalent ribosomal proteins from the cracked bacteria. By applying a high voltage, the ionized polypeptides and their fragments are accelerated and transferred to a flight tube in a high vacuum. At its end, a detector measures the impacting ions. Time to the detector depends on the charge and mass of the ionized polypeptides. The result is a specific mass spectrum, which is compared to a database of reference mass spectra within seconds. This comparison provides a reliable identification of the respective bacteria or fungus in a monomicrobial sample within minutes.

For direct identification of bacteria in clinical urine specimens by MALDI-TOF MS, different preparation steps have to be performed before the method can be applied. Human cells, mucus and salt need to be removed [72, 76, 77]. If more than one species is present, direct identification provides usually no meaningful results. Moreover, the accuracy of the results obtained by means of a direct MALDI-TOF MS-based identification of bacteria from urine specimen is by far inferior to that from bacteria grown on agar plate: bacteria concentrations of 10^4 CFU/mL (10^7 CFB/L) or higher are necessary to obtain reliable results directly from a specimen. This results in false negative reports in specimens with low uropathogen counts [78, 79]. Application of the MALDI-TOF MS to urine specimens without a preculture SHALL NOT be used for routine detection of bacteria in clinical laboratories.

RECOMMENDATION: Bacterial identification using Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is strongly recommended into medium-sized and large laboratories (> 100 specimens/day), to improve patient prognosis with accuracy and reliability of identification to the species level, and shortened delay of reporting. (1, A)

RECOMMENDATION: Limitations of the MALDI-TOF MS in detecting bacteriuria at low colony counts (less than 10^4 CFU/mL, or 10^7 CFB/L) must be understood in organising laboratory processes for urine specimens with a possibility of significant low bacteria counts. MALDI-TOF MS shall NOT be applied directly to urine specimens in routine laboratories without preculturing the specimen. (1, A)

7.4. Bacterial cultures

The bacterial culture procedures are structured into three performance levels based on the hierarchy of their diagnostic performance (see **Chapter 4** for general definitions based on accuracy): Qualified comparison methods (*Level 3*), quantitative field methods (*Level 2*), and ordinal scale or rapid methods (*Level 1*).

Individual laboratories and their customer clinicians must decide - based on local patient populations and resource - the way in which urine cultures should be organised locally. Common sense is needed in a clinical bacteriological laboratory to ensure both high clinical sensitivity and high specificity of routine reports. This may be influenced by the microbiology tradition and costs of health care in different countries. The ideal analytical process may not be attainable.

7.4.1. Choice of culture conditions

7.4.1.1. Culture media

No single culture medium allows growth of all uropathogens. Chromogenic medium is strongly recommended as the primary routine agar. As compared to other media such as Cystine-Lactose Electrolyte Deficient (CLED) agar, it allows rapid identification of the most frequent microorganisms causing urinary tract infections (particularly *E. coli*). It also supports detection of polymicrobial growth thanks to the hydrolysis of different chromogenic substances by species-specific enzymes [80, 81, 82]. Thus, using chromogenic agar allows to reduce workload of the laboratory technicians, material required for bacterial identification (no need for large supplementary tests to identify *E. coli*), and to improve turn-around time for patient results with lower costs [83, 84, 85].

7.4.1.2. Special urine cultures

Clinical microbiologists should additionally consider necessity of special procedures (**Figure 1-2**), such as culturing urine specimens on blood agar under 5% CO₂ atmosphere for 48 hours. These clinical cases may include patients with defined urological diseases [9], or cases of positive leukocyturia with negative culture results [42], and needs to detect emerging fastidious Gram-positive pathogens [15, 50]. For urine specimens collected during urological procedures (e.g., cystoscopy, nephrostomy) or from prostatic secretions, a chocolate agar is suggested as an optimum approach [41]. Columbia colistin-nalidix acid agar could be seeded and incubated under 5% CO₂ atmosphere, or even in anaerobic atmosphere in specific clinical needs.

Urine samples showing the presence of yeast on microscopy can be inoculated on chromogenic yeast culture medium. This may allow a direct presumptive identification of clinically important *Candida albicans*, *C. tropicalis* and *C. krusei*.

RECOMMENDATION: Chromogenic agar is strongly recommended as the primary agar medium to identify *Escherichia coli* (most frequent uropathogen) easily, quickly, and inexpensively (no need for a panel of tests to define the species). A second agar (such as blood agar) is recommended in clinically defined cases and for fastidious organisms. (1, B)

7.4.2. Manual routine culture (*Level 2*)

Statistics of particle counting

Poisson distribution is used to estimate imprecision of particle counts with the following parameters:

Standard deviation, s	$s = \sqrt{n}$, where n = number of counts
Coefficient of variation, CV	$CV = s/n = \sqrt{n}/n$

The limit of 10 colonies/plate for a reproducible detection of growth is derived from Poisson distribution of counting, where standard deviation $s = \sqrt{n}$ (see above). Three standard deviations typically define the analytical sensitivity = limit of detection (LoD). At the total count $n = 10$, $1s = \sqrt{10} = 3.1$, and $3s = 9.3$. The limit of detection is then above the range 0-9, i.e., 10 is the first count detectable above a negative result.

Imprecision of colony counts in clinical specimens is larger than that of the theoretical Poisson distribution, since it is influenced by the variability of bladder incubation time (urgency), diuresis, homogeneity of urine suspension, technical fluid volume caught into the inoculum, and culture conditions. Because of these additional factors, a colony count of 10^3 CFU/mL (10^6 CFB/L) remains a borderline quantity, and first 10^4 CFU/mL (10^7 CFB/L) is diagnostically reproducible even with a 10- μ L inoculum.

Procedures

Volume of inoculum: The volume of urine that is inoculated onto a culture medium affects the limit of detection of bacteriuria (see **Chapter 7.5** for diagnostic significance).

At least 10 colonies/plate are needed for a statistically reproducible detection of growth. A minimum of 10 colonies/plate corresponds to 10^4 CFU/mL (10^7 CFB/L) using a 1- μ L inoculum, but at 10^3 CFU/mL (10^6 CFB/L) a 10- μ L inoculum is needed for 10 colonies/plate. If a reproducible colony count at 10^2 CFU/mL (10^5 CFB/L) is needed, a volume of 100 μ L must be inoculated [15, 16, 41, 42]. Specific microbiology advice is necessary in locations with minimal resource for the practical inoculation volumes. External quality assessment has shown that there is a great variation in the methodology of performing conventional urine cultures despite attempts at standardisation.

Inoculation procedure: After mixing the urine gently, the end of a sterile 10 μ L calibrated loop is dipped in the urine just below the surface and removed vertically without carrying urine on the shank. This is then inoculated on the agar medium and spread by using one of the recommended methods described in **Figure 7-1 (a,b)**. For urine samples collected by invasive procedures (e.g. SPA), 100 μ L must be inoculated over the entire surface of the plate using a sterile spreader as described in **Figure 7-1 (c)**.

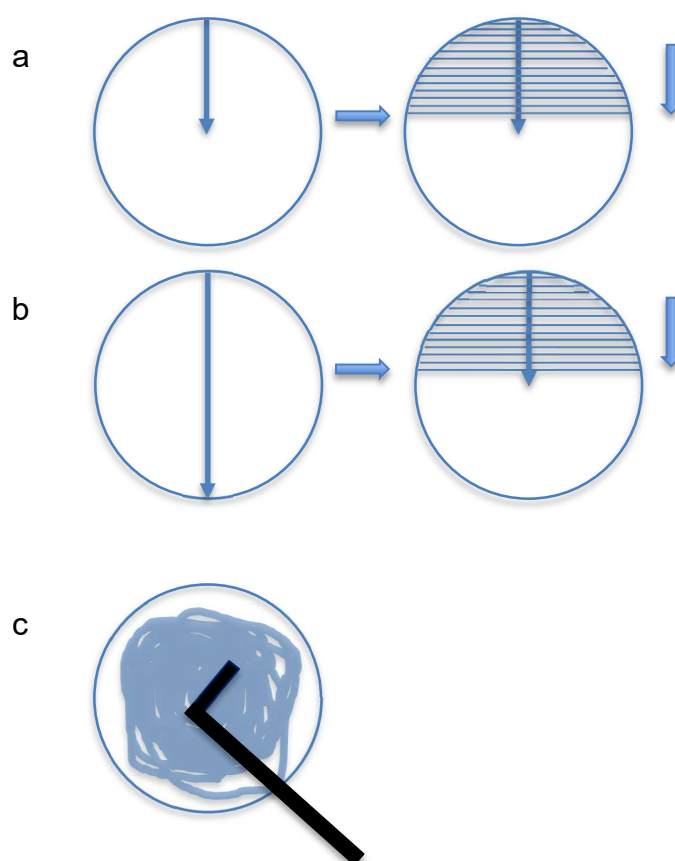


Figure 7-1. Inoculation of a culture plate

The images were modified from [41]. Both method (a) or (b) of streaking urine for colony count using a 10- μ L calibrated loop are recommended. These include dragging the loop over the radius (a) or the diameter (b) of the agar plate and streaking perpendicularly from top to bottom. For inoculation of a 100 μ L volume, the spreader method (c) is recommended because of large urine volume. Spread the inoculum over the entire medium surface back and forth while rotating the plate.

Cultures: Quantitative culture should be performed on a relatively non-selective agar plate as a minimum process (see **Chapter 7.4.1**). Incubation for 16-24 hours is sufficient for primary uropathogens. Aerobic incubation at $35 \pm 2^\circ\text{C}$ is recommended [15, 41, 42].

The routine culture procedure is generally less reliable for inpatients with a larger variety of uropathogens in their specimens, for invasively collected specimens, and for infections caused by fastidious organisms. Agar plates from these urine specimens without bacterial growth after 24 hours, but with leukocyturia and clinical signs indicating a UTI, might benefit from longer incubations. An additional 24 hours may confirm either sterility, or find out a possible fastidious uropathogen [15, 41, 42]. An increase of 8-10% in the frequency of isolations has been documented in 2-day cultures as compared to one-day culture [86, 87]. Local practice for special cultures shall be decided together with clinical customers based on specific cases and specimens arriving in the laboratory.

To improve growth of fastidious organisms, e.g., some Gram-positive species, blood agar media need to be incubated under a 5% CO₂ atmosphere for 48 hours, in addition to aerobic conditions.

The uncertainties of the routine process should be controlled by the advanced comparison method (see **Chapter 7.4.4**) when the procedure is established, as well as with regular internal comparison focusing on critical steps as needed (**Chapter 7.8.1**). Depending on the success of these adjustments, the routine process is considered to represent a quantitative procedure (*Level 2*) or a ordinal scale procedure only (*Level 1*).

RECOMMENDATION: Reproducible detection of low colony counts at 10³ CFU/mL (10⁶ CFB/L) requires an inoculum of at least 10 µL, adopting one of the recommended methods of inoculation. (1, A)

RECOMMENDATION: Aerobic incubation at 35 ± 2 °C for 16-24 hours is sufficient for primary uropathogens. For special urine specimens, blood agar plates are recommended to be incubated under 5% CO₂ atmosphere for 48 hours in addition to aerobic conditions, to detect possible fastidious organisms. (1, A)

7.4.3. Automated urine cultures

7.4.3.1. Total laboratory automation in bacteriology

Automated systems have been introduced for culturing clinical blood specimens, identification of pathogens and antimicrobial susceptibility testing around 30 years ago, but total laboratory automation (TLA) appeared only recently in microbiology laboratories. For large microbiology laboratories, automated urine culture is now an available option.

TLA in clinical microbiology laboratories is defined as instrumentation that mechanises the steps from specimen processing to discarding plates when results are final, and delivery of plates to workbenches [88, 89]. Two automation systems are currently available: BD Kiestra Work Cell Automation (WCA) or TLA (Becton Dickinson, B. V., Drachten, The Netherlands), and Copan WASPLab (Copan Diagnostics Inc., Italy). The systems are modular and customizable to the space and needs of a diagnosis laboratory, e.g., according to the specimen types and their numbers.

Besides the preanalytical steps (opening of specimen containers, sample preparation, and microbial streaking), the system may include automated aerobic and CO₂ incubators with plate readers, as well as conveyors for transferring plates between these instruments. Furthermore, automated colony pickers coupled to automated antimicrobial susceptibility testing (AST) and preparation for MALDI-TOF MS-based identification (ID) system are available. According to the number of samples received in a given laboratory, partial or full configurations are offered. The advantages of automation and the impact on the laboratory workflow vary according to the level of automation. In all cases, automation helps in elimination of repetitive manual tasks, reduces patient identification errors, and improves standardisation and reproducibility of culture [90, 91].

7.4.3.2. Improving urine bacterial culturing with automated processes

Automation of urine cultures has several technical benefits as compared to manual culturing [89]. Automated instruments improve isolation of colonies, even from mixed growth, reducing the need of subcultures [92, 93]. The culture plates are reviewed at regular intervals on high-resolution monitors with different illumination technologies and multiple angles, to allow earlier detection, identification, and thus earlier reporting of growth [90, 94, 95]. Additionally, incubation in a standardised temperature and atmosphere of the automated incubators increases sensitivity to detect bacterial growth already after 18 hours [59, 60].

Several studies have described a significant reduction of the turn-around time (TAT) down to 5 hours from arrival of urine specimens to the final report of negative urine cultures [90, 94, 96]. However, since a shorter incubation time reduces the recovery of slow growing-species, it is necessary to find a trade-off between TAT reduction and sensitivity of early readings [96, 97].

The TAT reduction is dependent on organisation of preanalytical, analytical (screening and confirmatory tests) and postanalytical phases. Shortening of TAT is not as significant with positive urine specimens as in negative specimens in the automated culturing process. The outcome depends more on the level of automation and organisation of the workflow including post-analytical steps, and finally adaptation of working shifts of the personnel in the laboratory to support a 24/7 service, or service at least in two shifts [89, 98, 99]. Digital imaging with quantitative algorithms allows both quantitative detection of growth and identification of bacterial species grown on chromogenic agar plates [100, 101, 102]. A decreased TAT of 14 hours from sample arrival to reporting was achieved by using tailored rules in detecting growth of *E. coli* [95, 97]. Identification and classification of other micro-organisms still needs to be improved [100]. Software algorithms may help in distinguishing negative from non-negative urine specimens [95]. The performance of these algorithms depends on microbial load, type of species, image contrast of the colonies and related technical factors, and interpretation criteria of primary cultures [100]. The future expectation of automated systems is an autorelease of negative routine and chromogenic culture results with a fully automated urine workflow between all instruments connected, challenging organisation for additional cultures to slowly growing species.

In implementing bacteriology automation, several factors need to be assessed in addition to expected improvement of efficiency, accuracy, and reduced TAT [89, 98, 99]. Some of these are summarised in the **Table 7-4**. Shortening the TAT can positively improve patient management and outcome. However, this needs to be further evaluated as this benefit is strongly linked to the local antibiotic stewardship programme.

Table 7-4. Important factors in adapting urine bacteriology automation

Number of tests (annually and daily)
Prevalence of positive urine specimens
Expected sample throughput and turn-around times
Workflows and staff working hours

7.4.4. Advanced reference procedure for bacterial culture (*Level 3*)

7.4.4.1. Purpose and scope of a reference measurement procedure (advanced comparison method) for bacterial cultures

Levels of accuracy of the measurement procedures used in this guideline is described in **Chapter 4**. An advanced comparison method (*Level 3*), called officially a reference measurement procedure, is *a well characterised procedure with a small measurement uncertainty to provide measurement results fit for their intended use* [103]. This guideline uses the term “measurement” occasionally also for qualitative (nominal scale) examinations, such as identification of bacterial species, together with the compared measurements in Chemistry or Particle counting.

The VIM term 2.26 Measurement uncertainty is a key concept of quantitative measurements, expressed as a quantitative result = a measurement quantity value with its uncertainty ($x + u$). It is applicable both for actual quantitative values (ratio scale) and for ordinal scale quantities. It is explicitly said that it is not applicable to qualitative (nominal scale) examinations, such as nomination of grown species that encompasses qualitative variability, i.e., uncertainty of nomination (= classification) without a quantity. In bacterial cultures, quantitation of colony counts represents an official “measurement”.

An advanced reference procedure (*Level 3*) is principally required for bacterial culture

1. To verify initial performance of routine quantitative bacterial culture (at *Level 2*) in local clinical epidemiology (in the existing facility and variety of specimens), or to confirm the acceptable performance after essential changes in the modified procedure, e.g., by new reagents, materials, or equipment.
2. To assess any (automated) instruments in bacteriology intended to detect, quantify, or identify bacterial species for clinical diagnostics, when verifying the device(s) according to manufacturer’s instructions. Comparison is preferably carried out three-way, by comparing results from automated instruments to the current culture procedure (at *Level 2*). See **Chapter 7.8.1** for detailed perspectives.

In both cases, the verification by the end-user laboratory needs a focused plan for analytical verification, including specimens, procedures, materials and equipment, personnel and assessment of results that provide evidence needed to substantiate reliability of the laboratory's routine cultures.

Rapid examinations (*Level 1 or Level 2*) used to screen for the presence of clinically significant bacteriuria need to be compared against routine bacterial cultures at *Level 2*. A *Level 3* procedure may occasionally be chosen for studying specimens of individual patients or patient groups based on specific clinical needs, e.g, to confirm detection or identification of fastidious species.

7.4.4.2. Normative references

BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML. International Vocabulary of Metrology – Basic and general concepts and associated terms (VIM 3rd ed, 2012) [103].

International Organization for Standardization (ISO). ISO standard 15189:2022 [104].

European Union. Regulation (EU) 2017/746 on in vitro diagnostic medical devices [105].

7.4.4.3. Principles of a reference examination procedure in urine bacterial culture

The contents of *Chapter 7.3 Examination processes* in the ISO15189:2022 standard were used to create a structure to this proposed reference procedure for urine bacterial culture. At least the following features are important for a bacteriology reference procedure:

a) Principles of the procedure

The principle of an advanced procedure for urine bacterial culture is a culture-based procedure with a maximum sensitivity to detect clinically significant uropathogenic species at sufficient reproducibility even at low colony counts, and with a maximum specificity to isolate and identify them correctly. MALDI-TOF MS is a tool for species identification of cultured colonies.

b) Specimens

(i) Standard American Type Culture Collection (ATCC) or equivalent other control strains of representative uropathogens are needed to confirm commutability of the obtained results between laboratories, and to verify theoretical performance of the procedure.

(ii) Clinical urine specimens obtained from routine diagnostics from mixed patient populations shall be tested, considering needs of the served clinical units, and different ways of urine collection and preservation. The total number of clinical specimens sufficient for validation of the reference procedure itself is dependent on the number of used ordinal scale quantities (categories) of growth, and coverage of sufficient variety of bacterial species.

c) Preanalytics

Specimen collection and preservation shall be adapted from local practice, considering requirements in **Chapter 3** of this guideline. Both non-preserved and preservative containers should be investigated, as used in local practice, and confirmed to comply with the European Union IVDR regulation 2017/746 (specimen receptacles and containers) and the EU MDR regulation 2017/745 (devices for invasive urine collection). A verification of preanalytical procedures or devices is usually separate from the verification of an analytical examination procedure, but the analytical verification needs to collect a representative sample of clinically relevant specimens from its customer units (see the ISO 15189:2022, chapter 7.3.2).

d) Required equipment and reagents

Calibrated pipettes (10 µL and primarily 100 µL volume) minimise inaccuracy related to inoculation. Inoculation with a loop does not provide precise volumes.

Culture media and used equipment shall comply with the EU IVDR regulation 2017/746. High-quality culture media and their storage method shall be verified to guarantee sterility before use, recovery, detection, and isolation of uropathogenic bacteria.

e) *Process of inoculation, incubation and reading of cultures*

In addition to accurate inoculation, streaking practice shall be standardised in a laboratory. Stability of temperature and designed atmosphere of incubators should be measured and followed. An additional 24-hour incubation time is required after the routine incubation for 18-24 hours in aerobic atmosphere, and an incubation in 5% CO₂ atmosphere for 48 hours for detection of all clinically relevant organisms. Standardised reading of quantities is recommended for reproducibility of categories. Both quality and quantity of isolated colonies shall be considered.

f) *Performance specifications and analysis of errors*

The summary of performance and error analysis should provide an estimate on uncertainty of the derived reference procedure. See the description of the details below.

7.4.4.4. Detailed characteristics of a reference procedure

The features of the procedure were developed from [91, 93, 106], by using expert consensus.

Specimens

(1) At least four standard ATCC or equivalent control strains of uropathogens from other sources are needed to verify quantitation of colony counts (**Table 7-5**). These may include, e.g., *E. coli*, *E. faecalis* and *P. aeruginosa* that represent aerobic growth, while *S. pneumoniae* and *E. faecalis* grow in 5% CO₂ atmosphere. *S. pneumoniae* also represents fastidious species despite not being a uropathogen. Moreover, *E. coli* and *E. faecalis* also serve assessment of colour of colonies on chromogenic agar.

Prepare 0.5 McFarland suspensions of the strains and dilute into 10⁶ CFU/mL (10⁹ CFB/L) with physiological 0.9 % NaCl (saline). Finally, monomicrobial 1:10 dilution series at 10² – 10⁵ CFU/mL (corresponding to 10⁵ – 10⁸ CFB/L in SI units) shall be prepared, as well as at least 4 representative polymicrobial combinations. The reference procedure should be validated using containers both without and with preservatives if fastidious organisms need to be tested.

(2) 50-100 clinical urine specimens from mixed patient populations should be selected after routine diagnostics, reflecting the variety of specimens received from the clinical customers of the laboratory. These should include different ways of urine collection, representative variety of isolated groups of species and polymicrobial specimens, and locally used preservatives, the extent depending on the type of the actual verification in question.

Table 7-5. Control strains for urine bacterial culture

Agar medium	Species	ATCC nr	Incubation atmosphere	Expected reaction ^a
Chromogenic	<i>E. coli</i>	25922	35°C ± 2°C, aerobic	Growth and colour
Chromogenic	<i>E. faecalis</i>	29219	35°C ± 2°C, aerobic, and 5% CO ₂ -enriched	Growth and colour

Chromogenic	<i>P. aeruginosa</i>	27853	35°C ± 2°C, aerobic	Growth
Blood agar	<i>S. pneumoniae</i>	6305	35°C ± 2°C, aerobic, and 5% CO ₂ -enriched	Growth, α-haemolysis

^a Growth is assessed after incubation for 18-24 hours. For fastidious organisms, assess additionally after a 48-hour incubation.

Inoculum procedure

For the reference procedure, 100 µL pipetted volume is used to detect 10 – 1000 bacterial colonies/plate at 10² – 10⁴ CFU/mL (corresponding to a range of 10⁵ – 10⁷ CFB/L), respectively, with a highest precision of visual counting of 100 colonies/plate at 10³ CFU/mL (10⁶ CFB/L). A 10 µL inoculation provides detection of growth up to 10⁵ CFU/mL (10⁸ CFB/L), with a highest precision at 10⁴ CFU/mL if needed. After inoculation with a calibrated pipette, streaking pattern (a) or (b) must be chosen and standardised, as shown in **Chapter 7.4.2**.

Repeatability of colony counts is measured by duplicate inoculations from serial 1:10 dilutions of ATCC or equivalent strains, or duplicate inoculations of clinical urine specimens without dilutions. Mix the specimens upside down at least 10 times to create an even suspension before inoculation.

Culture media

Primary agar of the reference procedure is recommended to be a chromogenic agar. The French Society of Microbiology and the ESCMID recommend blood agar as a secondary medium [107, 108].

Defective culture media may lead to false results. At least the following defects should be excluded:

- (i) Deterioration of the chromogenic compound due to inferior storage conditions before use, or inside the automated incubator. This possibility is detected by comparing the colours of colonies of standard ATCC or equivalent strains grown on chromogenic agar with the expected colours of those colonies.
- (ii) Loss of growth-promoting capacity: The capacity of the used agar to promote growth of all major uropathogenic species is confirmed from frequencies of isolated species over time, to ensure detection of full epidemiology of uropathogens requested from the laboratory.
- (iii) Contamination: A random contamination of a given batch is rare but possible. Examine each media batch visually upon receipt and before use.

Culture conditions

Laboratory grade incubators should be maintained both in aerobic atmosphere, and in 5% CO₂ atmosphere for fastidious species growing on blood agar.

Specimens on primary chromogenic agar are to be cultivated in aerobic conditions. Specimens on secondary agar should be cultivated both in aerobic conditions and in 5% CO₂ atmosphere. Detection of growth is carried out after incubation at 35 ± 2 °C for 18-24 hours. In addition, fastidious organisms shall be detected after a 48-hour incubation.

Reading of growth

The bacterial growth is classified into negative and 3 or 4 positive ordinal scale quantities (ranks) with approximate colony counts according to **Table 7-6**. Since enumeration of dense colonies is inaccurate due to their merging, locally designed enumeration grids are recommended for reproducibility [106]. Both polymicrobial and monomicrobial growth shall be reported as colony counts in the reference procedure.

Table 7-6. Quantitative interpretation of growth

Volume of inoculum	Number of colonies on plate	Colony count (CFU/mL)	Colony count (CFB/L)
10 µL	10	10 ³	10 ⁶
	100	10 ⁴	10 ⁷
	1000	10 ⁵	10 ⁸
100 µL	10	10 ²	10 ⁵
	100	10 ³	10 ⁶
	1000	10 ⁴	10 ⁷
	10000	10 ⁵	10 ⁸

At least 3 similar discrete colonies (to avoid exceptional variants) need to be present for additional working steps, as a definition of successful isolation of species [109].

Identification of species

Some species, such as *E. coli* may be identified directly on chromogenic agar with a rapid test. MALDI-TOF MS analysis is required for a reference identification of species.

Operator training

Laboratory professionals performing the reference procedure in practice shall be familiarised and trained in advance to learn the key principles of a reliable and standardised operation procedure. Inter-observer variability shall be compared in advance to assess the levels of human uncertainty in manual work and interpretation.

7.4.4.5. Performance specifications for a reference bacterial culture

Trueness of identification (nomination): The reference procedure is recommended to identify desirably all inoculated colonies in the mixed ATCC or equivalent reference strain suspensions. It should also identify at least 95% of the uropathogenic species from clinical specimens at 10^3 CFU/mL (10^6 CFB/L), and at least 90% of them at 10^2 CFU/mL (10^5 CFB/L).

Quality of isolation: At least 3 similar discrete colonies shall be grown on plates to allow additional tests, such as MALDI-TOF or AST.

Trueness of quantitation (counting): Agreement of quantitative colony counts between two parallel inoculations from 100 μ L (and 10 μ L in addition) clinical specimens using the reference procedure should be compared in a cross-table, using negative and usually three or four positive ordinal scale quantities, i.e., 10^2 to 10^5 CFU/mL (corresponding to 10^5 to 10^8 CFB/L, respectively). Less than 30 % of specimens should yield a negative result in culture. Relevant statistics shall be applied in addition to clinical judgment of results. A possibility of Cohen's *kappa* statistics is described in **Chapter 5.2.3.3** for ordinal scale results with urine test strips [110, 111].

Precision of quantitation: The repeatability coefficient of variation (CV) of colony counts is obtained from 10 replicate cultures from standard ATCC or equivalent reference strains, using mean and standard deviation of results. Note that colony counts have inherent variability usually modelled by Poisson distribution (**Chapter 7.4.2**). An example for interpretation is as follows:

The repeatability CV should approach theoretical imprecision derived from Poisson distribution, $CV_{theoretical}$. Since the standard deviation of Poisson distribution $1s = \sqrt{n}$, where n = number of colonies on a plate, the 95% confidence interval may be approximated with $\pm 2s$ limits. For a colony count of 10/plate, $1s = \sqrt{10} = 3.1$. Then, a 95% confidence interval is ± 6 colonies/plate (4-16 colonies/plate). The observed CV with patient specimens is desirably $< 2 \times$ Poisson $CV_{theoretical}$.

Analysis of sources of variation

Colony counts in culture follow imprecision of Poisson statistics. Both systematic errors (bias) and extra random variation (increased imprecision) increase the variability of obtained colony counts in culture, thus increasing *measurement uncertainty (MU)*.

The observed uncertainty should be reviewed in the summary of the validation of the derived reference procedure for bacterial culture. The VIM term 2.33 Uncertainty budget is “a statement of a measurement uncertainty” applicable to quantities only. For nominal examinations, such as identification of species in bacterial culture, an estimate of combined misclassification rate of grown species should be attempted from clinical specimens, including at least the following possible sources of uncertainty:

- specimens (such as types of reference strains, or bacterial species in clinical specimens, way of collection with polymicrobial background, and storage conditions),
- tools and methods of inoculation,
- properties and preservation of used culture media,
- instruments and conditions used in incubation of plates,
- ways of reading, isolating colonies and identifying the grown species, and
- human operator-related differences (shown with inter-observer comparisons after training).

RECOMMENDATION: A qualified reference examination (Level 3 procedure) is recommended to be used for bacterial cultures

(1) to verify a required performance of routine bacterial culture (at Level 2), or

(2) to assess any instruments in bacteriology intended to detect, quantify, or identify bacterial species for clinical diagnostics against the suggested performance specifications as needed. (1, A)

7.5. Bacteriuria quantitation

7.5.1. Background for limits of clinically significant bacteriuria

7.5.1.1. Unit recommended for expressing bacterial concentrations

Automated counting of different particles in urine specimens [112, 113] recalls the international standardisation of quantities and units [114]. The SI unit for volume in particle concentrations is particles/litre (L), e.g., a leukocyte count in urine and other body fluids is expressed as WBC $80 \times 10^6/L$ [115, 116] (see **Chapter 6.2.2**).

Bacteria concentrations counted directly in body fluids differ from those obtained as colonies after bacterial culture. The traditional unit of reporting bacterial concentrations after culture has been colony-forming unit/millilitre (CFU/mL). The SI unit with a litre volume is CFU/L (colony-forming unit/litre), as adopted by the UK Standard for Microbiology Investigations [42]. The primary ECLM European Urinalysis Guidelines suggested to replace U with B, proposing a litre-based unit, CFB/L (colony forming bacteria/L), to avoid confusion between exponentials if only mL volumes were changed to L volumes [39]. The term “bacteria” also refers to visible discrete objects. The adoption of the SI units has remained optional for urine, mostly in scientific writing on particle concentrations.

Clinical microbiology laboratories mostly express culture results in CFU/mL units similar to other body fluid specimens, e.g., sputum, broncho-alveolar lavage, or catheter specimens with quantitative Brun-Buisson technique. Changing the unit for different cultures to SI units (per litre) must be decided and standardised at national level of healthcare, to avoid confusions for clinicians, and risks to patient safety. In this guideline, conventional units (CFU/mL) and previously adopted SI units (CFB/L) appear in parallel for clarity.

NO RECOMMENDATION is given to the unit for reporting urine bacterial cultures. A national harmonisation is recommended to avoid confusion among professionals and patient risks.

7.5.1.2. Significance of low bacterial concentrations and leukocyturia

The classical concept of significant bacteriuria is based on the finding that uropathogen counts of $\geq 10^5$ CFU/mL ($\geq 10^8$ CFB/L) are associated with the presence of a urinary tract infection (UTI) at a significantly higher probability than lower bacteria counts [117, 118]. However, this “Kass number” was based on studies on exclusively premenopausal women with pyelonephritis. Despite this fact, the “Kass number” has been used widely even for diagnosing symptomatic lower urinary tract infections. However, increasing clinical evidence has shown that colony counts far below the limit of 10^5 CFU/mL (10^8 CFB/L), down to 10^2 CFU/mL (10^5 CFB/L) are associated with urinary tract infections, too. This is particularly true for premenopausal women with symptomatic lower urinary tract infections [119, 120, 121].

Low thresholds for significant bacteriuria reduce the specificity of UTI diagnostics if the low bacteria numbers are considered to indicate UTI independently of urinary tract symptoms. In fact, the presence of bacteria in low count often represents contamination or colonisation. Thus, a non-

selective additional processing of all urine samples with low bacterial concentrations creates unjustified workload for the microbiology laboratory, followed by a large number of unnecessary antimicrobial therapies. A practical solution to the workload is explained in **Chapter 7.5.2**, discussing laboratory-related decision limits for significant bacteriuria (**Fig. 7-2**).

Lower bacterial counts may be significant in paediatric UTI as well [17, 122, 123, 124]. However, bacterial contamination with the faecal microbiota occurs in infants frequently. Therefore, other laboratory findings, such as leukocyturia, and clinical picture need to be considered, to minimize false-positive, solely culture-based diagnoses of urinary tract infections in childhood.

Detection of **leukocyturia** in obtained urine specimens guides evaluation and further diagnostic procedures in the laboratory in general, as an increased concentration of WBC indicates an active inflammatory response. Thus, concentrations of 10^3 to 10^4 CFU/mL (10^6 to 10^7 CFB/L) of uropathogens tend to be clinically relevant when associated with corresponding clinical symptoms OR leukocyturia [125, 126, 127, 128].

7.5.1.3. Level of significant bacteriuria depending on way of collection

The threshold for significant bacteriuria depends on the way of urine collection and on the detailed procedure of collection (see **Chapter 3.2**).

For urine cultures from **suprapubic aspiration (SPA)** specimens, any number of uropathogens is considered clinically significant. Therefore, urine cultures from SPA should be prepared in such a way that even very low pathogen concentrations, e.g. **10^2 CFU/mL** (10^5 CFB/L) can be detected with certainty. The same applies to urine specimens obtained from punctures of kidney pelvis or pyelostoma openings, or those for the diagnosis of chronic bacterial prostatitis obtained after a prostate massage (Meares & Stamey procedure, see **Chapter 3.2.9**).

In the case of **single in-and-out catheterisation**, bacterial counts from 10^3 CFU/mL (10^6 CFB/L) are considered to be infectious when symptoms indicate a urinary tract infection [14].

Specimens for urine bacterial culture are NOT recommended to be taken from **indwelling catheters** due to rapid development of bacterial biofilm in urine catheters, creating difficulty in assessing significance of observed species (**Chapter 3.2.4**). Instead, a specimen is to be collected after removing the old catheter and taking the sample through the new one.

Regarding **mid-stream urine (MSU)**, the threshold is defined according to uropathogenic group, monomicrobial or polymicrobial growth in culture, and clinical presentation. Clinical data are often difficult to obtain accurately for microbiological laboratories. A threshold of 10^3 CFU/mL (10^6 CFB/L) from MSU collection is suggested to be significant in women presenting corresponding symptoms related to UTI and low-count bacteriuria with a class I uropathogen, *E. coli* [121] or *S. saprophyticus*. The same applies to patients with severe renal insufficiency or dialysis treatment, as well as many urological patients [9].

In addition to clinical diagnosis, several other reasons may result in low bacterial counts in urine culture (**Table 7-7**).

Table 7-7. Causes of low bacterial concentrations in mid-stream urine

Early stage of infection
High volume rate (diuresis)
Urgency symptoms (short bladder incubation time)
Presence of antibiotics in urine
Low pH in urine
Contaminated specimen
Presence of resident bacteria in the bladder (urobiome)

7.5.1.4. Polymicrobial growth

Composition of detected bacteria is informative for evaluating significance of the quantitative culture result. Pure culture of a single typical uropathogen indicates a causative role. The detection of more than one organism from a urine specimen needs to be interpreted in the light of

- presence of one dominant organism,
- way of collection, and success in collecting the specimen,
- presence of features indicating a true infection (presence of WBC), and
- clinical signs, symptoms, and patient’s clinical history.

True infections with two species may occur. When two uropathogens are identified, the colony count must be reported for each species. In most cases, the presence of more than two species in a urine sample is interpreted as contamination with no diagnostic value. The patient should provide a new specimen after detailed advice how to minimise the risk of contamination. Colonisation of the urinary tract is also frequently found. It may result in mixed growth even after successful collection.

If a polymicrobial culture is dominated by one pathogen (i.e., with colony counts at least two exponentials higher than those of the other species), this pathogen is considered as the infectious agent more likely than the other species. In polymicrobial cultures with smaller differences in counts of grown species, none of the detected species is likely a causative agent. This "leading pathogen concept" has been substantiated only by a few molecular biological studies [129]. However, it has pragmatically become commonplace in many laboratories. A prerequisite for the leading pathogen concept is a strict compliance with the given pre-analytical procedures: inadequate conditions may lead to secondary growth of different bacteria in the specimen subverting detection of a leading pathogen. See **Chapter 3.2** and **Annex I.1** for collection details of single-voided urine specimens.

7.5.2. Laboratory workflow-related decision limits for significant bacteriuria

The bacteriological diagnostics of urine specimens from patients with suspected UTI include detection of uropathogens, determination of their quantity, and performance of additional tests needed for exact identification of the pathogen (usually at species level), and evaluation of its antimicrobial susceptibility, based on locally agreed criteria.

Laboratory assessment of significance of bacteriuria includes the following factors:

- (i) Species: Uropathogens (typical and potential), with colony counts starting from 10^2 - 10^3 CFU/mL (10^5 to 10^6 CFB/L), see **Table 7-3 (Chapter 7.2.2)**.
- (ii) Leukocyturia
- (iii) Way of specimen collection
- (iv) Symptoms or signs of localised or general infection, or medical history in specific cases, as transferred with (electronic) requests
- (v) Asymptomatic bacteriuria (**Chapter 1.2.2**)

Based on the listed factors, a general flowchart is suggested for bacteriology process of routine urine specimens (**Figure 7-2**) [7, 15, 41]. The main purpose of this chart is to advise planning of routine workload, and to allow reducing turn-around times with mostly mid-stream specimens, allowing then extra time for specific specimens and those from high-risk patients (see **Chapter 1.2**).

Purposely, details concerning intermediate categories, such as specimens from single in-and-out catheters (**Chapter 3.2.3**) or those from indwelling catheters (**Chapter 3.2.4**) are not included in the **Figure 7-2** to keep clarity. An adaptation to local patient populations, all ways of collection, and preanalytical and analytical processes is recommended, leading into more detailed operating procedures. For specific specimens, such as that obtained by punctures of urinary bladder (suprapubic aspiration), kidney pelvis, or urine voided after prostatic massage (Meares and Stamey procedure, **Chapter 3.2.9**), a full assessment with ID and AST is advisable, starting from colony counts $\geq 10^2$ CFU/ml ($\geq 10^5$ CFB/L) (see also **Chapter 7.4.1.2**). Identification (ID) of bacterial species has to be performed in all cases, except when 3 or more species are seen in culture (contamination). The suggestion also includes criteria for antimicrobial susceptibility testing (AST) of the isolated species.

Presence of leukocyturia should be assessed at a cut-off of about $30 \text{ WBC} \times 10^6/\text{L}$, with a grey zone $10\text{-}30 \text{ WBC} \times 10^6/\text{L}$ [130], keeping in mind that leukocyturia can be absent in patients with neutropenia (See **Chapter 6.5.1** for analytical variation of leukocyte counts). A test strip measurement (using esterase and nitrite test, see **Chapter 5.2.2**) is also possible, depending on the verified sensitivity and specificity among the served patients in local practice. The laboratory flowchart shown in **Figure 7-2** supports interpretation of results from primary urine cultures and guides further examination procedures.

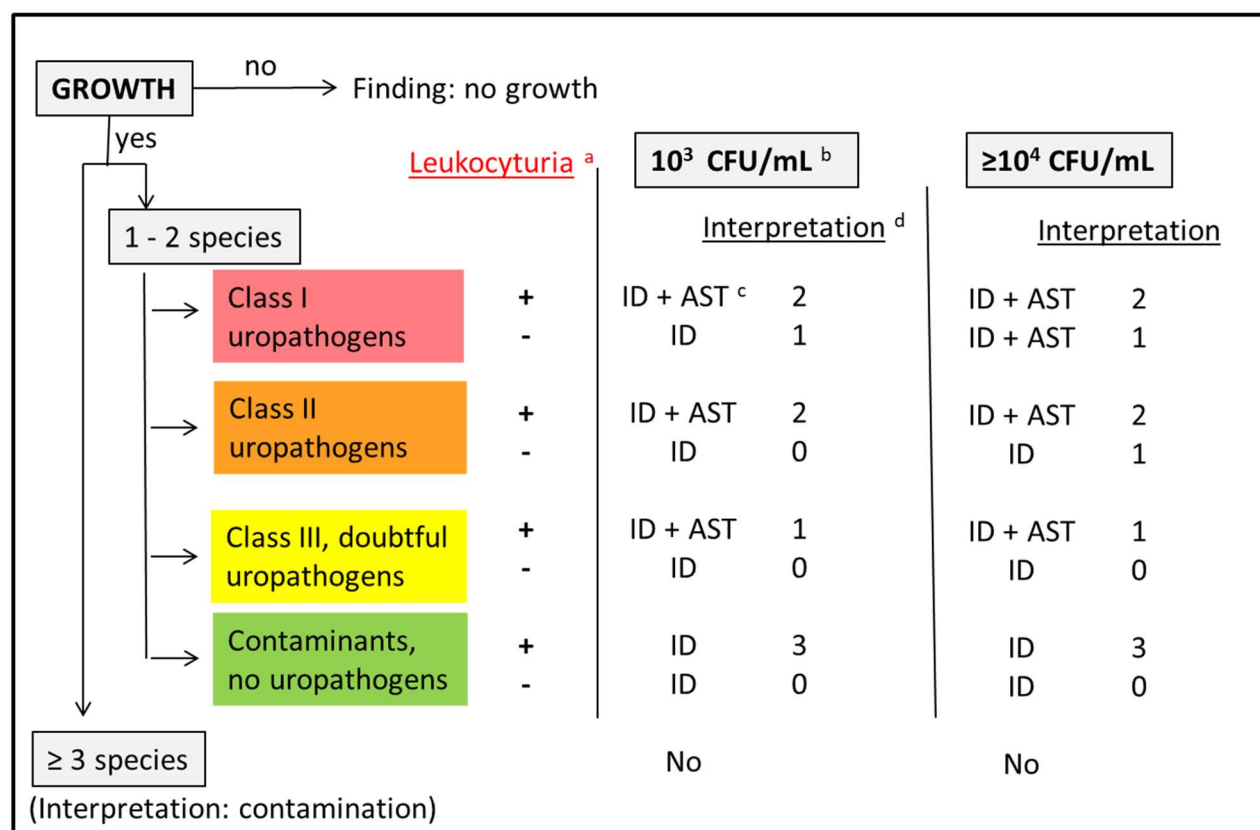


Figure 7-2. General workflow of primary bacterial cultures from routine urine specimens

The figure provides an outlook how to organise routine workflow of most urine cultures. Local adaptations or additional details may be considered as needed.

Explanations to the footnotes:

^a Presence of leukocyturia at WBC > 30 x 10⁶/L, with a grey zone at 10-30 WBC x10⁶/L (measured with particle counting or test strip, depending on health care setting).

^b Limiting colony counts divide the process, expressed using the conventional colony-forming unit CFU/mL. Alternative SI units are colony-forming unit/L (UK recommendation), or colony-forming bacteria, CFB/L may be adopted after national decisions. 10³ CFU/mL (equal to 10⁶ CFB/L) represents a borderline quantity of significant growth in routine cultures.

^c ID = identification to species level; AST, antimicrobial susceptibility test.

^d Clinical interpretation codes 0-3 are explained further in the text below.

Step 1: Contamination

After an incubation for 16-24 hours, the workflow is divided into cases with no growth and those with growth on agar plates. In the latter, it is further differentiated into two possibilities:

- Growth of three or more distinguishable bacterial isolates. In practice, this situation usually suggests contamination during the collection process with hand skin microbiota, periurethral or genital tract, and sometimes gut microbiota. To distinguish between a contamination of the specimen by the periurethral or genital microbiota and a genuine bladder urobiome is a not easy for the microbiologist since some species are shared by both communities. Commonly, a recollection is required. Reporting “a polymicrobial culture potentially corresponding to a contamination” is intended to raise awareness, and empower patients

and the clinical team to improve preanalytical step. An example report is: “More than 2 detected species suggesting contamination of specimen. If a UTI is suspected, a careful collection of a new specimen is recommended.” The leading pathogen concept is discussed in **Chapter 7.5.1.4**.

- The presence of 1 or 2 distinguishable isolates. Go to step 2.

Step 2: Uropathogenic group

Identify the bacterial isolate(s) to species level. Assign the identified species to one of the four groups of **Table 7-3**. Process workflow within Class III is recommended, except for yeast.

Step 3: Leukocyturia

After assignment to a uropathogenic group, the presence of leukocyturia (≥ 30 WBC $\times 10^6/L$) is considered, because it increases the probability of a UTI. At the borderline WBC $10\text{--}30 \times 10^6/L$, a statement “consider density of urine and clinical picture related to dysuria” is possible.

If information on leukocyturia cannot be arranged in the laboratory, it is necessary to decide locally, whether to assume presence of leukocyturia and to carry out further investigations (AST) accordingly. The report should then be supplemented with a statement, e.g.: “Consider clinical picture and leukocyturia in the interpretation of the result of urine culture”.

Step 4: Colony count

The subsequent workflow is divided in the samples with a borderline colony count of 10^3 CFU/mL (10^6 CFB/L), and those with higher colony counts $\geq 10^4$ CFU/mL (10^7 CFB/L).

Step 5: Antimicrobial susceptibility test

Examinations may include continuation to an AST, as suggested in the **Figure 7-2**. Interpretative texts of examinations are recommended to be harmonised in clinical reports by using coded statements. An example list of statements is given below:

0 = Detected microorganisms **probably do not cause** a UTI (even with corresponding symptoms).

1 = Detected microorganisms **possibly** cause UTI in selected clinical presentations (immunocompromised patients, early infection...) with appropriate clinical picture.

2 = Detected microorganisms with significant colony counts. UTI is **probable** with appropriate clinical picture.

3 = No microorganisms detected with the used culture procedure. Antibiotic treatment? In presence of appropriate clinical picture, consider tests specific for **other microbes**, e.g., *Chlamydia*, *Mycoplasma*, *Ureaplasma*, *M. tuberculosis*, *N. gonorrhoeae*.

The value of routinely reporting of statement 3 in case of no growth needs to be considered locally.

RECOMMENDATION: A flowchart for routine urine specimens is recommended as a practical advice to bacteriology laboratories to organise their workflows, starting from mid-stream urine specimens. It is open for modifications based on specific specimens or patient populations, as well as local epidemiology of uropathogenic species in the laboratory. (1, B)

7.6. Identification of bacterial species

Bacteria and yeast from urine specimens of patients suffering UTI need to be identified to the species level. The exact species identification is important to affiliate the isolated bacteria to one of the different groups of uropathogenicity, and need to be included in the released microbiology report (see **Chapter 7.5.2**). In addition, this is particularly necessary in order to perform correct AST standardised by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST provides breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing, and functions as the breakpoint committee of the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC). EUCAST also publishes widely used microbiology standards, such as the annually updated clinical breakpoints for AST [131].

The EUCAST-conformed AST of different antibiotics for UTI requires identification of the uropathogen to the species level, e.g., within the order *Enterobacterales*. For instance, some suggested AST breakpoints are only applicable to certain species within the *Enterobacterales*, e.g., *E. coli*, *Klebsiella* spp. (except *Klebsiella aerogenes*), *Raoultella* spp., *Citrobacter* spp., *Enterobacter* spp. or *P. mirabilis*. This regards for instance commonly used antibiotics such as the orally applied cefuroxim, mecillinam and temocillin as well as orally applied fosfomycin, nitrofurantoin and nitroxolin.

7.6.1. Biochemical identification of cultured bacteria and yeast

The traditional way to identify bacteria and yeast cultured from urine samples is by means of biochemical tests that rely on the ability or failure of the isolated species to metabolise each single substrate tested. These metabolic reactions take place in individual reaction chambers of a diagnostic device. Colour indicators enable detection of the outcome of each test. The series of yes-no results provides a biochemical code that identifies each species. Clinical laboratories typically use semi-automated or fully automated instruments to identify bacteria and yeast from urine specimens, comparing obtained results to the database of biochemical codes provided by the instrument.

If laboratories decide to use manual biochemical tests, commercial kits are available. In addition, single biochemical tests, e.g., in connection with chromogenic agar, may enable species identification of some uropathogenic bacteria on plate. An international manual is recommended to confirm current practice, e.g., an update of the Clinical Microbiology Procedures Handbook of the American Society for Microbiology, in addition to possible national guidelines. The laboratories that solely carry out biochemical tests, need to ensure that they are able to identify not only common uropathogens to the bacteria species level, but also to identify novel uropathogens, such as *Aerococcus urinae*, *Actinotignum schaalii* and *Corynebacterium urealyticum*, or to arrange identification of novel uropathogens in another laboratory (see **Chapter 7.2.3**).

7.6.2. MALDI-TOF MS for identification of cultured bacteria and yeast

MALDI-TOF MS-based systems have replaced traditional biochemical methods for identifying microorganisms in many microbiology laboratories. They have several advantages over traditional biochemical identification: ease of use, reliability, accuracy, low unit cost, and - above all - the speed, which all together can help to improve patient prognosis. The current MALDI-TOF MS has the capability to identify more than 2000 species, including clinically significant uropathogens within minutes [75, 132]. In practical work, the laboratories need to keep their MS libraries updated to be able to identify the established uropathogens.

Identification of bacteria by MALDI-TOF MS requires biomass that is less than a single bacteria colony on an agar plate. Therefore, the incubation time on agar plates can be reduced to a few hours, still retaining reliable identification [133]. The identification of MALDI-TOF MS is limited only with availability of suitable reference spectra. The current databases of the commercial MALDI-TOF MS systems allow identification of almost all uropathogenic bacteria at the species level [132]. Even the earlier incomplete databases of the MALDI-TOF MS correctly identified 93% and 82% of Gram-negative bacilli to the genus and species levels, whereas the biochemical system correctly identified 83% and 75%, respectively [134]. Currently, only MALDI-TOF MS is able to identify reliably emerging uropathogens such as *Aerococcus* spp., *Actinotignum schaalii* and *Corynebacterium urealyticum* in clinical laboratories with reasonable cost, since the available molecular methods are not suited for diagnostic laboratory with high throughput. To avoid misidentification between *E. coli* and *Shigella* spp. that may occur with MALDI-TOF MS, *E. coli* can easily be differentiated from *Shigella* using a biochemical test [132].

Several factors influence the quality of the spectra in the MALDI-TOF MS measurements. Poor spectra lead to insufficient identification of the bacteria in the specimen. Most important factors include improper protein extraction before MALDI-TOF MS analysis, and mixed microorganisms in the sample applied to the MALDI-TOF MS [135]. In case of uncertain identification on species level, e.g., low level of agreement with reference library, users should consider possible interfering factors and ensure proper protein extraction. For some closely related species of a certain genus, specific identification of the respective species may be difficult by MALDI-TOF. This holds also true for the identification by biochemical tests or by sequencing of ribosomal DNA. Thus, in those cases the reporting of a species group or species complex is suggested (e.g., *Enterobacter cloacae* complex).

RECOMMENDATION: Bacteria and yeast detected from urine specimens need to be identified to the species level to satisfy proper clinical diagnostics, and to be able to assess their antimicrobial susceptibility. Limitations of different identification methods are recommended to be considered to avoid deficient identifications or misclassifications. (1, A)

7.7. Antimicrobial susceptibility testing

The goal of AST is to allow the clinician to choose the correct antibiotic for individual urinary tract infections, and to help in investigating the reason for treatment failure. The antibiotic sensitivity of pathogenic organisms is important if they cause urinary tract infections with high probability. To reduce the workload in laboratories and to avoid unnecessary or harmful antimicrobial treatments, limited continuation with AST after specimen identification (ID) is recommended based on uropathogenic groups and colony counts as shown in **Chapter 7.5.2, Figure 7-2**. The antibiotic sensitivity patterns of individual species vary considerably according to geographic location, patient populations, and background antibiotic usage.

The laboratories should consult the annually revised and updated documents of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in selection of their practices [136]. National recommendations, including minimum selection of antibiotics based on EUCAST recommendations are encouraged to increase clinical effectiveness of AST and to direct clinicians to the use of inexpensive and effective antibiotics least likely to generate resistance in regional health care.

7.7.1 Procedures available

The antibiotic susceptibility may be assessed by using phenotypic or genotypic procedures. **Phenotypic methods**, e.g., disk-diffusion method, or broth dilution assays, provide a direct information on the susceptibility of a given microorganism to antimicrobial agents at defined concentrations. One of the limitations of phenotypic culture-based methods is the speed as they may require 48 hours to complete, depending on growth and resistance mechanisms of the bacteria tested. **Genotypic methods**, e.g., polymerase chain reaction (PCR)-based methods or genome sequencing, are sometimes used to detect known genomic markers that predict antimicrobial resistance.

With increasing bacterial antibiotic resistance, there is a need for reliable and timely AST reports. These reports should ideally be available to antimicrobial stewardship in less than 8 hours to optimize treatment reducing empirical antibiotic prescriptions. This will help to prevent the spread of resistant bacterial infections, which are associated with high morbidity, mortality and healthcare costs.

7.7.2 Choice of procedure

Automated or semi-automated broth dilution procedures are widely used in addition to standardised disk diffusion procedures [137]. Commercial broth dilution and diffusion methods have shown good correlation with broth microdilution methods used to define minimum inhibitory concentrations (MIC) [138, 139, 140, 141]. All AST results can be influenced by many factors such as pH, and variation in quality of disk and agar media [142, 143].

The advantages of the standardised disk diffusion method include the following [144]:

- it is cheap with no need for special equipment,

- it is suitable for a vast majority of bacterial species, even for slowly growing or fastidious ones,
- the panel of tested antibiotics can be easily adapted to epidemiological needs,
- the presence of polymicrobial cultures can be recognised, and
- in case of polymicrobial culture, individual pathogens can be identified and isolated more quickly than with automated methods.

The major disadvantages of the disk diffusion procedure are, however, that it lasts for 16-20 h, and it does not provide minimum inhibitory concentration (MIC) values [145]. Those are very important in prescribing antibiotic treatments to severe infections, or infections caused by multidrug resistant bacteria. For urgent purposes, broth microdilution is the reference method for MIC determination [146].

Automated dilution procedures have the advantages to simplify workflow, reduce turn-around time [147, 148], and yield quantitative AST results (MIC). Their limitations include restricted panels, and inability to test some fastidious bacteria, e.g., Gram-positive emerging pathogens. There are uncertainties associated both with MIC determination and disk diffusion methods [149]. Both AST methods are deficient for certain pairs of microorganisms and antimicrobials [150, 151, 152, 153]. Some of the problems may be solved by using additional tests or alternative methods [151].

Rapid antibiotic susceptibility testing

A rapid phenotypic AST (RAST) based on disk diffusion has recently been developed, which provides results yet after 4-8 hours using specific breakpoints [154, 155]. However, RAST has been established only for positive blood cultures and the test performance requires adherence to a strict protocol [156]. Currently, no rapid AST can be recommended for routine workflow with urine bacterial cultures. The text below is intended to describe the present limitations to substantiate this statement.

A non-standardized disk diffusion method, so called “direct AST” (dAST) has been applied to positive urine specimens, combining bacterial information from urine flow cytometry [157, 158] or microscopy examination [159, 160, 161], or direct AST from primary urine specimen combined with identification of uropathogens with MALDI-TOF MS [162]. A beneficial impact on the selection and the use of narrow-spectrum antimicrobial agents has been demonstrated [159, 161]. Several major drawbacks with dAST remain still to be solved: lack of standardization and reproducibility, and no correlation established to reference methods. Sensitivity of detection is not yet sufficient for a large portion of specimens in clinical UTI diagnostics, the type of inoculum cannot be properly controlled, mixed growth leads to useless results, urine characteristics (e.g., antimicrobial agents in urine or variable pH) may reduce reliability of the inhibition zones, and the lack of early detection of resistant mutant isolates [163]. Furthermore, urine specimens with Gram positive species have not been suitable for dAST [157]. It is also important to emphasize that there are no breakpoints for determining resistance directly from urine as the EUCAST (and CLSI) breakpoints are applicable only to defined pathogen concentrations, e.g., McFarland 0.5. As a conclusion, current dAST is not recommended in clinical routine.

Rapid immunochromatic (ICT) or chromogenic testing

Commercially rapid tests are available to detect specific antimicrobial mechanisms, such as methicillin resistance due to PLP2a production in *Staphylococcus aureus*, resistance to 3rd generation cephalosporins in Enterobacterales, production of extended spectrum betalactamases, and production of carbapenemases. A few of them have been evaluated on urine specimens. These tests cannot replace AST. In order to warrant performances of the test, medical relevance and efficient laboratory workflow, the indications of these tests should be limited to certain situations where pre-test probability is high and after consultation with the clinician of the impact of a result on antibiotic strategy.

Genotypic procedures

Genotypic procedures, e.g., PCR-based or isothermal amplification methods, provide detection of antimicrobial resistance genes. These are more rapid and specific than phenotypic methods. However, they mainly remain auxiliary procedures, due to major drawbacks such as

- absence of complete correlation of genotype with phenotype,
- detection of non-expressed genes leading to false positive resistance results,
- lack of MIC reporting,
- exclusive detection of genes that are already known to be associated with resistance, but lack of sensitivity when the genetic mechanism for resistance of the species has not yet been defined [147, 148, 164, 165, 166],
- comprehensive development of genotypic assays is very expensive - or impossible - due to the wide variety of antimicrobial resistance genes, and
- common molecular tests identifying solely bacterial DNA sequence do not identify the resistance mechanism at all if it is based on the level of expression of a commonly present gene, such as enhanced expression of export pumps in the cell membrane in case of resistance to β -lactam antibiotics.

Results from AST must be interpreted in a wider perspective, as knowledge of an underlying resistance mechanism in each bacterial isolate may allow prediction of resistances to other antibiotic agents not tested so far. It is crucial to understand detected resistance mechanisms, as well as to validate the expected phenotypes by using expert rules published by national or international committees such as the EUCAST [167], when adapting AST reports to clinical conditions. To prevent antibiotic misuse and to promote prescription of narrow-spectrum antimicrobial agents, the European microbiology laboratories are recommended to report selective antimicrobial susceptibility panels only, considering patient's sex and age as well as the resistance profile of the uropathogen isolated [168, 169].

Whichever method is chosen, the AST procedure must be standardized according to the procedure recommended by the EUCAST [145, 146, 167, 170, 171, 172, 173], applying the ISO 20776-1:2019 standard [174]. The critical technical points include the following: preparation and storage of media, storage of different reagents - particularly disks and AST cards, preparation of a pure and standardized inoculum, and incubation conditions [145, 146]. The performance of AST depends on tested strains or species, antimicrobials, and the actual procedure used. It must be

periodically evaluated with different methods and quality control strains used in the laboratory, also following performance in external quality assessment programmes.

7.7.3. New technologies for AST

Many new technologies address limitations of current methods such as slow speed, need of precultivation or identification of species before AST, low sensitivity, lack of portability, absence of distinction between living and dead cells, and difficulty to detect heterogeneous populations within a given isolate. The main objective of these new technologies is to develop platforms for rapid and reliable detection of antimicrobial resistance, in order to support antimicrobial stewardship programs in their fight against the resistance.

In addition to genome sequencing and metagenomics, the emerging methods for AST are based on spectroscopy, or miniaturisation, such as microfluidics. Microfluidics platforms are capable of single-cell growth rate measurements of bacteria exposed to antibiotics in microchambers or channels within a chip. Depending on the type of optical sensor coupled to the microfluidic device, AST report can be obtained from within 5 h to less than 45 min - even from urine specimens with low colony counts if using integrated pre-treatment steps [147, 148, 175, 176, 177, 178].

Multicenter evaluations or several single site evaluations should address numerous technical details, such as calibration to reference MIC methods, treatment of non-culturable pathogens, standardised operating procedures, and software issues, before these technologies reach a level of procedures recommended in routine laboratory guidelines.

RECOMMENDATION: This guideline recommends documents of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for procedures of antimicrobial susceptibility testing (AST), including reminders of limitations of each method. No rapid or direct AST can be recommended for routine workflow at the moment. The microbiology laboratories shall adhere to national antimicrobial stewardship in their AST reports. (1, A)

7.8. Performance evaluation in urine bacteriology with performance specifications

Improvements in laboratory technology aim finally to improve control of diseases. Point-of-care units are encouraged to consult their serving laboratory units when verifying their rapid tests. Small laboratories need support from larger national laboratories to proceed with verification of intended new instruments. Risk assessment of clinically inadequate results is a key feature when assessing the needed extend of verification in each clinical or laboratory environment.

Accreditation according to the ISO 15189:2022 standard [104] provides extensive technical and administrative guidance for verification and validation of measurement procedures for medical laboratories. Implementation of new equipment and consumables (devices) is regulated by the EU Regulation on *In Vitro Diagnostic Medical Devices* IVDR 2017/746 (*in vitro* equipment) [105] and the EU Regulation on Medical Devices MDR 2017/745 (medical devices include tools and containers used for specimen collection) [179].

7.8.1. Analytical performance of urine bacterial culture

Any new manual or automated procedure for urine bacterial culture is recommended to be validated against the described *Level 3* reference method (**Chapter 7.4.4**). In specific adaptations or in the verification by the end-user laboratories, the investigators must describe their focus and extent of their verification or in-house validation, and the corresponding comparison method in detail, including calculations of analytical performance and traceability according to the ISO 15189:2022 standard. Existing national standards or similar requirements need to be followed when new manual or automated procedures to urine bacterial culture are adopted, e.g., the MiQ 30 Quality Management in Germany [180], the UK Standards for Microbiology Investigations [181], the Qualité en microbiologie médicale (QUAMIC) in France [182, 183, 184], or American Society of Microbiology guidance to the ISO 15189 [185].

In addition to the cited standards and guidance, some practical remarks to the verification studies of routine procedures for urine bacterial culture are given below.

Purposes: The intended clinical use or specific needs guide application of the reference procedure for urine bacterial culture in the end-user laboratories. The scope may include an instrumental analysis against the described reference procedure, and an assessment of clinically required variety of patient specimens and specific important species to confirm the diagnostic performance in a local end-user environment. A comparison against a reference procedure may be needed to verify another essentially different manual examination procedure as well.

A reference procedure for urine culture may applied for a limited scope as well, to evaluate individual steps of the examination process in preanalytical, analytical or postanalytical phase. It may consist of performance evaluation with different sources of urine specimens, alternate equipment used for urine collection or storage, alternate ways of inoculation of homogenised specimens, comparison of 2-3 culture media, incubation equipment, atmosphere or time, or reading of the culture plates only. A limited application of the reference procedure of urine culture may be needed if the routine bacterial culture is not considered sufficiently accurate for the intended use, or the validation material provided by the manufacturer does not satisfy local use.

The chosen critical steps of verification should have a clear impact on patient treatment, to avoid use of excessive resource. Regional cooperation is highly recommended to share the tasks.

A reference procedure is needed to define and confirm the performance of special cultures for detection of fastidious uropathogens from clinical specimens by using extended incubation time (48 hours) and 5% CO₂ atmosphere, as described in **Chapter 7.4.4**. Verification assessment may include different levels of identification of species in the laboratory, or assessment of auxiliary tests, such as urine particle counting (both bacteria and WBC), when planning them to be parts of the routine workflow (**Chapter 7.8.3**).

7.8.1.1. Evaluation protocol and planning

Detailed written protocol (operating procedure) and resource should be created both for analytical testing, for training of the personnel, and storage of the experimental data.

Analytical performance evaluation should consider all key features described for the reference procedure of urine bacterial culture in **Chapter 7.4.4**. In case of limited adaptation, selection of used features of the reference procedure shall be mentioned. If the verification is intended to confirm diagnostic reliability of the candidate measurement procedure, a special attention shall be paid on sufficient clinical variability of specimens from different patient populations, ways of urine collection, as well as clinically important species in patient care. Differences between an optimised routine (candidate) procedure and the reference procedure create systematic errors that must be considered in the final assessment of performance. On the other hand, the major scope is not a preanalytical verification of different ways of specimen collection, devices, transportation or storage, which is practical to assess separately (see also **Chapter 7.4.4.3**).

Analytical and diagnostic performances are supported from data on internal quality control, results from external quality assessment, personnel training, and description of local computerised interfaces and data transfer between laboratory and hospital information systems.

A verification study of urine bacterial culture may be compared to the verification required for clinical blood culture, where a practical advise is to start with analysis of the diagnostic process, focussing on critical impact of the different workflow steps to patient outcome [Lamy B et al, CMI 2018]. The difference between blood and urine cultures is that parallel inoculations are possible from the same urine specimen, allowing direct comparisons of instruments and procedures using patient specimens, while that is not easily possible with specimens collected for blood culture. In addition to reference examinations with patient specimens, key performance indicators should be established for the follow-up of the found critical steps in urine cultures, similar to those for blood cultures [186].

Total laboratory workflow analysis, alternative procedures and verification steps, turn-around times, and risk management need to be described. Moreover, required human resource and training shall be estimated, as described in the ISO 15189:2022 standard.

7.8.1.2. Specimens

If a routine (*Level 2*) manual or automated procedure is to be compared against the reference (*Level 3*) procedure, about 100-1000 selected clinical specimens may be practically required, including Gram positive species. Primarily, it is important to cover clinically essential critical points

with acceptable uncertainty in the evaluation of analytical performance, rather than collect a defined total number of specimens.

The selected groups of specimens shall reflect local prevalence of species (see **Table 7-3**), aims of the intended analytical comparison, and needed accuracy of the results [90, 187]. Less than 30% of specimens should remain negative in culture, to leave > 70% of specimens to 3 (or optionally 4) positive ordinal scale categories of polymicrobial and monomicrobial growth, to be compared between the candidate and reference procedure in 4x4 (or 5x5) cross-tables. Species with different growth requirements need different comparisons when clinically important.

Laboratories shall verify applicability of their devices to different types of urine specimens and transportation procedures, as necessary. ATCC or equivalent control strains should be recovered and tested, as modified from the reference culture procedure (**Chapter 7.4.4**), depending on the purpose of the verification.

7.8.1.3. Equipment, consumables, and environment

The used products shall comply with the MDR 2017/745 regulation (specimen collection with invasive devices) or IVDR 2017/746 regulation (instruments, other equipment, and consumables such as primary collection containers and test tubes), as proven or assessed in separate studies together with the manufacturer. An example citation is related to an imaging device, to be validated by the manufacturer [188].

Calibration and metrological traceability of measurements should follow the principles given in chapter 6.5 of the ISO15189:2022 for equipment, as needed, to support consistency of reported results. For analytical environment, i.e., incubators, traceability and follow-up of temperatures and specific atmospheres must be documented. In chapter 6.6 of the ISO 15189:2022, principles of acceptance testing of reagents and consumables, including pipettes, are described.

Quality of used *culture media* is to be verified in separate inspections or experiments, as needed, despite the certificate of the manufacturer. Growth-promoting capacity of the media used for routine and reference cultures may need a confirmation, comparing the ability of different media to isolate the same organism, using ATCC or equivalent control strains representative of the uropathogens. Stability of media under environmental conditions needs an assessment as well.

Process data should not only be collected from analytical outcomes, but also from provision of enough material for identification of species and AST. Service data include turnaround times in the facility, including pre- and postanalytical steps.

7.8.1.4. Practical remarks to verification of a routine procedure for urine bacterial culture

Inoculation

The analytical sensitivity (limit of detected colony counts) is directly dependent on volume inoculated onto the media (**Chapter 7.4.4.4**). A laboratory must decide the volumes (1-100 µL) and types of inoculation in routine, depending on applied equipment and clinical specimens. A manual inoculation procedure is less vulnerable to cross-contamination than an automated serial inoculation since specimens are processed one by one (under a safety cabinet). Automated inoculation devices need be assessed with respect to frequency of cross-contamination they

produce. Streaking patterns must be experimentally determined to guarantee the highest number and reproducibility of discrete colonies from the inoculation of pure and mixed bacterial suspensions.

Uncertainty of colony counts

Multiple variables affect the obtained colony counts in addition to statistical imprecision (**Chapter 7.4.4.5**), such as way of specimen collection, specimen preparation, transportation time, culture media, inoculation process, incubation temperature and plate reading, and differences between human operators. It is important to verify the candidate procedure using specimens with colony counts close to the defined diagnostic range of quantification (10^3 through 10^5 CFU/mL, or 10^6 through 10^8 CFB/L). Application of enumeration grids minimises variability in estimation of observed counts [106]. Reproducibility of counts can also be used to train technical staff and to confirm its competency. The obtained estimates of reproducibility must be discussed in the summary of the verification study.

Trueness of identification (nomination)

The candidate procedure must be compared to the reference procedure to obtain an estimate of accuracy of bacterial identification (misclassification rate). Training of technical staff to use mass spectrometer must be documented.

Identification to the genus or species level shall be evaluated using ATCC or equivalent reference strains or clinical strains identified by reference molecular procedures. Analytical specificity of identification procedure is defined as the ability to not affiliate a strain to a taxon to which it does not belong. Analytical sensitivity is the ability to affiliate a strain to the taxon to which it belongs to. The definitions apply both for manual and automated procedures against the reference procedure.

Specificity can be affected by quality of the colony picking for identification, and by the cleanliness of the MALDI target plate. Inaccurate identification results from a mix of colonies, and from a poor-quality deposit on the MALDI target plate. Contamination is due to handling errors of the operator only. Both interferences and contaminations are resolved by staff training.

Sensitivity is affected by the presence or absence of the genus/species in the database and by the number and accuracy of reference mass spectra for each species in the MALDI TOF MS library. Specificity and sensitivity tests for rare species can be supplemented by a bibliographical review.

The laboratories that solely carry out biochemical tests need to ensure that they are also able to identify novel uropathogens, such as *Aerococcus urinae*, *Actinotignum schaalii* and *Corynebacterium urealyticum*, e.g., to organise their detection from another laboratory (see **Chapter 7.2.3**).

Robustness of performance

Robustness of performance shall be tested if automated identification is applied in conditions not recommended by the manufacturer, concerning sample preparation, age of obtained colonies, culture media, or stability of reagents.

Follow-up

Periodic reviews of results from the routine culture procedure are needed to maintain the performance in isolation and quantitation of diagnostic findings [59, 60]. Reviews also support problem solving of established routine workflows.

7.8.1.5. Performance specifications for routine bacterial culture (Level 2)

Performance specifications for routine urine bacterial culture (Level 2) are compared against the reference procedure (Level 3; Chapter 7.4.4) as applicable.

Trueness of identification: After a 10 µL inoculation, a described Level 2 culture identifies desirably all species in the *mixed* ATCC or other reference strain suspensions, and at least 95 % of the uropathogenic species from clinical specimens against the Level 3 reference procedure at 10⁴ CFU/mL (10⁷ CFB/L). A sensitivity > 90% at 10³ CFU/mL (10⁶ CFB/L) is required for routine specimens against the reference procedure. A separate assessment is needed for specific specimens, requiring a sensitivity > 90% at 10² CFU/mL (10⁵ CFB/L).

Specificity to detect uropathogenic bacteria is evaluated by using polymicrobial specimens, and quality of isolated colonies as compared to the reference procedure. Analytical specificity is desirably > 95% and minimum > 90% at any positive category 10³ – 10⁵ CFU/mL (10⁶-10⁸ CFB/L).

Causes of misclassification in practice: Frequencies of misclassification of identified species (false positive and negative results, or erroneous nominations of species) need to be described, as compared against the reference procedure. Their significance shall be assessed based to annual prevalence of specimens and different isolated species in the laboratory.

Examples of misclassification against the reference procedure

The examples below intend to provide some practical reasons to false positive or false negative results, to be considered during verification of a routine culture procedure:

- *False positive results* may derive from contaminants of perineal or external genital microbiota (transient or resident urogenital mucosal microbiota) in common urine specimens, including mid-stream, indwelling catheter, and single-catheter urine, defective preservation during transportation, or in obscure analytical steps with untrained technical staff.
- *False negative results* may derive from
 - (i) fastidious pathogens not growing on routine media, e.g., *Aerococcus* spp. or *Actinotignum schaalii*, or in routine aerobic atmosphere
 - (ii) technical problems in the collection, transport, or culturing process, such as improper manufacturing or storage of culture plates
 - (iii) too high concentration of preservative in a low specimen volume
 - (iv) freezing of the specimen during transportation
 - (v) non-standard conditions in the atmosphere, temperature, or time of incubation
 - (vi) antibacterial substances (inhibitors of growth) in patient's urine that may not be detected easily on agar culture, or
 - (vii) improper classification of detected isolates due to technical or human errors.

Quality of isolation: At least 3 discrete colonies shall be grown on plates to allow additional tests, such as MALDI-TOF or AST. The fraction of low-quality isolations in studied clinical specimens shall be documented.

Precision of quantitation (counting): Repeatability CV of colony counts from 10 replicate cultures may be tested with chosen standard ATCC or equivalent reference strains, counting at least about 10 and 100 colonies/plate equal to 10^3 and 10^4 CFU/mL (10^6 and 10^7 CFB/L), respectively (after a 10 µL inoculum), both with the candidate procedure and the reference procedure. Mean counts of both procedures and their coefficients of variation (CV) should be reported.

If needed in the assessment, there is a possibility for an average imprecision of colony counts from patient specimens by using from duplicate inoculations of 20-30 specimens and the following equation to calculate the standard deviation s [189]:

$$s = \sqrt{[\sum(x_{i1} - x_{i2})^2 / (2n)]},$$

where n = number of duplicate pairs, and x_{i1} and x_{i2} are paired observations from specimens $i = 1$ to n . Then $CV = s/x(\text{mean})$. Theoretical imprecision, $CV_{\text{theoretical}}$, is derived from Poisson distribution (see **Chapter 7.4.2**).

Trueness of quantitation: Agreement of ordinal scale quantities of colonies in clinical specimens between the candidate and the reference procedure should be compared with a crosstable, using 10 µL inoculations, or 100 µL inoculations to reach 10^2 CFU/mL (corresponding to 10^5 CFB/L). Disagreement between observations needs to be evaluated, using applicable statistics.

Operator-related uncertainty: After primary training and familiarisation with both the verified and reference procedure, an agreement between human operators shall be documented using cross-tabulation of agreement, or classification of identified / misidentified species, as appropriate. Interpretation of significant disagreement may be carried out statistically, but at least clinically, based on the collected data. Individual performance is usually followed in internal quality control (IQC) reviews or in EQA schemes of the laboratory.

7.8.1.6. Analysis of sources of variation

Systematic errors (biases) and random variability (increased imprecision) exceeding Poisson imprecision should be described as components of *measurement uncertainty (MU)* of counts. The considered extra uncertainties include those described already for the reference procedure (**Chapter 7.4.4**). Some practical examples are given below:

- Variability between the human employees is more important in clinical practice than in limited technical verifications. Describe both internal comparisons and results from external quality assessment schemes.
- Causes of increased imprecision in clinical urine specimens include leukocytes or other particles if appearing as clumps, amorphous precipitate or mucus that create uneven distribution of bacteria in urine.
- Testing environment includes variability at least with respect to employees, processes of specimen collection and transportation, reagents, materials, and analytical processes.

7.8.2. Evaluation of automated bacteriology workstations, process management, and economics

Implementation of automation into bacteriology working environment has several other features than analytical performance to be considered. Automated processes are generally better standardised, traced, and secured than manual processes, but some risks of manual processes are increased, and some new risks are encountered. Frequency, severity, detection and correction of errors in automated procedures differ from those observed with manual procedures. Furthermore, risks with automation depend on the applied systems [89], and degree of automation. Thus, a candidate equipment must be assessed thoroughly to confirm that it meets the expected specifications with minimum downtime periods.

7.8.2.1. Specific targets of verification in bacteriology workstations

In addition to analytical performance, the following *features of an automated bacterial culture system* with several instruments and conveyors are given as a provisional checklist. Other features may also be important as judged by the professionals of the laboratory.

- (i) Quality of colony separation on agar plates from pure cultures and mixed bacterial suspensions, covering a range of clinically important colony counts from 10^2 to 10^5 CFU/mL (10^5 to 10^8 CFB/L)
- (ii) Repeatability of specific robotic procedures, as applicable
- (iii) Correctness of digital plate imaging and reading, including ability to detect polymicrobial growth
- (iv) Triggering of interpretation rules, e.g., accuracy of segregation of plates
- (v) Proper triggering of picking assignments of colonies for identification and AST
- (vi) Measurement uncertainty around decision limits for significant bacteriuria, and uncertainty related to digital images and software algorithms
- (vii) Cross-contamination during automated inoculation, using specimens at high bacterial concentrations against water (saline)
- (viii) Non-conforming samples, ability of equipment to identify them
- (ix) Robustness, stability and reliability of reagents and media (storage conditions outside and inside the instrument)
- (x) Software and middleware performance in process control, user interface, and details of interface to laboratory information system, including bi-directional connections
- (xi) Management of pre- and post-analytics: equipment and procedures of specimen collection and delivery to the automated laboratory, storage after analysis and recall to further analysis

Risk management of an automated system is more critical than that of manual procedures. At least the following views need to be addressed:

- (i) Management of most frequent error flags and malfunctions by laboratory operators

- (ii) Supplier's service (24/7), procedures of contacting, service agreement with ability and delays to intervene on-site and remotely; availability of spare parts.
- (iii) Definition of a back-up procedure, including alternative plates or other consumables when facing shortage in the vendor's stock
- (iv) Increased risks based on the level of automation (major risks of human error if the laboratory has incubators, but not an automated inoculation system)
- (v) Triggering alarms for technical errors, including robotics, outcomes of automated plate reading, error flags of the instruments and analysing software
- (vi) Warning flags of false results due to features of bacterial species and patient specimens
- (vii) Environmental conditions, such as temperature of laboratory, electricity and pneumatic air supply, power and heat from computers, and air conditioning
- (viii) Computer hardware and interfaces to analytical instruments and robotics, connections to automatic conveyor, LIS and hospital information system
- (ix) Reliability, measured as % downtime (service breaks) from total working hours

Human resource planning

Thorough staff training is a key factor for successful implementation of automation, including planning of new workflows and employee organisation, and training of new skills to available professionals. Shared planning with the personnel supports motivation and well-being in the middle of change. Increased availability of staff is needed during the verification and training periods despite finally needed human resource.

7.8.2.3. Clinical and economic impact of new workflow in urine bacterial culture

Cost/benefit assessment is a requirement for laboratory leadership, including all costs, already described in the purchase tender of the instruments and reagents, maintenance and service, data management, and estimated human resource. Often, verification and full-scale implementation of new system creates transitional costs, despite reduced costs of the new process. Indirect costs, e.g., related to obligatory changes in the working space, power supplies, or air conditioning, may become as surprise. Customer co-operation also takes time from the responsible personnel.

Impact of reduced turn-around times and new laboratory reports may change outcomes in clinical units, which may be a major driver towards automation. Changes in the requisition of urine bacterial culture must be discussed with clinical units and hospital leadership, to maximise benefits and minimise costs with optimised workflows.

7.8.3. Analytical performance specifications for rapid tests in detecting bacteriuria

Non-culture determination of bacterial concentration with rapid tests, e.g., with particle counting (*Level 2* methods), and test strips (*Level 1* methods) are being used in point-of-care and other diagnostics of symptomatic bacteriuria by means of leukocyte or bacteria detection, before the results from bacterial cultures are available. They may also improve workflows within bacteriology laboratories.

A combination of automated particle counting with bacterial cultures has become popular in microbiological diagnostics of UTI [190, 191, 192, 193, 194, 195]. It is then important to define the performance specifications needed in such a rapid diagnostics (see outcomes in **Chapter 6.3.3.1**).

In general, a diagnostic sensitivity of 80-90% in the selected patient population is considered adequate, while a specificity of 90-95% should be maintained in diagnostic reports. However, when the rapid examination is used for diagnostic screening (ruling out negative specimens) before a confirmatory test as a part of laboratory workflow, a sensitivity >95% with a specificity of at least 50% should be the target against $\geq 10^3$ or $\geq 10^4$ CFU/mL in culture ($\geq 10^6$ or $\geq 10^7$ CFB/L, respectively) based on local practice, as improved with clinical and preanalytical information. In addition, application must be economically viable for the diagnostic workflow, or for the clinical patient management [196].

Desirable specifications for rapid ruling out of bacteriuria at $\geq 10^5$ CFU/mL (10^8 CFB/L) and $\geq 10^3$ CFU/mL (10^6 CFB/L) in the laboratory workflow are suggested for common specimens in **Table 7-8**. A higher than 50% specificity provides better rapid diagnostics in emergency cases, indicating that other, high-specificity limits with lower sensitivity should be applied for emergency services additionally [190].

Comparisons are recommended to be organised into crosstables that compare results from rapid procedures to those with quantitative bacterial culture of the same specimens, using, ordinal scale statistics. Assessment of diagnostic significance should be included in the interpretation of those comparisons.

Table 7-8. Analytical performance specifications for laboratory screening of uropathogens in ruling out negative cultures

Uropathogens in culture	Sensitivity	Specificity at the given sensitivity
$\geq 10^5$ CFU/mL ($\geq 10^8$ CFB/L)	> 95 %	> 50 %
$\geq 10^3$ CFU/mL ($\geq 10^6$ CFB/L)	> 80 %	> 50 %

It is to be reminded that the chosen patient populations, symptoms of patients, interpretation of leukocyturia and criteria used to define significant growth greatly affect the performance characteristics of rapid tests. Given this variability, results from rapid tests can be used in all laboratories to target diagnostic work on clinically more significant specimens based on results

from rapid tests, in particular leukocyturia (see **Chapter 7.5.2**). Modern particle counting is more sensitive and specific than a chemical test strip measurement (**Chapter 5.2.1.1**).

RECOMMENDATION: The suggested practical procedures or tools for verification of routine bacterial examinations aim to help in the assessment of various changes in routine workflows. The level of satisfactory assessment is case-dependent. It needs to focus on critical diagnostic steps, and must be judged against relevant references, including the ISO 15189:2022 standard. (1, B)

7.9. RECOMMENDATIONS FOR BACTERIOLOGY

No	Recommendations	SoR (1-2), and LoE (A-D) ^a	Chapter discussed
49	Commensal urogenital microbiota are not recommended to be sought nor treated from asymptomatic individuals (Asymptomatic bacteriuria).	1, A	7.1.1
50	Suspicious of sporadic uncomplicated lower urinary tract infections in otherwise healthy women are recommended to be screened for the presence of infection by using a validated questionnaire, to reduce routine workflow in bacteriology laboratory. Rapid tests for leukocytes and bacteria are recommended into diagnostics of unclear and other cases.	1, A	7.1.1
51	Urine specimens from most routine patients suspected for UTI are recommended to be sent to quantitative urine culture and possible antimicrobial susceptibility testing. Sensitive screening procedures are encouraged to reduce the number of specimens from the routine workflow. Special cultures of specimens from special patient groups are recommended to be organised as nationally or locally defined.	1, A	7.1.2
52	No control cultures are recommended from patients with lower UTI if becoming asymptomatic after an antimicrobial treatment.	1, A	7.1.3
53	Classification of uropathogens has been slightly updated. In addition to uropathogenicity, predisposing host conditions, quality of specimen collection, results from particle analysis (leukocytes and bacteria), and quantity and types of species grown in culture are recommended to be considered when assessing the diagnostic value of detected bacteriuria.	1, A	7.2.2
54	New species <i>Aerococcus spp</i> and <i>Actinotignum schaalii</i> and <i>Corynebacterium urealyticum</i> are proposed into the list of class II uropathogens if detected in monomicrobial culture.	2, B	7.2.3
55	Bacterial identification using Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is strongly recommended into medium-sized and large laboratories (> 100 specimens/day), to improve patient prognosis with accuracy and reliability of identification to the species level, and shortened delay of reporting.	1, A	7.3.3
56	Limitations of the MALDI-TOF MS in detecting bacteriuria at low colony counts (less than 10 ⁴ CFU/mL, or 10 ⁷ CFB/L) must be understood in organising laboratory processes for urine specimens with a possibility of significant low bacteria counts. MALDI-TOF MS	1, A	7.3.3

	shall not be applied directly to urine specimens in routine laboratories without preculturing the specimen.		
57	Chromogenic agar is strongly recommended as primary agar medium to identify <i>Escherichia coli</i> (most frequent uropathogen) easily, quickly, and inexpensively (no need for a panel of tests to define the species). A second agar (such as blood agar) is recommended in clinical defined cases and for fastidious organisms.	1, B	7.4.1
58	Reproducible detection of low colony counts <u>at</u> 10^3 CFU/mL (10^6 CFB/L) requires an inoculum of at least 10 μ L, adopting one of the recommended methods of inoculation.	1, A	7.4.2
59	Aerobic incubation at 35 ± 2 °C for 16-24 hours is sufficient for primary uropathogens. For special urine specimens, blood agar plates are recommended to be incubated under 5% CO ₂ atmosphere for 48 hours in addition to aerobic conditions, to detect possible fastidious organisms.	1, A	7.4.2
60	A qualified reference examination (<i>Level 3</i> procedure) is recommended to be used for bacterial cultures (1) to verify a required performance of routine bacterial culture (at <i>Level 2</i>), or (2) to assess any instruments in bacteriology intended to detect, quantify, or identify bacterial species for clinical diagnostics against the suggested performance specifications as needed.	1, A	7.4.4
61	<i>No recommendation</i> is given to the unit for reporting urine bacterial cultures. A national harmonisation is recommended to avoid confusion among professionals and patient risks.	Not given	7.5.1
62	A flowchart for routine urine specimens is recommended as a practical advice to bacteriology laboratories to organise their workflows, starting from mid-stream urine specimens. It is open for modifications based on specific specimens or patient populations, as well as local epidemiology of uropathogenic species in the laboratory.	1, B	7.5.2
63	Bacteria and yeast detected from urine specimens need to be identified to the species level to satisfy proper clinical diagnostics, and to be able to assess their antimicrobial susceptibility. Limitations of different identification methods are recommended to be considered to avoid deficient identifications or misclassifications.	1, A	7.6
64	This guideline recommends documents of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for procedures of antimicrobial susceptibility testing (AST), including reminders of limitations of each method. No rapid or direct AST can be recommended for routine workflow at the moment. The	1, A	7.7

	microbiology laboratories shall adhere to national antimicrobial stewardship in their AST reports.		
65	The suggested practical procedures or tools for verification of routine bacterial examinations aim to help in the assessment of various changes in routine workflows. The level of satisfactory assessment is case-dependent. It needs to focus on critical diagnostic steps, and must be judged against relevant references, including the ISO 15189:2022 standard.	1, B	7.8

^a Strengths of Recommendations (SoR) are: 1= strong, 2 = weak recommendation.

Levels of Evidence (LoE) are: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts. Laboratory modification of the GRADE rating is described in the Introduction.

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ANNEX

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ANNEX I. Detailed instructions for specimen collection and preservation

I.1. Instructions for collection of urine specimens

Nurses and laboratory personnel usually instruct patients how to obtain an adequate urine specimen. Health care personnel should first understand the requirements of standardised specimen collection, and then empower patients to take care of their own diagnostics. Since the compliance of the patient or his/her parents is usually needed to obtain an adequate specimen, both oral and written guidance, often with illustrations or videos, is necessary. Each institution is encouraged to modify the texts given below to make their local practice as good as possible. Pictures showing the basic procedures for females, males, and children should be used. The enclosed *illustrations on specimen collection can be freely copied*. They may be the only means of understanding by individuals unfamiliar with the native language. Use of training videos is also encouraged. Professional “hands on” assistance is often needed for small children and elderly people.

I.1.1. Collection of Mid-Stream Urine (MSU) specimens

(Models for Patient Instructions)

The following instructions and illustrations are provided for mid-stream specimens. These illustrations may be translated for local clinical practice as a public resource from non-profit Finnish hospital districts (originally drawn at Tampere University Hospital (TAUH), Tampere, Finland). Collection of MSU specimens is still suggested after cleansing for both sexes, see **Chapter 3.2.1.1**.

Adults: Females Figure I-1
 Males, Figure I-2

Children, using potty chair: Figure I-3

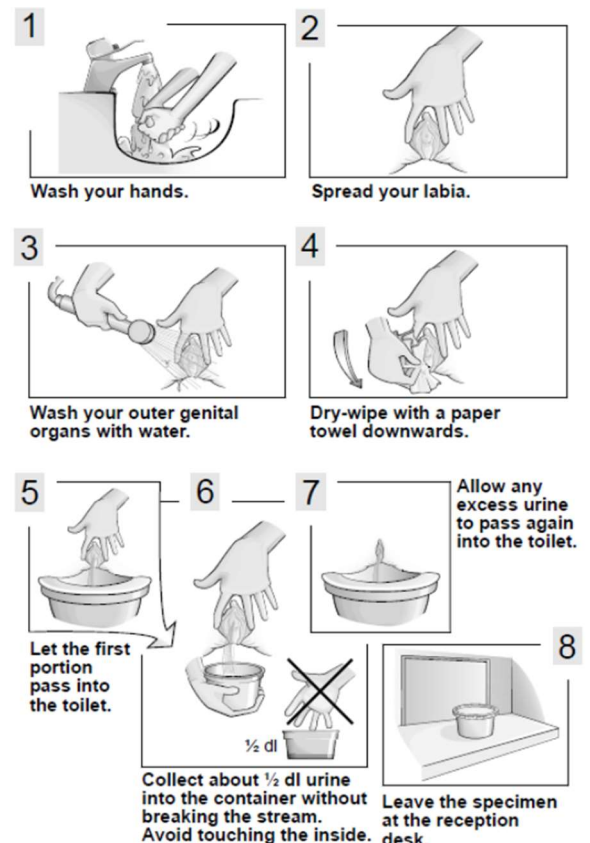
Figure I-1. Collection of mid-stream urine specimen, females*Females*

Wash your hands with soap and water or a towelette. Dry-wipe them. Take the clean collection container with you. Avoid touching the inside with your fingers. While sitting on the toilet wash your outer genital organs including the opening where the urine comes out with a hand shower or with lukewarm water and wet paper towels (or sterile towelettes) without using any disinfectants that would inhibit bacterial growth.

Dry-wipe. When urinating, let the first portion pass into the toilet (bedpan). Collect the mid-portion into the container. Allow any excess urine to pass again into the toilet.

After urination, dry-wipe the outer surface of the container, secure the lid or transfer the urine to the tube(s) provided, and write or check your name and the date and time when you produced the specimen on the label on the container.

Then proceed as advised (local explanation) ...
If there are any problems, please consult your local clinical attendant...

Collection of mid-stream urine, females

TAUH Department of Clinical Chemistry / T.Heittola

Figure I-2. Collection of mid-stream urine specimen, males**Males**

Wash your hands with soap and water or a towelette. Dry-wipe them. Take the clean collection container with you. Avoid touching the inside with your fingers. Uncover the urethral opening by withdrawing the foreskin if necessary. Wash the end of your penis, to include the opening where the urine comes out, with a hand shower or with lukewarm water and paper towels (or sterile towelette) without using any disinfectants.

Dry-wipe. When urinating (either standing or sitting), let the first portion pass into the toilet (bedpan). Collect the mid-portion into the container. Allow any excess urine to pass again into the toilet.

After urination, dry-wipe the outer surface of the container, secure the lid or transfer the urine to the tube(s) provided, and write or check your name and the date and time when you produced the specimen on the container label.

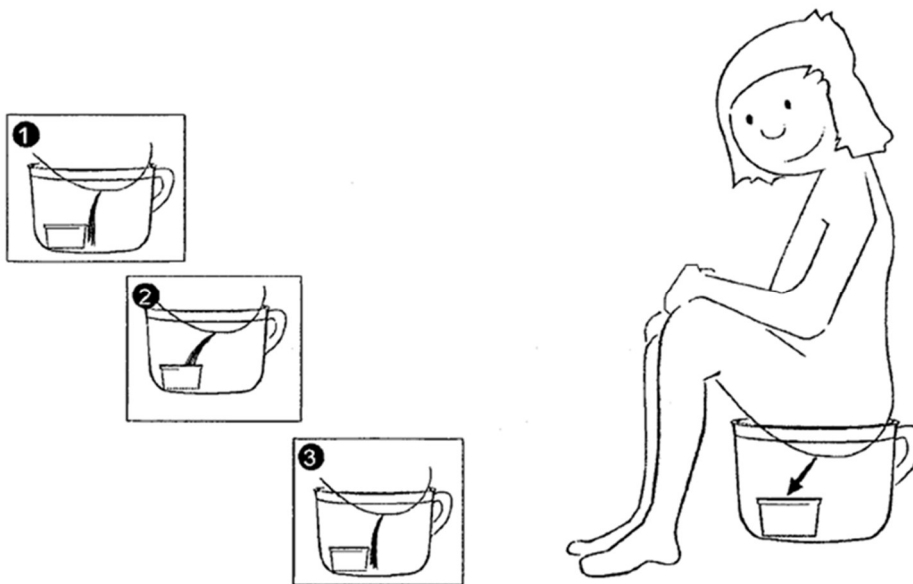
Then proceed as advised (local explanation)....
If any problems occur, please consult the clinical attendant.....

Collection of mid-stream urine, males

Figure I-3. Collection of mid-stream urine specimen, children

From infants and toddlers being able to control their urination, a container inserted into a potty chair helps in getting a mid-stream specimen.

Children (capable of controlled micturition)



After appropriate explanation, reasonably adequate mid-stream specimens can be collected from children old enough to sit on a potty chair. This can be achieved by inserting the collection container into the potty chair.

Older children may follow the same advice as given to adults.

After producing the sample, dry-wipe the outer surface of the container, secure the lid or transfer the urine to the tube(s) provided, and write or check the child's name and the date and time when the specimen was produced on the container label.

Then proceed as advised (local explanation)...

If any problems occur, please consult the clinical attendant....

I.1.2. Collection of sequential urine specimens (Meares and Stamey procedure)

For diagnosis of prostatitis, sequential collection of first and middle portions of a single-voided specimen is of diagnostic value, as well as drops expressed with prostate massage, and urine after prostatic massage. A modified procedure with two specimens has also been described (see **Chapter 3.2.9**). The results are better if the patient has not ejaculated at least for 3 days before the collection of the specimen, since ejaculate microbes are not representative for diagnosis of prostatitis. The given instructions are to be followed with the assistance of the physician performing the examination.

Patient instructions

- (1) Half an hour before specimen collection, drink 400 mL of water (or juice). When you want to void, the examination starts.
- (2) Label four sterile collection vessels (A-D) and remove the closures from them. Avoid touching the inside of the vessels or closures.
- (3) Wash your hands with soap and water or a towelette. Dry-wipe them.
- (4) Take the clean collection container with you. Uncover the urethral opening by withdrawing the foreskin. Wash the end of your penis, to include the opening where the urine comes out, with a hand shower or with lukewarm water and paper towels (or sterile towelette) without using any disinfectants. Dry-wipe.
- (5) Urinate 10-15 mL into the first container (A) in a standing position.
- (6) Urinate 100-200 mL into the toilet (bedpan). Without interrupting the stream, urinate 10-15 mL into the second container (B). Allow any excess urine to pass again into the toilet.
- (7) Bend forward and hold the sterile specimen container (C) to catch the prostate secretion while the physician massages the prostate. Several drops are needed.
- (8) If no secretion is visible during massage, the physician collects a specimen with a 10 µL loop from urethral orifice for direct culture.
- (9) After prostatic massage, try to urinate additionally 10-15 mL into the container (D).

The containers A-D should be sent for bacterial culture.

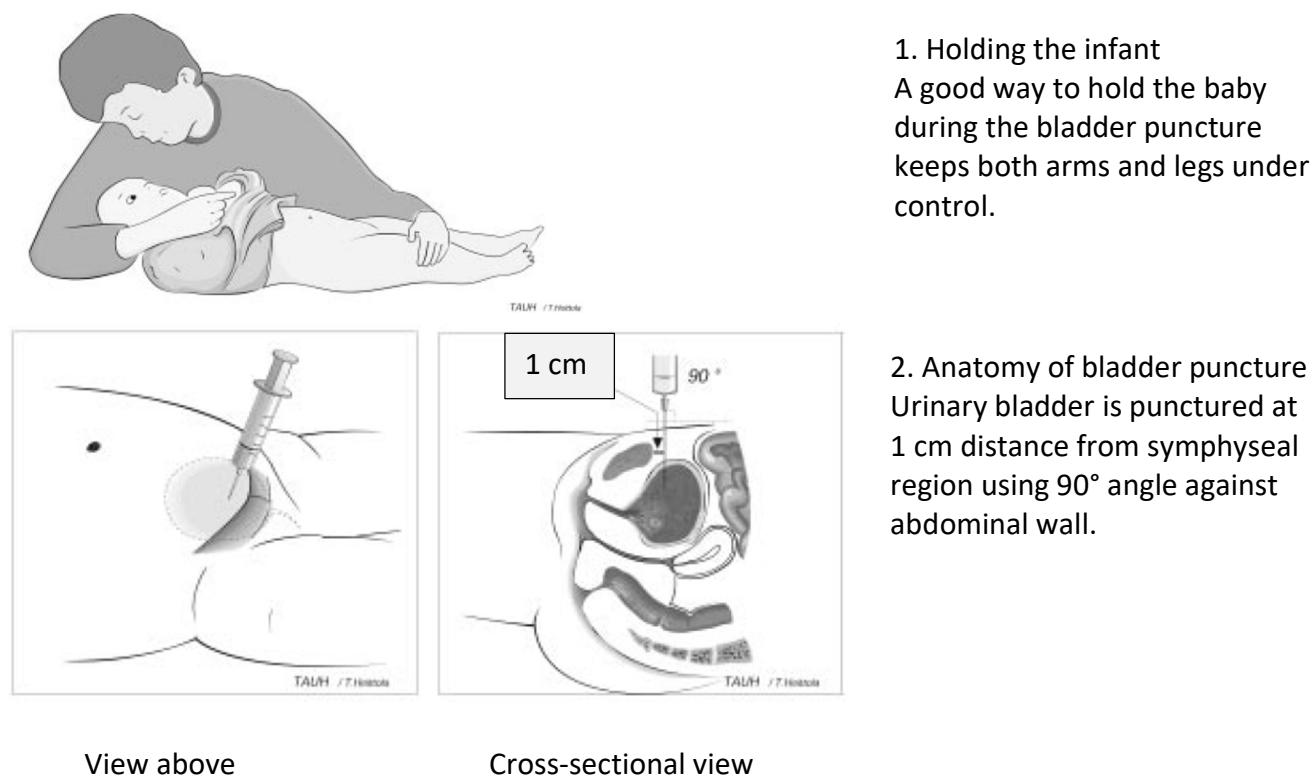
If possible, particle analysis is also of diagnostic value after inoculation of the plates.

I.1.3. Collection of Suprapubic Aspiration specimen

A container inserted into a potty chair helps in getting a mid-stream specimen from infants and toddlers being able to control their urination, See **Figure I-3, Chapter I.1.1.**

For incontinent infants, suprapubic aspiration (SPA) should be attempted when the diagnosis or exclusion of urinary tract infection is crucial, and a spontaneous urine, bag or pad specimen do not apply (**Figure I-4**). This is because SPA specimens result in remarkably lower occurrence of mixed growth than those obtained with bags, pads or spontaneous specimens, and even those obtained by in-and-out catheterisation, see **Chapter 3.2.5.**

Figure I-4. Illustrations for suprapubic aspiration specimen



Aseptic measures should be taken to avoid skin contamination. Specimen collection and washing tools should be prepared ahead, including a 5 (-10) mL syringe used for aspiration. It is possible to wait up to 2 h for the bladder to fill, possibly using ultrasound imaging. However, the urgency symptoms may lead to loss of the specimen by spontaneous voiding if not followed carefully. Dehydrated febrile children should take in fluid to the extent needed to start diuresis. Anaesthetic skin cream containing lidocaine or prilocaine is recommended before the puncture.

The bladder is punctured by simultaneous aspiration. The site is chosen to avoid both periosteal damage (1 cm distant from the symphyseal region) and intestinal contamination. Aliquots of urine to different laboratory tests need a local agreement. For bacterial culture, 0.5 to 2 mL is usually sufficient for inoculation, and another 1 mL for visual microscopy.

I.1.4. Timed collection of urine

A 24-hour urine is the most common example of a timed collection. Instructions must be provided for each patient or guardian to support the collection, and modified for local use. An example for patient instructions is given below. *Different preservatives* to be used in timed urine collections have been listed in **ANNEX I.2, Table I-3**. The table was compiled for analytes requested from outpatients.

Patient instructions: 24-hour collection of urine

READ THESE INSTRUCTIONS CAREFULLY BEFORE YOU START THE COLLECTION.

You have been asked to collect a timed urine because the doctor wants to know the exact amount of (the examined substance) excreted into your urine as a part of your medical examination. You have been asked to collect for a 24-hour period.

- (1) Preparation: If you are not in hospital, select a peaceful day when you expect to be able to use the toilet where you keep the collection container throughout the continuous collection period.
- (2) Preservative: Your collection may need preservatives for reliable analysis. Your local advisor will tell you how to deal with these. Preservatives are usually added to the collection container before the start or immediately after the first voided portion.
- (3) Write down the date and time when you start the collection (you can choose when to start). Empty your bladder and discard that sample. All voided urine after this start is to be collected into the container. Keep the container refrigerated during the collection if no preservatives were advised, and you have that possibility.
- (4) Exactly 24 hours after starting the collection empty your bladder and add this to the collection container.
- (5) Close the container tightly, dry wipe and place the label provided on the container. Write or check the details of your collection times and your personal identification data.
- (6) Store and transport the container to the laboratory as advised,

OR (instead of items 5-6)

- (7) If a portion of the 24-hour specimen only was requested, close the container tightly, mix the complete collection thoroughly before pouring a small sample into the small container you have been given. Dry-wipe the small container.
- (8) Check or write your name, personal identification number and detailed collection date and times on the label. Place the label provided on to the small container. Store and transport the small container only, or both containers to the laboratory as advised.

If any questions arise, please contact your clinical attendant at....

I.2. Preservatives for urine collections

Criteria of preservation are discussed in **Chapter 3.3**. Stringent experiments show statistically significant changes in some measured components already within the first two hours after voiding at room temperature. Some flexibility to allowable time frames is obtained by using the criteria in **Chapter 3.3.1**, understanding the speed and type of diagnostic changes.

There is a clear need for preservation of urine specimens intended for chemical measurements and particle analysis at room temperature for at least 1-3 days. For bacterial culture, preservation at room temperature for 1-2 days after collection is available for centralised laboratory services. **Tables I-2** and **I-3** also provide data on preservation by refrigeration. Week-end and holiday service must be organised accordingly.

Table I-2. Preservatives for test strips, particle analysis, and urine bacterial culture

The figures express maximum documented stable time, when known, with the following abbreviations: h= hours, d= days, w = weeks, mo = months, y = years.

The table assumes non-infected urine (bacteriuria may dramatically affect the preservation of some analytes). Usually, about 1% final concentration of boric acid is used.

Analyte	Room temp (+20°C ± 5°C)	Refrigerated (+5 ± 3°C °C)	Boric acid, alone or mixed	References ^a
<i>Multiple test strip</i>				
WBC, Esterase / RBC, Pseudo- peroxidase	2-6 h (optimum, maximum)	4 h - 1 d (false negatives) ^c	6 h	[1, 2, 3] ^b
Nitrite	< 5 h	4 h – 8 h (false positives) ^c	< 5 h	[1, 3, 4]
Albumin (Protein)	1 d	4 h – 1 d (false positives) ^c	1 d	[1, 3]
Glucose and ketone bodies	< 5 h	< 5 h / 3 d	6 h	[1, 4]
Relative density (RD, SG)	1 d	3 d	3 d	[1]
<i>Particle analysis</i>				
RBC and WBC	2-6 h (optimum, maximum)	5 h	1-2 d ^d	[1, 3, 5, 7] ^b
Squamous epithelial cells (SEC)	3 d	8 h / 1-3 d		[1, 3]
Renal & transitional epithelial cells	1-3 d (optimum, maximum)	1-3 d		[7]

Casts		1-3 d		[7]
Bacteria counts	2-6 h (optimum, maximum)	1-3 d	1-2 d ^d	[1, 7] ^b
<hr/> <i>Bacterial Culture</i> <hr/>				
Bacterial culture	No	1 d	1 d	[8, 9, 10]

^a References are listed in the end of ANNEX I.

^b The BD Life Sciences has not validated the use of C&S tube (boric acid mixture) for particle counting, as applied by Kouri T et al. in their local studies [1, 7].

^c A tendency of change (false positives or false negatives) in extended storage is given in brackets.

^d There is no good evidence of preservation of WBC with boric acid alone for particle counting, buffered mixtures with supported osmolality are recommended. For bacteria preservation, a maximum of 2 days has usually been documented by manufacturers for boric acid-containing preservatives at room temperature.

Table I-3. Preservatives for 24-hour collection of quantitative chemical measurements ^a

The urine specimens should not be infected or contaminated, since bacteriuria may dramatically effect preservation of some analytes.

Analyte	Room temp (20°C ± 5°C)	Refrigerated (5°C ± 3°C)	Frozen ≤ - 18°C	HCl ^b 6 mol/L stock solution 5ml to 1 L	Boric Acid 1-2% final conc	Na ₂ CO ₃ 5 g/L	Links and References ^c
Albumin	7 d ^a	1 mo	6 mo*	No ^a	7d		*Concentration decreased by nephelometry up to -30%, by HPLC up to -50% [11, 12]
Alpha-1 microglobulin (Protein HC)	7 d	1 mo	6 mo*				*Depends on the procedure, similar to albumin
Alpha-2 macroglobulin	7d	7d					
Amino acids			Yes ^a				
Calcium	1-3 d §	4 d §	3 w	*		No	* HCl generally not needed [13, 14, 15]; § Some precipitation in patient specimens is prevented by HCl when receiving the collection
Citrate	3 d		4 w*	Yes			* If pH < 1.7 [15, 16]
Creatinine	3 d	5 d	6 mo		Yes		[12, 15]
Cystine		3 mo*	1 y*	7 d*			*Add HCl
Glucose	< 2h	2h	8h	No	Yes		*Azide [16]
Human chorionic gonadotropin (pregnancy test)		Yes					
Immunoglobulin, kappa & lambda, quantitative	7 d	1 mo	6 mo				

Immunoglobulins, intact, quantitative	7 d	1 mo	No*				*Cryoproteins may not redissolve
Magnesium	3 d	3d	1 y	*			* HCl not needed [14, 15, 16]
Novel kidney biomarkers (IL-18, KIM-1, L-FABP, cysC)	IL-18 labile	2 d					Final storage at -80°C [17]
Osmolality	3 h	7 d	3 mo				
Oxalate	3 d		4 mo*	Yes			EDTA addition in the laboratory helpful; * If acidified [15, 16]
Phosphate (inorganic)	3 d			>3 d*			* If acidified; HCl is not needed if analysed within 3 d [15]
Protein, immunofixation and electrophoresis	7 d	1 mo	6 mo				
Protein, total	1 d	7 d	1 mo	No	Yes		Depends on the procedure
Urate	3 d			No	No	>3 d*	* pH >8 with Na ₂ CO ₃ ; not needed if analysed ≤ 3 d [15]

^a Abbreviations: "Yes" is used for a probably successful preservation, "No" suggests lack of preservation; both of these to be confirmed if applied. Data on the details are not available.

The figures express maximum stable time: h= hours, d= days, w = weeks, mo = months, y = years.

^b Addition of HCl in the laboratory after reception of the collected specimen is acceptable [1].

^c Link to an option on the same line (measurand) is shown with an asterisk* or a paragraph[§] sign.

References are listed in the end of ANNEX I.

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ANNEX II. Morphological details of urine particles

Table II-1. Morphology of urine particles by phase contrast microscopy

The differentiation is based on visual microscopy (magnification x 400), using phase contrast optics. The details are derived from the handbook by Dr. G.B. Fogazzi if not otherwise stated [1]. Occasional modifications are based on Core Curriculum 2019 for American nephrologists [2], or additions by the authors of this guideline. Polarised light is needed to see birefringence.

Table II-1A. Blood cells in urine

Nucleus	Cytoplasm	Other features
Red blood cells (RBC)		
Absent	Biconcave discs with a diameter of about 6-7 μm . The diameter of RBC may range from microcytes to macrocytes, within an interval of 4-10 μm due to the osmotic variability of urine.	Small RBC appear in hypertonic urine, while RBC swell in hypotonic urine. "Ghost cells" are grey shadows with sharp margins in phase contrast optics due to hemoglobin leakage. Small RBC with abnormal shapes (dysmorphism) result from glomerular bleeding and interaction of renal tubular cells with RBC leakage into the kidneys. Acanthocytes or G1 cells (ring-shaped RBC with blebs), are a specific subgroup of dysmorphic cells.
White blood cells (WBC) / Granulocytes		
Multi-lobular or rod-shaped nucleus	7-15 μm in diameter. Granulocytes contain cytoplasmic granules.	Morphology varies due to degeneration, activation by inflammation, and density of urine: when high, the nucleus may not be clearly visible, and when low, the cytoplasm becomes swollen, and the nuclei are easily seen, until cells are lysed [3]. Pseudopod extensions of the cytoplasm are rarely formed in activated granulocytes, creating a possible confusion with fungi. Granulocytes occur often in clumps.
WBC / Macrophages		
More than a single nucleus located either centrally or peripherally in the cytoplasm.	The cytoplasm contains vesicles, granules, or ingested material, e.g., pieces of RBC. Vesicles may mask the nucleus.	Round cells with a diameter of 13 to 100 μm . "Oval fat bodies" are formed when the macrophages ingest a large number of lipid droplets [4]. "Oval fat bodies" may also originate from lipid absorption by renal tubular epithelial cells.

WBC / Lymphocytes

The nucleus is smooth, round, mononuclear (without lobuli), and occupies most of the cytoplasm.

Cytoplasm is scarce and without granules [5].

Differentiation of lymphocytes from granulocytes and small epithelial cells may be improved by staining procedures (**Table II-2**). Degeneration of nucleated cells may make the differentiation difficult or impossible.

Activated lymphocytes have an increased cytoplasmic volume. Rarely, the major leukocyte population in urine.

Table II-1B. Epithelial cells in urine

Nucleus	Cytoplasm	Other features
<i>Squamous epithelial cells, SEC</i>		
Central nucleus	Their shape is polygonal and the average diameter is about 55 µm. Nucleus-to-cytoplasm ratio is relatively small.	May detach in clumps of cells, creating confusion with casts because of the total size or folded shape of the particle. Free nuclei in urine are possible.
<i>Transitional epithelial (Urothelial) cells, TEC</i>		
Transitional epithelium forms the multi-cellular surface of the urinary tract from the renal pelvis to the bladder in the female, and to the proximal urethra in the male.		
<i>TEC / Superficial urothelial cells</i>		
Single nucleus, nucleoli are not always seen	Usually round to oval with a mean diameter of about 30 µm. They have pale halo around the nucleus.	Occasionally, cells may have two or more nuclei. The uppermost multinucleated cells are called facet or umbrella cells, as they maintain the surface integrity of uroepithelium [6].
<i>TEC / Deep urothelial cells</i>		
Central or peripheral nucleus, with 1-2 nucleoli	Smaller than superficial cells (mean diameter about 17 µm). They exhibit club-like, polygonal or spindle-like shapes, and a thin granular cytoplasm.	Atypical shapes of urothelial cells may be caused by infection or urothelial cancer. Low-grade urothelial cancers are not detected by cytology. High-grade cancer cells typically exhibit aberrant nuclei and exceptional nuclei. Atypical cells may appear in clumps caused by catheters, stones, or tumours. Atypical nuclei and nucleoli are possible. Systematic detection of atypical cells is a responsibility of cytopathology laboratories.

Renal tubular epithelial cells, RTC

RTC derive from the single-layered columnar epithelium of proximal or distal tubuli in the kidneys, showing different morphological features when intact. May appear in fragments in tubular necrosis. Tubular damage creates apoptosis and degeneration of RTC, making their identification sometimes impossible without immunochemical staining (as performed in research laboratories).

RTC, proximal tubular cells

Round to ovoid nuclei with 1-2 nucleoli if intact	The average diameter is about 14 µm (range 9-25 µm, up to 50 µm). They are larger than granulocytes. Their cytoplasm is most often granular.	Proximal RTC originate from proximal tubules of kidneys. They occasionally detach in clumps resembling honeycombs.
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RTC, distal tubular cells

Central or basal nuclei	Rectangular, polygonal or even columnar cells with a granular cytoplasm.	Distal RTC originate either from distal tubules or collecting ducts. Rarely, they detach in clumps that resemble casts, but without a typical matrix.
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Table II-1C. Casts

Type of cast	Key features
Hyaline cast	Composed of a matrix with low refractive index. They are best identified by phase-contrast microscopy.
Granular cast	Contain either fine or coarse granules, accentuated by phase-contrast optics.
Waxy cast	Usually large, with clear-cut edges or indented borders, and refractile. Waxy casts have a homogeneous appearance, resembling wax.
Fatty cast	Contain translucent or birefringent lipid particles.
Cellular casts	Classified according to the cells contained in: erythrocyte, leukocyte, and renal tubular epithelial cell casts
Hemoglobin cast, and Myoglobin cast	Both brownish in colour with a granular surface. They cannot be differentiated from each other by morphology.
Bilirubin cast	Yellow-brown due to water-soluble (conjugated) bilirubin excreted into urine.
Bacterial cast, and Yeast cast	Contain bacteria or yeast. They are seen in patients with bacterial or fungal infection affecting the kidneys.
Artefacts	Artefacts may resemble casts (then called “pseudocasts”). These may be pieces of toilet tissue with indented borders, pieces of hair, aggregated crystals, various synthetic fibres, or artefactual lining of any urine particles when preparing the specimen for microscopy.

Table II-1D. Crystals in urine

Type of crystal	Key features
Uric acid	Rhomboids, barrels, needles, rosettes or other variable shapes, with a typical amber colour and birefringence under polarized light. They precipitate in acidic urine only (pH < 5.8).
Calcium oxalate dihydrate	Typically bipyramidal. They can appear also in aggregates. Only large crystals show birefringency.
Calcium oxalate monohydrate	Ovoid, dumb-bell or biconcave discs, always brightly birefringent. They may be confused with RBC especially by automated instruments if appearing ovoid and close in size to RBC. The hard, broken structures of crystals as compared to RBC often distinguish the two.
Calcium phosphate	Prisms, needles or rosettes that polarize light. When occurring in plates, calcium phosphate is not birefringent.
Triple phosphate (Magnesium ammonium phosphate)	Transparent birefringent prisms, usually with a "coffin lid" appearance.
Amorphous urates and phosphates	Granular particles, often in clumps. Urates are found in acid urine, phosphates in alkaline urine. Urates polarise light, while phosphates do not.
Cystine	Thin, hexagonal, non-polychromatic birefringent plates with irregular sides. May appear isolated, heaped upon one another, or in clumps and rosettes. Their precipitation is increased at low pH (< 6) and after an overnight incubation at +4°C.
2,8-Dihydroxyadenine (DHA)	Resemble urate, like other xanthine crystals. Birefringent like urate crystals.
Xanthine	Easily confused with urate [7].
Leucine	Forms oily-looking spheres with concentric striations like annual rings of trees.
Tyrosine	Thin needles, often aggregated in bundles or rosettes.
Cholesterol	Transparent thin plates with sharp edges and corners.

Table II-1E. Microbes in urine

Type of microbe	Key features
Bacteria	Rods or cocci. Seen on visual bright-field microscopy, but particularly visible with phase-contrast microscopy. Rods are typically identifiable, but cocci may be confused with amorphous precipitates if they are not motile.
Fungi	Cells of <i>Candida</i> spp. appear as ovoid or roundish elements not absorbing stain. They also appear as hyphae. Budding is the most typical feature.
Protozoa	<i>Trichomonas vaginalis</i> is easily identified due to the motility of the flagella and the rapid and irregular movements of the body, when alive. These become difficult to distinguish from leukocytes when dead.
Helminths	The eggs of <i>Schistosoma haematobium</i> measure about 140 x 50 µm. They are spindle-shaped with a round anterior and a conical posterior end tapering into a delicate terminal spine. They may be seen to hatch if the urine is dilute enough. The eggs of <i>Enterobius vermicularis</i> measure about 25 to 50 µm if found as contaminant or parasite in bladder.

Table II-2. Differentiation of nucleated cells in urine with Sternheimer staining

Staining of urine particles supravitaly, i.e., directly without fixation may help in identification of various nucleated cells. Simple stains such as toluidine blue may be used. The table below describes details of Sternheimer staining, showing nuclei as blue and cytoplasm as red in most cells [8]. It has been in clinical use in European countries participating in Labquality's external quality assessment scheme for urine particle identification since 1990's [9].

Cell type	Nucleus	Cytoplasm
Granulocyte	Bright blue if takes the stain (often unstained). Multilobular or rod-shaped.	Reddish or pink if stains. Granular and round cytoplasm. Degenerates and breaks easily.
Macrophage	Bluish, dark chromatin, broken in degenerated cells	Bluish or pink. Granular, containing vacuoles, RBC pieces (red) or lipid droplets. "Thin" fragile structure that breaks easily.
Lymphocyte	(Dark) blue nucleus fills the cell almost entirely. Chromatin usually not seen.	Bluish, smooth, and thin rim around the nucleus. Easily broken cell membrane.
Squamous epithelial cell (SEC)	Stains blue or remains unstained. Often degenerated and small. Central.	Stains pink if taking the colour at all. Polygonal shape resembling "fried eggs". Pale, large, slightly granular.
Transitional epithelial cell (TEC), superficial	Stains blue if taking the stain. Round, finely granular chromatin. Usually, visible 1-2 nucleoli.	Finely granular, staining pink. Degenerated forms may not stain at all. Large, round with clear perinuclear halo.
Transitional epithelial cell (TEC), deep	Stains blue usually. Well defined borders with evident 1-2 nucleoli. Variable location in the cell.	Many marked granules, often stains darker red and are smaller than those of superficial TEC. Remain unstained if degenerated.
Renal tubular epithelial cell (RTC), proximal and distal	Stains blue or purple. Homogenous, occasionally visible 1-2 nucleoli, chromatin usually clear. Often degenerated.	Granular, often dark red cytoplasm that appears "thick" with clear cytoplasmic borders. Ingested granules or vacuoles filled with lipids ("oval fat bodies"). Degenerated forms common.

The intensity of staining is dependent on the length of exposure to the stain as well as unknown factors related to the specimen. With the Sternheimer stain the nuclei are usually blue and cytoplasm red. The tint (hue) varies due to both by specimen and batch-related factors.

References, Annex II

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