European Urinalysis Guidelines

SUMMARY

These **European Urinalysis Guidelines** are given under the auspices of the European Confederation of Laboratory Medicine (ECLM).

Medical needs for urinalysis

Urinalysis should always be performed on the basis of medical need, and appropriate examinations for various clinical populations and presentations should be determined by cost/benefit analysis. On this basis, selection indications for urinalysis are suggested for detecting diseases of the kidneys or urinary tract (Page 6).

The referral for detailed urine examinations should include an adequate description of the specimen type, and should inform the laboratory of the clinical need in order to facilitate correct selection of examination procedures and interpretation of results (Page 6). This medical information is mandatory when the choice is being made between minimum and optimum procedures for different specimens. The minimum information needed is outlined (Page 48). Requirements for computerized information systems are discussed (Page 49).

Patient preparation

Preparation for a high-quality urine specimen should start on the previous evening and should be as uniform as possible to allow standard interpretation of results (Page 7). Details of patient preparation before specimen collection is given, ending with qualified specimens whenever possible (Page 8). First and second morning urine specimens are defined (Page 7).

Collection of specimens, preservation and transport

Recommended methods of urine collection are listed (Page 9), including detailed illustrations for the collection of mid-stream specimens (Page 50, 91 – 96). Specifications for collection and transport containers are given (Page 10), and preservation procedures for chemical measurements, particle analysis and microbiological examinations are listed (Page 51).

Classification of examiniations

Examinations have been re-classified into four hierarchical levels based on accuracy of measurements (chemistry on Page 12, particle analysis on Page 23, microbiology on Page 31). In addition, the previous literature on the visual appearance and odour of urine is presented (Page 13).

Chemical methods of examination

Principles and performance criteria of multiple test strips are reviewed (Page 13). A nitrite test should not be used alone in detecting urinary tract infections because of its low sensitivity. Qualified procedures for measurement are recommended (Page 56). Pregnancy examinations are reviewed briefly (Page 18).

Quantitative chemical measurements are discussed in detail, mainly with respect to protein measurements (Page 18). Measurements assessing volume rate (diuresis) are also summarized (Page 19). Reference intervals, existing reference materials (calibrators) and measurement procedures are quoted (Page 57).

Automation can be applied in centralized laboratories after appropriate evaluation of analytical equipment (and pre-analytical procedures). Local diagnostic requirements guide the manner of implementation of point-of-care methods, as well as manual or automated procedures in different laboratories.

Particle analysis

Clinically significant particles in urine are reviewed and classification is divided into basic and advanced levels (Page 20), one of which should be selected by each laboratory (or by its subunit, such as emergency services versus regular-hour working personnel).

For routine particle identification, a standardized procedure with phase-contrast microscopy or supravitally stained urine sediment is recommended (Pages 23, 62). Morphological criteria of particles are given as investigated under a cover-slip with a known volume of urine (Page 64). Urine cytology for investigation of cancer cells is

considered to require special expertise. For microbiology, different procedures apply because bacterial infection usually needs to be detected in ordinal scale. That is why the slide-centrifugation technique and Gram staining are recommended as the comparison method (Page 23).

Instruments supplement clinical particle analysis by reducing manual work (Page 25). Evaluations against an appropriate comparison method are recommended.

Microbiology methods

It is not necessary to request microbiological examination of urine in all clinical situations. Low-risk symptomatic patients consist of adult females with recurrent dysuria/urgency symptoms without fever, and without known diseases predisposing to urinary tract infection (Page 26). Urine cultures from these cystitis patients are discouraged for routine purposes – they are necessary, however, for epidemiological purposes. Rapid methods for detecting bacteriuria can be used in acute treatment decisions (Page 26) after appreciating the possibility of false-negative findings. All other symptomatic patients belong in the high-risk group, whose specimens should be sent for bacterial culture, including identification of species and antimicrobial susceptibility testing.

We provide a new classification of bacteria based on the uropathogenicity and frequency of bacteria in the aetiology of urinary tract infection (Page 26, Table IX). Limits of significant bacteriuria are defined more precisely than previously depending on the type of specimen, clinical presentation and organism (Page 30, Table XIII). For selected patient groups, large 10 µL or 100 µL inocula are needed to reach the required sensitivity. A new standardized unit for reporting quantitative cultures is recommended based on the developing particle identification techniques, adapting international standardization of nomenclature and volume: colony forming bacteria/litre (**CFB/L**) (Page 28).

Details of different culture procedures are given (Page 68). Procedures for species identification are quoted for routine microbiology laboratories (Page 72). Antimicrobial susceptibility testing is reviewed, with acceptance of direct susceptibility testing for rapid test positive cases (clear positives) in experienced hands (Page 32).

Automation of bacterial cultures may be considered in large microbiological laboratories based on evaluation of instrument performance against an appropriate comparison method.

Stepwise strategies in urinalysis

Cost-benefit analyses should guide screening policies. Multiple examinations recommended for sieving of general patient populations should include measurements of leukocytes, bacteria, erythrocytes, albumin (protein), and of quantity for volume rate (= diuresis; such as creatinine or other) (Page 34). In acute cases, measurements of glucose, ketone bodies and pH can be of additional value. A flow-chart is given for additional investigations in the case of positive findings (Page 36). It includes questions of clinical need.

Use of a flow-chart is suggested to reduce the number of less important urine cultures (Page 38). After clinical evaluation, a highly specific laboratory examination should be undertaken as a first step. Remaining negative specimens shall then be examined with a sensitive procedure to exclude true negative cases. The development of new examinations with high sensitivity (and specificity) for rapid bacterial detection is encouraged to reduce the number of routine cultures (Page 34). Asymptomatic individuals should not be screened for bacteriuria, with the exception of pregnant women and some other specified patient groups (Page 38). Increased local co-operation with clinical units and detailed information from individual specimens is needed to first define and then select the minimum operating procedures or the enhanced procedures for each specimen in routine work-flow.

Increased detection sensitivity for early renal damage is recommended because of the prognostic significance in diabetes mellitus, probably also in hypertension, and in monitoring patients receiving nephrotoxic drugs (Page 39). New patient groups should be incorporated into strategies using these sensitive measurements when prevention of long-term deterioration of renal function is anticipated.

Quality assurance, including analytical quality specifications

Procedures for quality assurance and new analytical quality specifications are suggested

for rapid (ordinal scale) examinations (Page 42), particle analysis (Page 44) and microbiological examinations (Page 45). For ordinal-scale examinations they are based on detection and confirmation limits, as compared with a quantitative reference procedure (Page 53), or calculation of agreement based on κ statistics (Page 55). Analytical quality specifications for protein and other quantitative chemical measurements are summarized based on earlier literature (Page 44). Implementation of elements of a structured quality system (good laboratory practice (GLP) with standard operating procedures (SOP) as documented in a quality manual) is encouraged even in small laboratories, based on national co-operation (Page 40).

These guidelines should impact on the validation of local working practices. In addition, the principles of quality embodied in these guidelines may assist independent authorities charged with accrediting laboratories.

1. INTRODUCTION

1.1. Foreword

An effective diagnostic strategy from urine should be based on standard procedures for collection, transport and analysis. These standardized procedures are required to produce consistent reference intervals and decision limits for the harmonized interpretation of results. Europe has no consensus standard procedures. Standardization is essential not only for interpretation of results in individual patients, but also for epidemiological studies, for determining which populations should be screened for urinary abnormalities, and for the procedure to be followed when an abnormal result is found [1]. In addition, laboratories want to accredit their urine diagnostics by comparing their methods with acceptable references. These guidelines therefore intend to fill this gap by summarizing available knowledge into one consensus practice for urinalysis (or urine analysis) in Europe.

Selected list of analytes: The terms "urinalysis" and "urine analysis" are synonymous. The combination of analytical procedures used in practice is changing and varies in different clinical situations. To form a basis, this document is limited to the *most commonly requested examinations.* Some analytes are discussed in terms of specimen collection only, since the precise medical needs and actual analytical methods concerned would have made this document too large for everyday use. The present recommendations have made use of some of the existing national guidelines [2 – 7] as appropriate. Certain recent analytical techniques, such as flow cytometry and nucleic acid amplification, have widened the perspectives of information obtainable from urine. The latest technology is referred to but not described in detail.

Target audiences: These 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. The text is divided into a general section and an appendix containing detailed methodological descriptions, to make it accessible to clinicians and others not directly concerned with such detail. Wherever the location, the performance of examinations must be determined by clinical requirements and this means close co-operation between the traditional laboratory disciplines and clinicians. The large number of specimens and the need for emergency reports increase the pressure for rapid and reliable measurements. At the same time, opportunities for cost-containment must be identified. These guidelines will help establish qualified procedures for laboratory examinations in these environments.

Process: This document is based on the work of the European Urinalysis Group (EUG), established under the auspices of the European Confederation of Laboratory Medicine (ECLM) in 1997 [8, 9]. The EUG has collaborated with the Working Party on Urinalysis of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) in the preparation of the guidelines. The "European Urinalysis Guidelines" are based on both medical needs and available methods and technologies. In collaboration with many experts from most European countries (see the list below) the present paper attempts to create consensus practice for urinalysis. The EUG wishes to express its gratitude to the various sponsors listed in

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1.3. Abbreviations

AACC American Association for Clinical Chemistry
AMA Antimicrobial activity
ASM American Society for Microbiology
BIPM International Bureau of Weights and Measures
BSAC British Society for Antimicrobials and Chemotherapy
CAMP Christie-Atkins-Munch-Petersen factor
CAP College of American Pathologists
CDC Centers for Disease Control (U.S.A.)
CEN European Committee for Standardization
CFB Colony-forming bacteria (instead of the traditional colony-forming unit, CFU)
CLED Cystine-lactose electrolyte deficient (agar)
CNS Coagulase-negative staphylococci
CRM Certified Reference Material
CV Coefficient of variation (relative standard deviation)
EC Enzyme Commission (number of classification)
ECCLS European Committee for Clinical Laboratory Standards
ECLM European Confederation of Laboratory Medicine
EN European Standard
EQA External Quality Assessment
EQAS External quality assessment scheme (or survey)
ESCMID European Society of Clinical Microbiology and Infectious Diseases
EUG European Urinalysis Group
FDA Food and Drug Administration (U.S.A.)
FESCC Federation of European Societies of Clinical Chemistry
FN False negative
FP False positive
GBS Group B streptococci
GFR Glomerular filtration rate
GLP Good Laboratory Practice
hCG Human chorionic gonadotropin
HPF High-power field (in a microscope)
IEC International Electrotechnical Commission
IFCC International Federation of Clinical Chemistry
IQC Internal quality control
ISO International Organization for Standardization
IUPAC International Union of Pure and Applied Chemistry
IUPAP International Union of Pure and Applied Physics
IVD *In Vitro* Device
JCCLS Japanese Committee for Clinical Laboratory Standards
kDa Kilodaltons
KIA Kligler Iron Agar

L_C Confirmation limit
L_D Detection limit
NCCLS National Committee for Clinical Laboratory Standards
NIST National Institute of Standards and Technology
NRSCL National Reference System for Clinical Laboratories
OIF oil immersion field
OIML International Organization of Legal Metrology
RBC red blood cells
RBP retinol-binding protein
RCF relative centrifugal force
ROC receiver operating characteristic (curve)
RPM rotations per minute
SOP Standard Operating Procedure
SRM Standard Reference Material
SRGA-M Swedish Society of Medicine's Reference Group for Antibiotics
TQM Total quality management
TSIA Triple Sugar Iron Agar
URL Upper reference limit (of a specified reference interval)
VIM International vocabulary of metrological terms
VP Voges-Proskauer test
WBC white blood cells

2. MEDICAL NEEDS FOR URINALYSIS

After a long history of clinical urinalysis there is a need to update the medical relevance of different investigations of urine. Cost/benefit analyses should guide the implementation of examinations for various populations. The epidemiology of target diseases should be considered: for example, screening for incipient nephropathy by detecting albuminuria in patients with diabetes mellitus is recommended world-wide [10, 11]; on the other hand, that for schistosomiasis in children should be restricted to those countries where this parasite is endemic [12]. General indications for urinalysis are given in Table I.

Clinical presentations in these individuals can 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 turnaround time of less than 0.5–2 h, rather than a confirmatory examination the result of which comes too late for decision-making. The repertoire of local laboratory or point-of-care environments will also influence the selection of laboratory examinations that can be performed. Some of these considerations are discussed in Section 8 (Stepwise strategies in urinalysis).

Request and report information. The format of the request for and the report of urinalysis is influenced by the site of examination: at point-of-care, specimen collection and analysis results can be documented directly into the patient record, whereas a remote laboratory always needs a paper or computerized request. The request reaching the laboratory may initiate a stepwise procedure agreed locally for a particular patient group. Such pre-determined strategies maximize diagnostic yield while maintaining cost-efficiency.

The importance of adequate clinical and specimen-related information for correct selec-

TABLE I. Medical indications for urinalysis.[a]

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(1) Suspicion or follow-up of symptoms or situations suggesting the possibility of urinary tract infection
(2) Suspicion or follow-up of non-infectious renal disease, either primary or secondary to systemic diseases, such as rheumatic diseases, hypertension, toxaemia of pregnancy, or to the adverse effects of drugs
(3) Suspicion or follow-up of non-infectious post-renal disease
(4) Detection of glycosuria from specified patient groups, e.g., individuals admitted to hospital for various medical emergencies, or from pregnant women
(5) Follow-up of only selected diabetes mellitus patients, e.g., children at home, to detect morning glycosuria and ketonuria in addition to blood glucose measurements
(6) Detection or follow-up of selected metabolic states, e.g., vomiting and diarrhoea, acidosis/alkalosis, ketosis, or recurrent urinary stone formation

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[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 these guidelines which focus mainly on diseases of kidneys and urinary tract.

tion of examination procedures and interpretation is generally underestimated. Sufficient detail is seldom documented for urinalysis specimens. The minimum information is proposed in the Appendix (Annex 10.1).

The analysis report may be a structured note entered directly into the patient's record when examination is performed at point-of-care. The remote laboratories must use a standardized report format with defined units, reference intervals and report style (Appendix, Annex 10.1.3). The purpose of a report is to guide the clinician towards rational and evidence-based diagnostics and therapeutic management. It must be technically correct and must provide unambiguous information.

3. PATIENT PREPARATION

3.1. Definitions of urine specimens based on timing

The following timed types of urine specimens are described by modifying the definitions quoted in textbooks [13–15] and national guidelines [2–7]. The time of specimen collection must be recorded both on the examination request and on the subsequent report to aid in the correct interpretation of findings.

Random urine is a portion of single voided urine without defining the volume, time of day or detail of patient preparation. This is usually the unavoidable case in acute situations. Random urine specimens are associated with many false-negative and some false-positive results.

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. It is recommended that the early morning urine be voided after an 8-h period of recumbency, and after not less than 4 h storage time in the urinary bladder (even if the bladder was emptied earlier during the night). This has been traditionally recommended as the standard specimen for urinalysis, because it is more concentrated than day urine and allows time for possible bacterial growth in the urinary bladder. This specimen is most easily collected from hospitalized patients, but may be collected even at the patient's home if

compliance and rapid transportation to the laboratory can be organized.

Second morning urine is a single specimen voided 2–4 h 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. However, it may be more practical for ambulatory patients. To increase the sensitivities of bacterial culture and particle counting, the quality of the second morning urine should be improved by allowing ingestion of only 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 4 h is possible with this fluid restriction. Postural proteinuria cannot, however, be prevented and should be further investigated by examining a first morning urine sample if diagnostic problems occur. If these standardized collection instructions have not been followed, the second morning urine is classed as a "random" specimen.

Timed collection of urine is collected at a specified time in relation to another activity, e.g., therapy, meals, daytime or bed-rest. A *24-h urine collection* contains all portions voided over 24 h. A timed 24-h collection can be started at any time of the day by emptying the bladder and noting the time. All urine during the next 24 h is then collected and preserved as appropriate for each analyte (see Appendix, Annex 10.3.4).

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

3.2. Patient preparation before specimen collection

The patient should be told why a urine specimen requires to be examined and given instructions on how it should be collected (see Section 4.1). 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 Appendix, Annex 10.3.1). Because the same

specimen is often used for both microbiological and chemical measurements, the instructions should combine both requirements.

Transmission of pre-analytical information.
Adequacy of patient preparation and type and success of specimen collection can be coded on the label on the sample tube. When available, this information should ultimately be transmitted to the patient records together with the results of examinations, e.g., "qualified"/"standard", or "random"/"non-standard" specimen, to increase the reliability of interpretation. Thus, significant deviation from "standard" should be recorded on the request form or label added to the container as "non-standard".

3.3. Influence and interference factors

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. These are called *influence factors* (discussed in detail below).

In addition, there are other factors that technically interfere with the analytical method applied (called *interference factors*, 16). The latter are particularly important with non-specific analytical methods, e.g., those used in traditional test strip fields (see Annex 11.1), but also in other chemical measurements from urine (17). As a classical example from microbiology, the specimen for bacterial culture should be obtained before antimicrobial treatment is initiated to allow bacterial growth.

3.3.1. Volume rate (diuresis) and fasting.
Many urine constituents change in concentration when the urine volume rate (excretion rate of water = diuresis) alters due to variation in fluid intake, reduction of renal concentrating ability or ingestion of diuretic substances. If sensitive screening is needed, a low volume rate (20–50 mL/h) is desirable to produce highly concentrated specimens. This is best achieved in morning urine. Documentation of the urine concentration is recommended, e.g., density (specific gravity or weight), osmolality, or a related analyte, which allow calculations of analyte/reference ratios (see Section 5.4.2).

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 (16). In detecting glycosuria, a carbohydrate meal before the specimen collection improves the clinical sensitivity (postprandial urine). In general, fasting before urinalysis is designed to reduce diuresis. The preparation of patients for standardized fasting blood specimens can be combined with that for urinalysis.

3.3.2. 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 minimizes these influences. Urinary calcium excretion increases more than twofold on immobilization of a patient by bed-rest (18). Exercise may increase the amount of body constituents excreted by increasing glomerular filtration as a result of increased blood pressure (e.g., appearance or increase in albuminuria or haematuria).

3.3.3. Incubation time in the bladder.
To demonstrate reliable bacterial growth, classical microbiological advice has been to allow bacteria a log phase of growth by incubating the urine in the bladder for 4–8 h [19]. Urine is a good culture medium for many bacteria. Using the classical Griess's examination (applied in the nitrite examination pad on test strips), it has been shown that the first morning urine specimen is more sensitive in detecting asymptomatic bacteriuria among pregnant women than later specimens [20]. In most recent guidelines for the evaluation of new anti-infective drugs, no incubation times are mentioned [21]. The urgency of micturition associated with acute infection will often not permit sufficient bladder incubation time before voiding, resulting in false-negative cultures. For chemical analyses, no particular incubation time is necessary. In studying cell and cast morphology, the best results are obtained with a short incubation time of 1–2 h, provided that a high volume rate does not lead to false-negative results (rare particles are seen more often in concentrated urine specimens). For patients without urgency or acute symptoms, advice concerning bladder

incubation time, and a record of that time, is still recommended to reach the highest sensitivities [22].

3.3.4. Contamination. Urine specimens should be collected free of internal and external contaminating fluids. Sexual intercourse should be avoided for 1 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. Excretion of N-acetyl-β,D-glucosaminidase, a marker of renal tubular function, is increased for 3 days after ejaculation [23]. Seminal fluid may contaminate urine after normal ejaculation, but also in diseases associated with retrograde ejaculation to urinary bladder.

Vaginal secretions or menstrual blood may contaminate urine from females. This may be misleading and can be minimized by tamponing the vagina when acute symptoms necessitate examination of urine. Pregnancy is associated with physiological pyuria.

4. COLLECTION OF SPECIMENS, PRESERVATION AND TRANSPORT

Urinalysis may be requested on specimens obtained by voiding (micturition), by catheterization, needle puncture, through a postoperative urostomy, or by using different collection vessels, such as bags or special receptacles for bed-bound patients. The most commonly obtained specimen is the mid-stream urine. Requesting clinical staff must document the method of collection to allow correct interpretation of results.

4.1. Procedures for urine collection, specimen types

The microbiological requirements usually determine the details of any collection method, because urine intended for microbiological examination must avoid or minimize: (1) contamination of specimens by commensal bacteria, (2) growth of bacteria following specimen collection, (3) damage or death of diagnostically relevant bacteria, and (4) disin-

tegration of diagnostically valuable formed elements.

Mid-stream urine (or clean-catch urine) characterizes the middle portion of a voided specimen. The first portion of urine is not collected, because it is always contaminated by commensal urethral flora in both sexes (if not purposely collected, see below). Minimizing contamination of specimens by commensal bacteria is especially important, and requires detailed patient advice. It is important to wash the introitus around the urethra in females, and the glans penis in males with water only, before micturition. This reduces false-positive urine cultures by 20% or more [24]. The use of antiseptics – and soaps (with variable additives) – is not recommended as this may affect bacterial viability [25]. The last portion is also left over after collecting 50 – 100 mL of urine in the container. Detailed instructions for mid-stream urine collection are included in the Appendix (Annex 10.3.1). Technically satisfactory collection of urine from children and from women especially in the late stages of pregnancy can be difficult. The use of different sterile devices may help women to urinate more easily into a collection container.

First-void urine characterizes the first portion of urine voided at the beginning of micturition. This is the optimal sample for the detection of *Chlamydia trachomatis* by nucleic acid amplification. It is NOT suitable for routine microbiological culture as urethral organisms usually contaminate it.

Single catheter urine is collected after inserting a sterile catheter into the bladder through the urethra (straight or ''in-and-out'' catheterization). For children without urinary control, this is one of the methods of confirming or excluding the presence of urinary tract infection [26].

Indwelling catheter urine is collected at replacement or by sterile puncture of an indwelling catheter. Urine analysis specimens must not be taken from the collection bag of a permanent indwelling catheter.

Suprapubic aspiration 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 urinary tract infection [27]. Detailed instruc-

tions are provided in the Appendix (Annex 10.3.3). The risk of bladder colonization by suprapubic aspiration is lower than that by in-and-out catheterization. Miniaturization of measurement techniques by laboratories may nowadays allow several different examinations from the 5 mL of urine obtained typically by the suprapubic aspiration technique.

Bag urine is widely collected from small infants, but carries a 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. The collection bag should be *in situ* for a maximum of 1 h, after which the probability of contamination increases greatly. Negative culture results reliably exclude urinary tract infection [26]. Borderline results need to be re-investigated from a suprapubic aspiration or single catheterized urine specimen.

Diapers or nappies are sometimes suggested as collecting tools for clinical specimens from babies [28]. Despite occasional promising results, especially for chemical constituents measured by qualitative strip examinations, it is hard to recommend this method due to high variability in the fibre construction of different brands and inability to measure the urine volumes voided. Morphological and in particular microbiological investigations are impossible with this procedure.

Urostomy specimens (urines from *ileal conduit*) are frequently obtained after bladder surgery. Paediatric and adult patients with dilated ureters may be given bilateral ureterostomies. Chronic infection and bleeding at the site of the stoma are common. Cleaning the stoma and discarding the first portion of urine obtained through a sterile catheter of suitable size ensures specimen quality.

Specimens to localize the site of urinary tract infection. The collection of specific segments of urine flow may help in defining abnormal areas of the urinary tract that may need urological attention. Then, a dialogue between the requesting clinician and the laboratory is essential *before* the procedure in order to guarantee the following:

– origin of the specimen (left/right ureter, bladder, etc.)

– identification of any bacteria to low levels ($> 10^4$ CFB/L)
– large inocula of urine to ensure accurate plate counts (10 – 100 μL)
– report simultaneously relative concentrations at each anatomical site
– antibiotic sensitivities of any bacteria grown

The lower urinary tract may be the subject of investigation, typically the prostate. For diagnosis of chronic bacterial prostatitis, the Meares and Stamey collection procedure is still recommended: first and mid-stream portions of urine should be collected, then drops expressed with prostate massage, and the final specimen voided after prostatic massage [29, 30]; (see Appendix, Annex 10.3.2).

4.2. Containers

Sterile sampling of urine is important for urine microbiology, but may influence some chemical measurements. Sterility of collection vessels means that the interior of the unopened and unused container is free from interfering microbial contaminants. The European Pharmacopoeia defines sterility as reduction of bacterial growth to a probability of one survivor in one million, e.g. after irradiation [31]. Container manufacturers must document their product's compliance with the intended clinical use (see Section 4.2.1), rather than following strictly the Pharmacopoeia definition only. It is to be remembered that a particle analyser or a nuclear amplification method will detect even dead bacteria. Furthermore, since waste is an increasingly important problem globally, the development of new, environmentally safe materials is encouraged for all disposable containers. Finally, containers should comply with the European directive for *in vitro* medical devices [32].

4.2.1. Collection containers.

Single-voided specimens: The design of collection containers should enable detection of uropathogenic bacteria even in special situations, i.e. at as low as 10^4 CFB/L level (equivalent to 10^1 CFU/mL) [5, 33]. 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. The container and its cap should be free from interfering substances and should not absorb or change the urine constituents to be measured. Those parts of the container and its cap which come into contact with the urine 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-h or overnight urine collection should have a capacity of 2–3 L. The container should be constructed from materials that prevent (a) adherence of urine constituents, (b) exposure of urine to direct light, which might alter clinically significant metabolites, (c) 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 (Appendix, Annex 10.3.4).

Secondary containers (for basic urinalysis, usually examination tubes) should be easily filled from the primary container without risk of spillage. The tube should be translucent to allow a clear view of the sample.

4.2.2. Transport, storage and analytical containers.

Specimens can be transported in their primary containers or divided into aliquots that may range from 1 to 100 mL for chemical and morphological investigations. For microbiological analysis, 1–3 mL of urine in a *clean* container is sufficient. For large laboratories, a standardized vessel with a volume of 3–12 mL is essential for automated analytical systems.

Examination tubes for test strip measurement, particle counting or urine culture should keep the specimen suitable for analysis at $+20°C$ or at $+4°C$ as specified.

Traditionally, urinalysis tubes have been conical to allow decanting of supernatant after centrifugation. A more accurate sediment volume is obtained by suction of the supernatant. Therefore as an alternative to the conical shape, a round-bottom tube may be considered for easy resuspension of the sediment when using standardized equipment.

The examination tubes used for samples 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). The size, structure and length of the secondary container vary depending on the needs of the diagnostic procedures.

4.2.3. Labelling.

All specimen containers must be labelled with a tag that remains adherent during refrigeration. The label should include a bar code (if used), a code of the examination requested, patient identification and requesting unit, as well as details of collection time, method of collection, and any additional pre-analytical information in coded form. Details of any preservative should be shown on a separate label and include 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.

When body fluids are mailed to a distant laboratory, additional biohazard labels should be added and the packages must comply with the European standard EN829 (34).

4.3. 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.

For many **chemical constituents examined with test strips** no preservatives are needed, provided the analysis is performed within 24 h 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 may be critical, since some preservatives interfere with enzymatic measurements (see Appendix, Annex 11.1).

For **quantitative chemical measurements** it is known that several specific proteins are unstable

in urine but preservatives can inhibit their degradation [35 – 37]. The effect of storage may be method-dependent, since an earlier radioimmunoassay for albumin did not find any effect on storing specimens at $-20°C$ for up to 6 months [38]. In the present guidelines, previous data are quoted [3, 39] as modified where necessary by technological development (Appendix, Annex 10.3.4).

For **particle examination** the specimen should be refrigerated if not examined within 1 h, although precipitation of urates and phosphates will occur in some specimens. If precipitation disturbs interpretation, a new specimen should be examined at $+20°C$ to avoid their artefactual generation. The longer the delay, the more likely are elements to lyse, especially when the urinary pH is alkaline and the relative density is low (especially true with children, often producing a large diuresis). The WBC counts may be questionable after 2 – 4 h, even with refrigeration [40]. Traditionally, ethanol (50% volume fraction) is used to preserve the cells but this only partially prevents lysis of red and white blood cells. To avoid shrinkage, polyethylene glycol (2% mass fraction, low molecular mass such as Carbowax®) may be included in the fixative (this is called Saccomanno's fixative [41]). On mixing equal parts of sample and fixative, the particles should then be stable for 2 weeks. Alternative fixatives also exist [42]. Commercial preservatives, such as formaldehyde-based solutions, buffered boric acid and formate-based solutions, and mercuric chloride-based tablets are also available. They have gained renewed interest following the development of automated systems. Fixatives may be adapted after evaluation with the new technology.

Specimens requiring **microbiological investigation** must be collected in a clean container and examined in the laboratory within 2 h [5, 6]. They should be refrigerated at 4°C without preservative if delay >2 h is expected. Then they should be examined within 24 h. If delay is unavoidable and refrigeration not possible, containers pre-filled with boric acid preservative alone [43] or in combination with formate or other stabilizing media [44 – 46], ideally in a lyophilized form, may be used. Boric acid will stabilize white cell number and bacterial concentration in urine held at $+20°C$ for 24 h. Boric acid concentration may be critical

for successful preservation without bacterial inhibition. It is suggested that containers containing boric acid should be filled to the indicated line to achieve a correct borate concentration. The specimen should be examined within 24 – 48 h of production. It should be noted that borate may inhibit growth of *Pseudomonas* spp.

5. CHEMISTRY EXAMINATIONS

5.1. Hierarchy of methods (measurement procedures)

In these guidelines, laboratory methods are classified in four levels of performance based on accuracy of measurement. This hierarchy was adapted from earlier definitions of method performance in clinical chemistry [47] to help discussions aimed at better accuracy in urinalysis.

Level 1: Rapid methods that ideally provide the user with a fast, reliable response to an individual patient and easy handling of equipment, usually in primary care laboratories and at points-of-care. These typically include, for example, urine test strip measurement, simple urine microscopy or dip-slide culture. Results may be expressed on an ordinal scale.

Level 2: Routine or field methods used in clinical laboratories because of medical need for quantitative or specialized methods, which then require experienced personnel and often a centralized site. These are often mechanized or automated methods. Examples include for example routine identification of several uro-pathogenic bacterial species, immunochemical quantitation of numerous urinary proteins, or advanced urinary particle identification.

Level 3: An intermediate category of "qualified comparison methods" is proposed. The techniques are analytically more accurate and precise than Level 2 methods, but are not applicable for routine use because they may be too time-consuming or expensive. Level 3 needs to be considered and created when no Level 4 methods exist and methods at Levels 1 or 2 need to be evaluated. Sometimes the Level 3 methods may be designed *ad hoc* for evaluation purposes only. Often, Level 1 may be evaluated with Level 2 methods when all the pitfalls of the methods of Level 2 are under-

stood. Level 3 methods may be considered as an intermediate stage in reference method development.

Level 4: The most analytically accurate methods are called reference and definitive methods (systematically called primary reference measurement procedures). A definitive method is designed to give the true value of the measured component [48, 49]. It is "an analytical method that has been subjected to thorough investigation and evaluation for sources of inaccuracy, including non-specificity. The magnitude of the imprecision and bias (uncertainty) of the definitive method is compatible with its stated end purpose. The true value is obtained from a mean value of the measurements" [50]. Very few definitive methods are available for clinical laboratories [51].

A reference method is defined as a "thoroughly investigated measurement procedure, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have trueness of measurement and precision of measurements commensurate with its intended use and that can therefore be used to assess the accuracy of other measurement procedures for the same property (-ies), particularly in permitting the characterization of a reference material" [48, 52, 53]. The European Committee for Standardization (CEN) has also described guidelines for the characterization of these procedures [54]. Reference methods should be used in the evaluation of trueness of routine methods. For urinalysis and bacterial culture, these are usually lacking or are in the process of development. This is why a new lower level (=Level 3) in the hierarchy was introduced. Level 3 methods may be developed to Level 4 after adequate descriptions of performance.

5.2. Visual inspection (colour and turbidity) and odour of urine

The most traditional urinalysis was based on human senses. Visual inspection is often undertaken and reported by the patient but should not be omitted by the laboratory. A summary of the most common causes for abnormal appearance of urine is given in Table II (compiled from references 14, 55 and 56).

The odour of normal urine is aromatic of undetermined source. Infected urine may be ammoniacal or fetid. Some metabolic diseases have characteristic urine odours (Table III; from reference 14).

5.3. Rapid chemical examinations (Level 1)

Because many urinary tract diseases present acutely, there is a need for rapid diagnostics frequently at points-of-care. Then, the first urinalysis is often an ordinal scale ("semiquantitative") measurement. Depending on the local environment, these may be performed at points-of-care or in laboratories in primary to tertiary care hospitals as part of a more detailed examination of urine.

5.3.1. Multiple test strips. Multiple test strips (dipsticks; officially called "multiproperty" strips) have been designed to detect several of the following components: leukocytes, bacteria (nitrite), erythrocytes, protein (albumin), glucose, ketone bodies, pH, relative density, bilirubin, urobilinogen and ascorbic acid. A minimum combination depends on the intended use; general approaches are suggested in Section 8 (Stepwise strategies in urinalysis).

The technology and principles employed in test strips have been widely studied. Recently, an interesting British evaluation of multiple strips from seven companies was published, assessing suitability for point-of-care diagnostics by visual reading [57].

The limitations of strip technology are well known [15], but are compiled here in Appendix, Annex 11.1.1. However, any new drug should be considered as a source of interference. The need for quality of test-strip measurements is re-enforced in these guidelines by suggesting new analytical quality goals (Section 9.2) and evaluation tools (Annex 11.1).

5.3.1.1. Different properties of the strips (see also Appendix, Annex 11.1 for methodological detail).

Leukocytes (Esterase): The presence of leukocytes in urine is associated with both urinary tract infection and non-infectious renal diseases. Leukocytes are detected on the basis of indoxyl esterase activity (deriving from neutrophil granulocytes and macrophages) released from lysed cells on the test pad. The analytical

TABLE II.   Characteristic appearances of urine.

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	Rectovesical fistula possible
Milky	Pyuria	Infection
	Chyluria	Lymphatic obstruction
	Paraffin	Vaginal cream
Blue-green	Biliverdin	
	Pseudomonas infection	Small intestine infections
	Drugs: arbutin, chlorophyll, creosote, indicans, guaiacol, flavins, methylene blue, triamterene, enteral nutrition (if blue dye added)	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
	Fuchsin, 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	

sensitivity of the strip is about 80–90% at the detection limit of 20×10^6 WBC/L against chamber counting of fresh uncentrifuged specimens. 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%. Specimens with lysed cells, which are microscopically classified as negative, are partially

TABLE III. Characteristic odours of metabolic diseases.

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

responsible for reduced specificity. The esterase field does not detect lymphocytes. Subtilisin of known activity may be used as a quality control solution.

Bacteria (Nitrite): Nitrite examination is based on activity of the nitrate reductase that is present in most Gram-negative uropathogenic rods, such as *E. coli* (Griess's examination). Nitrate reductase is, however, lacking from some common uropathogens, e.g. *Enterococcus* spp. and *Staphylococcus* spp., and will therefore not be detected whatever their urinary concentration. The positive detection of bacteria requires, in addition, nitrate in the patient's diet (vegetables), its excretion into urine and sufficient bladder incubation time. The analytical sensitivity of the method is variably reported as being between 20% and 80% against

the culture method, depending on the patient population and the cut-off limit for positive culture (usually chosen to 10^8 CFB/L or 10^5 CFU/mL) [58, 59, 60]. The specificity of this field for bacteria is high ($>90\%$).

Performance of test strips in detecting bacterial urinary tract infection must be interpreted carefully, since the selected cut-off for significant counts in culture (decreased to 10^6 **CFB/L** when patients with acute symptoms are evaluated), type of specimens and the studied patient population affect the results obtained [61]. The combined positivity "either nitrite or leukocyte result positive" is generally useful [Table IV]. Specificity of the combination is reduced compared to the nitrite examination alone, because not all patients with leukocyturia have bacteriuria. The clinical sensitivity of the test-strip measurement improves if only patients with pyuria are considered to have infection [62–64]. This can be accepted in patients with a low risk for urinary tract infection only, since high-risk patients may have significant bacteriuria without leukocytes in the urine (see Section 8 for strategic approaches).

Erythrocytes (Pseudoperoxidase): The presence of red blood cells (RBCs), haemoglobin or myoglobin in urine is seen either in dotted (cells) or homogeneous appearance of colour on the reagent pad. The examination relies on

TABLE IV. Performance of multiple test strips in combined detection of bacteriuria (either leukocytes or nitrite positive).

Patient population (prevalence of bacteriuria)	Specimen	Cut-off limit CFB/L (CFU/mL)	Sensitivity (%)	Specificity (%)	References and notes
Meta-analysis (not given)	Not specified	10^8 (10^5)	80–90	80–60	66, 67; numerous publications exist
Mixed hospital patients (26%)	Morning urine	10^8 (10^5)	85	80	68
Mixed hospital patients (20%)	Not specified	Overall $>10^5$ ($>10^2$); only range 10^{5-6} (10^{2-3})	84 25	83 not given	63
Mixed hospital patients (24%)	Morning urine	10^6 (10^3)	85	50	69; using reflectometry
Ambulatory adult patients, symptomatic (38%)	Untimed	Overall $>10^5$ ($>10^2$); only range 10^{5-6} (10^{2-3})	65 40	98 90	70
Symptomatic children (10%)	Untimed	5×10^7 (5×10^4)	88	75	71, fairly high cut-off limit
Symptomatic children, age 1–24 months (5%)	Untimed, catheter specimen	5×10^7 (5×10^4)	79	98	72
Pregnant women, asymptomatic (2–5%)	Not specified	10^8 (10^5)	40–50	95	73 74

pseudoperoxidase activity shown by the haem moiety of haemoglobin and of myoglobin. Unfortunately, this activity degrades rapidly (in a few days) even when the specimen is refrigerated, and is sensitive to various preservatives. RBCs reflect pre-renal, renal or post-renal disease, but also occur in certain physiological conditions, such as menstruation or strenuous exercise. Myoglobin can be demonstrated in patients with muscle necrosis (crush syndrome), rhabdomyolysis (alcohol, neuroleptic malignant syndrome or cocaine abuse) or polymyositis. Haemoglobin without red cells may be detected in haemolytic states and in patients with pre-renal, renal and post-renal haematuria if the cells have been destroyed (either *in vivo* or *in vitro*) by delay in investigation. The analytical sensitivity of the test strip is $70-80\%$ at 10×10^6 RBC/L against chamber counting on fresh uncentrifuged specimens [65]. Specificity of RBC detection is reduced when compared with chamber counts because RBCs lyse easily in urine.

Protein (Albumin): Total urine protein is a mixture of high molecular weight proteins (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 from the kidney (Tamm-Horsfall protein) and those from the urinary tract. The different causes of proteinuria are listed in Table V. The traditional test-strip field is $90-95\%$ sensitive to clinical proteinuria (mostly albumin) at a concentration of about 0.2 g/L [75]. It is less sensitive to mucoproteins and low molecular weight protein, and insensitive to Bence-Jones proteins. The quantitative comparison method (Level 2 or Level 3) used to evaluate the performance of the test strip (Level 1) has a clear influence on the analytical sensitivity and specificity obtained.

For early detection of glomerular damage, sensitive immunochemical [76, 77] and other [78, 79] rapid albumin methods have now been introduced and may gradually be incorporated into multiple strips. They should allow detection of albumin at 20 mg/L concentration (so-called microalbuminuria) at points-of-care as an alternative to quantitative laboratory measurements if a rapid report is needed.

Relative volumic mass *(Relative density; specific gravity)*: Positive or negative results from all urinalysis examinations should be related to the state of water excretion (volume rate). The density obtained with the chemical test-strip field is an arbitrary result allowing a rough estimate of urine concentration only [81]. Osmolality should be measured from urines of patients in intensive care or under parenteral nutrition.

Creatinine: Creatinine measurement has been traditionally used to estimate excretion rates by relating urine concentrations of proteins [82], hormones [83, 84] or other analytes to that of water in single-voided specimens. Its limitations are discussed in Section 5.4.2. A new measurement based on a copper complex has been

TABLE V.   Causes of proteinuria (modified from 80).

Main groups	Classification	Examples
Intermittent	Functional	Fever proteinuria
		Exercise proteinuria
		Congestive heart failure
		Epileptic seizures
	Orthostatic	Occurs in upright position only
Persistent	*Pre-renal*	Immunoglobulin heavy and light chain excretion (= Bence-Jones proteinuria)
		Myoglobinuria (in rhabdomyolysis)
		Haemoglobinuria (acute haemolysis)
	Renal	
	Glomerular	Albuminuria in IgA nephropathy
	Tubular	Low-molecular-mass proteinuria caused by nephrotoxic drugs
	Mixed (glomerular and tubular)	Excretion of plasma proteins in advanced renal disease
	Post-renal	Urinary tract infection; prostatic or bladder disease; vaginal discharge

introduced in test strips to measure albumin/creatinine ratios from patient urines [79]. Knowledge on clinically important interferences has not yet accumulated.

Glucose: Examinations of urine glucose concentrations have largely been replaced by measurements of blood glucose concentration [85, 86]. Urine glucose measurements may be advocated to check for inappropriate use of blood examinations, or for those patients unwilling to use blood sampling in addition to laboratory monitoring of glycohaemoglobin HbA_{1c} concentrations [87]. In less favourable economical situations, urine glucose monitoring is better than no monitoring. Since the finding of glycosuria may reveal patients with juvenile or maturity-onset diabetes mellitus, a screening policy using test strips continues to be important, especially in acutely ill patients (if blood glucose is not used). Routine urine glucose screening of pregnant women is useful if combined with measurements of body mass index (BMI) [88]. The analytical performance of glucose screening by test strip is highly satisfactory, partially because in the important acute cases heavy glycosuria exists.

Ketone bodies: Ketone bodies (acetoacetate, β-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 β-hydroxybutyrate. Ketone bodies need not be measured as part of general urinalysis, but serve to classify or treat specified patient populations, such as patients admitted as emergencies (especially paediatric patients), juvenile-onset diabetics or patients with toxaemia of pregnancy. After institution of insulin and fluid therapy in diabetic hyperglycaemia and ketosis, tissue β-hydroxybuturate converts back to acetoacetate, leading to transient increase in urine acetoacetate excretion despite an improved clinical situation. Slight ketosis is detected even after overnight fasting, indicating an acceptable clinical sensitivity. Unspecific reactions occur with the traditional examination principle (see Appendix, Annex 11.1.1).

pH: Urinary pH varies between 5 and 9. Concentrated morning urine is usually acidic. Urines from children are often alkaline. Bacteria metabolizing urea to ammonia may also increase the pH of urine. Survival of leukocytes [89] is particularly reduced in dilute and alkaline urines, typically in children with urinary tract infection [40]. Casts are also lost in alkaline urine [90]. Measurements of urine pH are needed for the diagnosis of acid-base disturbances (such as hypokalaemic alkalosis with paradoxical aciduria), or when acidification or alkalinization of urine is associated with specific diseases (such as renal tubular acidosis, renal stone disease), or during the elimination of specific drugs (e.g. cytotoxic drugs).

Bile pigments: Measurements of urinary urobilinogen and bilirubin concentrations have lost their clinical significance in the detection of liver disease following the introduction of liver enzyme examinations from blood. They may be useful, however, in differentiating icteric patients or in detecting alcoholic liver disease in non-laboratory environments.

Ascorbic acid: Ascorbic acid (vitamin C) interferes with the measurement of several test-strip analytes (see Appendix, Annex 11.1.1). Because many patients ingest vitamin C in large quantities (>1 g/day), measurement of ascorbic acid concentration in urine helps in identifying those patients with false-negative test-strip results. The other, more direct, approach should be to try to develop test strips insensitive to interference by ascorbate.

5.3.1.2. Instruments used for multiple strip examinations. The manufacturer's instructions must be strictly followed for optimal results when reading test strips (Appendix, Annex 11.1.6). Instruments (rather than the naked eye) are recommended for reading a multiple property strip, whether in the laboratory or at point-of-care [91], because observer-related major errors occur frequently in practice and are not traceable afterwards. It is also clear that all laboratory examinations, including those performed at point-of-care sites, should meet the required quality [92].

The selection of different instruments is determined by the local diagnostic requirements. Centralized laboratories providing a 24-h service tend to automate the analysis of large numbers of specimens, while point-of-care sites show an increasing interest in improving the quality of single rapid patient investigations. Fully automated test-strip readers are frequently considered in large laboratories. Automated urinalysis aims to improve the precision

and accuracy of results at higher level than that achieved by visual or semi-automated methods. Turnaround time, cost containment and safety of the working environment are important issues in routine work-flow as well. A combination of results from rapid chemical measurements and automated particle analysis is under development [93].

5.3.2. Pregnancy examinations. Detection of pregnancy is achieved by specific measurements of human chorionic gonadotropin (hCG) secreted by the developing placenta [94]. The appearance and rapid increase in the concentration of hCG up to 10 000 IU/L in serum and urine during early pregnancy make it an excellent diagnostic marker. The examinations are performed from either blood or urine specimens. The sensitivity to detect extra-uterine pregnancies is recommended using blood specimens, while urine is used for rapid examinations to detect mainly normal pregnancies. Different defined sensitivities should be considered depending on the intended application, since the sensitivity at 25 IU/L is sufficient to detect pregnancy already after 3 – 4 days of implantation [95], i.e., before the missed menstrual period. A reduced sensitivity limit of 200 – 500 IU/L may be of value if only detection of normal pregnancies is needed. This will delay the detection of pregnancy but increase the specificity of obtained results. Detail of measurements is given in the Section 11.3.

5.3.3. Other rapid examinations. Test strips with isolated fields to show the presence of glucose and/or ketone bodies, albumin or albumin/creatinine ratio only are indicated in screening or follow-up of some defined patient groups, such as certain patients with diabetes mellitus, pregnant women or renal patients. Pre-eclamptic toxaemia patients should be screened by blood pressure measurements rather than by conventional urinary protein measurements [96]. However, proteinuria assessment is important in treatment decisions for pre-eclamptic patients. Rapid detection of bacteriuria is discussed in detail in Section 7.4. The visual reading of one or two fields on a strip is easier than of multiple test strips. Rapid examinations for drugs of abuse are not included in these guidelines.

5.4. Quantitative chemical measurements

For many urine components, a quantitative result helps in the diagnosis and follow-up of patients. This used to involve timed 24-h or other collections of urine with calculated excretion rates of the analytes. As a practical alternative, a reference measurement to adjust for water excretion (osmolality, creatinine or equivalent) and calculation of analyte/creatinine or analyte/osmolality ratios has already been devised for measurements of protein excretion. This quantitative approach is typically performed in centralized laboratories (Level 2 methods), but may be used in primary care laboratories or even point-of-care sites with methods suitable for Level 1 (e.g., albumin/creatinine ratio) when the cost/benefit ratio is favourable. Several analytes measured quantitatively in specific clinical situations are listed in the Appendix (Annex 10.3.4) in relation to specimen collection. Detailed discussion was considered to extend these guidelines too much.

5.4.1. Proteins. Measurement of total protein excretion has been traditionally recommended for detecting renal disease [97]. In most cases the urine contains albumin that may be measured quantitatively or detected by test strip. Glomerular nephropathies are characterized by increased excretion of albumin, transferrin and in the advanced stage by high molecular mass proteins such as IgG [98]. It is already widely accepted that an early glomerular disease, such as incipient nephropathy in diabetes mellitus, can only be detected with sensitive measurements of (micro)albuminuria (analytical sensitivity down to 5 – 10 mg/L [10, 99]). Albumin excretion rate is elevated years before reduction in glomerular filtration rate is detected by an increase in serum creatinine concentrations [100]. Increased urinary albumin is now considered predictive of mortality and morbidity in both non-insulin-dependent and insulin-dependent diabetes mellitus [101]. Albuminuria is the strongest known predictor of cardiovascular disease in non-insulin-dependent diabetes mellitus [102]. The detection of albuminuria is a risk factor in non-diabetic hypertension [103 – 105], vascular disease [106] and a predictor of heart failure [107]. Albuminuria also predicts mortality in elderly age in general [108].

For detection of tubular disease, a sensitive strategy has also been proposed using measurements primarily of α_1-microglobulin [109], also called protein HC [110, 111]. Tubular dysfunction can also be detected with other excreted markers, e.g., retinol-binding protein (RBP) [112–115] and enzymes such as N-acetyl-β,D-glucosaminidase (EC 3.2.1.30) originating from kidneys [116–118]. The earlier β_2-microglobulin is too labile in acid urine. Enzyme measurements have gained only limited clinical value because the interpretation of variably elevated excretion rates has been difficult among large patient populations. For example, in the common paediatric urinary tract infections, elevated excretions of N-acetyl-β,D-glucosaminidase, β_2-microglobulin or α_1-microglobulin have no additional diagnostic information after measurements of body temperature [119, 120]. This may reflect the dynamic metabolic states of tubular cells, high regenerative capacity of kidneys as well as their ability to adapt despite the destruction of some nephron segments [121]. Renal tubular enzymes may, however, be useful in assessing individuals exposed to nephrotoxic drugs or heavy metals [122].

Clinical specificity and prognostic significance in follow-up must generally be assessed to allow targeted application. A sensitive measurement is justified with specific suspicion of renal disease, i.e., for patients with chronic diseases associated with renal complications, such as diabetes or hypertension, and similar states. Since tubulo-interstitial damage may cause end-stage renal diseases that have currently remained unnoticed by using mostly screening with albumin strips, a revised screening strategy should be considered for other patient populations as well [123].

5.4.2. Quantities of volume rate (diuresis). Osmolality. Assessment of renal concentrating capacity is classically evaluated in kidney diseases. The preferred quantity related to volume rate (diuresis) is urine osmolality because it is a solute property [124]. Osmolality should also be measured when other components in urine need to be related to variable excretion of water in order to estimate excretion rates. This is essentially true for both renal and lower urinary tract diseases, and both formed elements and dissolved chemical constituents. Because a separate instrument is required for osmolality measurements, very few laboratories have applied analyte/osmolality ratios in their clinical practice [125]. Urine osmolality is also related to diet and ingestion of salts.

Relative volumic mass (old terms: relative density; specific gravity). Relative volumic mass (traditional name: relative density [126]) is the preferred term over specific gravity, since the redefinition of litre in 1964 to equal 1 cubic decimetre of water. Since the reference density (reference volumic mass) is the maximum density of water at $+4°C$ ($\rho_0 = 0.999972$ kg/L), there is no practical difference between volumic mass and relative volumic mass of urine within clinical medicine. A more significant uncertainty of clinical results is derived from inaccurate measurement procedures in clinical laboratories. Urine relative volumic mass is closely related to osmolality [127]. The correlation between relative volumic mass and osmolality decreases in disease [128] because relative volumic mass 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.

Creatinine. Correction of diuresis using urinary creatinine concentration to calculate analyte/creatinine ratios has gained general acceptance despite certain theoretical problems [10, 11]. Creatinine measurement is easily performed and only minimally affected by protein-containing diet. Creatinine excretion suffers from inaccuracies related to body weight, age, gender [129, 130] and renal function, i.e. tubular secretion in uraemia [131]. Chronic diseases such as hypo- and hyperthyreoidism may also affect it. Analyte/creatinine ratios should always be measured as part of quantitative measurements if timed collections overnight or for 24 h are to be avoided.

Conductivity. Conductivity is a new parameter that is brought to clinical laboratories and intensive care units with novel instruments. It is related to osmolality, since both are dependent on concentration of salts in urine. Conductivity seems to correlate to osmolality fairly well, even better than creatinine [69,

128, 132]. However, the exact clinical role of this measurement awaits further clinical experience.

5.4.3. Diagnosis and secondary prevention of renal stone formers.

Some quantitative measurements from urine help in diagnosing and classifying patients with a recurring tendency to renal stone formation [133]. After analysis of the stone by X-ray or the infrared method, quantitative measurements of urine volume, relative density, pH, calcium, urate, citrate, oxalate and creatinine are recommended as minimum measurements from 24-h collection [134–136]. Optionally, measurements of urinary magnesium, inorganic phosphate, ammonia and cystine are recommended in addition. New technology to detect lithogenicity of urine has also been proposed based on X-ray microscopy [137, 138]. Serum (or plasma) concentrations of intact parathormone (primary hyperparathyreoidism), calcium, urate, inorganic phosphate and creatinine are of value, as well as examination of acid-base homeostasis in blood. Dietary background must be known and understood when interpreting these results. Microscopic analysis of urinary crystals is discussed under particle analysis (Section 6.1). The German experience suggests the importance of investigations of urolithiasis patients not responding to non-specific treatment, since new stone attacks could be prevented by 46% in the follow-up [139].

5.5. Electrophoretic examinations

Electrophoretic techniques are essential in detecting the clonality of serum paraproteins (M components) and monoclonal immunoglobulin light chains in urine (Bence Jones proteins). Immunofixation of the urine or serum helps in defining the isotype of the myeloma clone. Some authors recommend immunofixation electrophoresis of all specimens because of the sensitivity of the method [140]. The specificity must then also be understood because of the existence of monoclonal gammopathies of undetermined significance. As an alternative to screening and in monitoring, turbidimetric quantitation of urinary κ or λ chain excretion is also suggested [111], combined with immunofixation in case of a positive

finding with unknown clonality. Test strip is usually negative for the light chains.

Size fractionation of urinary proteins on sodium dodecyl sulphate-acrylamide gel electrophoresis [141–143] has been used to classify pre-renal, renal and post-renal proteinuria before quantitative measurements of specific proteins became widely available. Small laboratories may use this examination if a quantitative result is not needed.

6. PARTICLE ANALYSIS

6.1. Clinically significant particles in urine

Leukocytes: Granulocytes are the most frequent leukocytes detected in the urine of patients with urinary tract infection due to common organisms, and may also be seen in other conditions such as glomerulonephritis, interstititial nephritis and aseptic cystitis. The appearance of lymphocytes in urine is associated with chronic inflammatory conditions, viral diseases and renal transplant rejection. Macrophages (mononuclear phagocytes, histiocytes) appear fairly often in urine of patients with urinary tract infection. They are also suggested to reflect, e.g., inflammatory activity of renal disease [144]. Eosinophil granulocytes may occur in several disease states; they are no longer seen solely as markers of acute interstitial nephritis caused by drugs such as beta-lactamic antibiotics [145].

Erythrocytes: Haematuria remains a major sign of urinary tract and renal diseases. It may also reflect a general bleeding tendency. Haematuria for physiological reasons (strenuous exercise) and vaginal contamination (menstruation) should be avoided – if possible – during careful patient preparation. The appearance of RBC in urine reflects the origin of bleeding: dysmorphic erythrocytes (red cells with abnormal size or shape) suggest renal disease, whereas RBC with normal morphology usually originate from the lower urinary tract [146–148]. The morphology of RBC in urine is valuable in evaluation of patients with isolated haematuria, because it can determine if the subsequent diagnostic work-up is urological or nephrological [149]. The examination technique needs special training and is best performed with phase contrast microscopy [147]. A subgroup of

TABLE VI. Levels of microscopy differentiation in clinical urinalysis.

Basic level	Advanced level in addition
Red blood cells (RBC)	*Detailed subclasses erythrocytes*: dysmorphic erythrocytes
White blood cells (WBC)/Granulocytes	*Differentiation of leukocytes*: Granulocytes, lymphocytes, macrophages (monocytes and eosinophils)
Epithelial cells:	*From non-squamous epithelial cells*:
Squamous epithelial cells	Squamous epithelial cells
Non-squamous = small epithelial cells	Renal tubular epithelial cells
	Transitional epithelial cells (superficial and deep)
	Intestinal epithelial cells (occurring after bladder surgery)
	Atypical cells (experienced cytopathologist)
Casts:	*From non-hyaline casts*:
Hyaline casts	Erythrocyte, granulocyte casts
Non-hyaline casts	Renal tubular cell casts
	Hyaline, granular, waxy, fatty casts
	Bacteria and yeast-containing casts
	Haemoglobin and myoglobin casts
	Bilirubin casts
Bacteria	Gram-staining characteristics of bacteria (microbiology laboratories)
Yeasts, Trichomonas	*Schistosoma haematobium* (in appropriate geographical locations)
Spermatozoa	Spermatozoa
Artefacts (hair, paper and textile fibres, starch, glass) and mucus	Artefacts and mucus as on the left
Lipids:	*Lipids, in addition to droplets*:
Droplets (isolated and aggregated)	Oval fat bodies (lipid-laden tubular cells), cholesterol crystals
Crystals:	*Additional rare crystals*:
Urate, oxalate (mono- and dihydrated), phosphate and cystine	Drugs, cystine, leucine, tyrosine, 2,8-dihydroxyadenine, xanthine

abnormally shaped RBC (**acanthocytes** or G1 cells = urine red cells with blebs) has also been described recently [150–152]. Because of their characteristic shape, identification of acanthocytes is helpful in defining a glomerular haematuria.

Epithelial cells: Detached epithelial cells in the urine may help localize urinary tract disease. The appearance of **squamous epithelial cells** from the outer genitalia or distal urethra serves, however, as a marker of a poor collection technique, except during pregnancy when epithelial cell exfoliation is increased.

Transitional epithelial (urothelial) cells derive from the multilayered epithelium lining the urinary tract from the calyces of the renal pelvis to the bladder in the female and to the proximal urethra in the male. These cells are frequently found in urinary tract infection and in non-infectious urological disorders.

The detection and definitive diagnosis of atypical cells is difficult [153] and should be undertaken by experienced cytopathologists. A **suspicion** of atypical or malignant cells may be considered and reported by a general laboratory examining urine cells at an advanced level. A repeat urine specimen freshly collected and fixed according to a standardized procedure is needed in cytopathological investigation. Rapid examinations for bladder tumour cell antigens may enter clinical practice when problems with their specificities are solved.

Renal tubular epithelial cells are mainly found in the urine of patients with acute tubular necrosis, acute interstitial nephritis for any cause and acute cellular rejection of renal allograft [154, 155]. Tubular cells are also found in patients with active proliferative glomerulonephritides [156], glomerular diseases associated with nephrotic syndrome and some metabolic storage diseases, such as Fabry's disease [157].

Since renal tubular cells are difficult to distinguish from transitional cells by unstained bright-field microscopy, many laboratories have used the term "small round epithelial cells" to describe both. This guideline recommends the use of correct cytological terminology for cell types if possible after appropriate training and

experience. If a laboratory decides to remain at the basic level of differentiation for practical reasons, the term **small epithelial cell** is accepted (Table VI).

Casts: Casts are formed in the distal tubules and collecting ducts [158] from aggregation and gel-transformation of the fibrils of Tamm-Horsfall glycoprotein (uromucoid). This material is produced by the cells of the ascending limb of Henle's loop and forms the hyaline matrix of most casts [159]. A precipitate, i.e. a cast, is formed when the concentration of dissolved organic and inorganic material exceeds the saturation point of the normally colloidal solution. Within casts, plasma proteins, lipids, different types of cells, microorganisms (bacteria or yeasts), pigments (haemoglobin, myoglobin, bilirubin) and crystals may be found. Some **hyaline casts** may be present in the concentrated morning urine of healthy individuals. Casts, however, usually reflect the presence of renal disease. They are specific but not sensitive markers of renal disease.

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 of patients with heavy proteinuria. Lipids are usually detected as droplets, isolated or in clumps, lipid-laden tubular epithelial cells ("oval fat bodies"), cholesterol crystals or lipid-containing casts.

Microbes: Different microscopy methods vary in sensitivity and their ability to detect and permit the identification of bacteria. All methods are relatively insensitive for the detection of bacteria at concentrations below 10^8/L (10^5/mL), although the presence of bacteria below this concentration is important in selected groups of patients, whether symptomatic or not [160, 161]. A separate Gram stain may be performed in microbiological laboratories capable of interpreting it. Other organisms, such as yeasts and *Trichomonas vaginalis* are common in the urine of women with vaginitis. *Schistosoma* may also be found on urine microscopy [162]. Urine microscopy is not sensitive enough to exclude urinary tract infections.

Crystals: In most instances, the finding of crystals is not clinically relevant, since they may occur as a consequence of transient supersaturation caused, for instance, by food rich in urate or oxalate, or by *in vitro* changes in the temperature (refrigeration) or pH of urine.

Detailed investigation for crystals in all specimens is unwarranted. However, detection of crystals has clinical value in subpopulations of recurrent renal stone formers [163] (see also Section 5.4.3). They may also be significant for some patients with acute renal failure [163]. In such cases, crystalluria is the marker of a major disorder and is diagnostically important. The most typical examples are acute uric acid nephropathy and ethylene glycol poisoning, which is associated with calcium oxalate monohydrate crystalluria. Therapeutic drugs possibly crystallizing in urine include sulphadiazine, triamterene, acyclovir, indinavir and vitamin C (see Annex 12.1.3 for details). 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 of their relevance, a specific request should be made to the laboratory for investigation of urine crystals.

Cystinuria can be detected by finding hexagonal plain crystals in urine (prevalence from 1:2000 in England to 1:100 000 in Sweden [133, 164]). A rapid procedure (cyanide-nitroprusside examination) is also available [165]. To see cystine crystals by microscopy, the specimen should be acidified with acetic acid (at pH < 6) and kept refrigerated overnight to allow crystallization to occur. Rare 2,8-dihydroxyadenine crystals occur in a genetic deficiency of adenine phosphoribosyltransferase enzyme [166]. Another very rare xanthine crystalluria occurs in deficiency of xanthine oxidase; the crystals are easily confused with urate [135]. Tyrosine and leucine crystals are associated with a severe liver disease. Measurements of urinary (and plasma) concentrations of amino acids are recommended for confirmation of inborn errors of amino acid metabolism.

Levels of differentiation Differentiation of the above-mentioned particles by microscopy can be divided into **basic and advanced levels** (Table VI). The basic level for routine urine microscopy is a positive, specific identification of the usual formed elements (left column). Some laboratories or clinical units report only leukocytes, erythrocytes and bacteria. This should be said clearly in the specifications given by the laboratory. The advanced level

of urine microscopy usually provides evidence of renal damage (right column, usually in chemical laboratories or nephrological units), or specifies microbes in more detail, e.g. based on Gram staining characteristics (microbiological laboratories). The suggested order of process is that laboratories and nephrological units first decide between these two levels and then define the local detailed procedures to be followed depending on the needs for urine microscopy, be that in a clinical nephrology unit or in a general or specialized laboratory.

6.2. Different techniques of visual microscopy

6.2.1. Hierarchy of microscopy methods. **Reference method** for urine microscopy (Level 4) should provide both correct identification of different particles and their accurate quantities when measuring urine. Currently, no such method exists. Standardization of the method used is essential for improving accuracy and limit of detection [2, 167, 168], independently of the intended level of final performance. Special attention should be paid to different sources of error [169, 170]. The centrifugation step with removal of supernatant is a major tool for concentration of the urine specimens, but also a major source of errors.

6.2.2. Principles of comparison methods (Level 3). *6.2.2.1. Particle counting.* **Identification:** Particle identification needs an optical method to discern formed elements from their fluid background and a differentiation method to allocate these elements into correct classes (Table VI). Bright-field microscopy of unstained preparations is inadequate for detection of bacteria, RBC and hyaline casts, and therefore for advanced differentiation. For this reason, either supravital staining [171, 172] or phase-contrast microscopy [56], or both, is recommended. Detection of microbes by Gram staining and other special procedures, such as nucleic acid amplification methods, are needed in the microbiology laboratory. Identification of elements such as eosinophils, monocytes or all macrophages may require specific methods as well as experienced evaluators. The latter may, however, be beyond the general need of rapid differentiation of urinary particles.

Counting: Concentrations of urinary particles should be related to the original volume of urine (in litres, L). Counting of native urine avoids the error created by centrifugation. A sufficient volume is needed to detect rare particles related to renal damage (see Section 9.4). Particle concentrations at the low positive range or upper reference limit in health can vary up to 100% owing to the effect of pre-analytical variables. In regard to this figure, a reliable comparison level of enumeration of particles has a negligible bias and imprecision to allow correct classification of patients' results (see Annex, Section 12.1.1 for further detail).

Automated instruments have improved precision by counting many more cells than visual methods. They may be used for enumerating elements for which their method has been validated.

6.2.2.2. Bacteria counting (Slide centrifugation + Gram staining procedure). The slide centrifuge method with Gram staining offers the highest analytical sensitivity and reproducibility in the rapid detection of bacteria in urine, although it is not a quantitative method. It can therefore be used in Level 3 comparisons. When a cut-off value of 10^8 colony-forming bacteria/litre (CFB/L) in culture is taken, this method has a sensitivity of 98% and a specificity of 90% against the culture [173]. These performance figures were confirmed with specific patient populations in later studies, showing an overall sensitivity of 63% and specificity of 91% at 10^6 CFB/L [174]. The main disadvantage to this robust and consistent method is that it is very labour-intensive. Details of the method are described further under microbiological methods (Appendix, Annex 13.5.1).

6.2.3. Routine identification methods for urine particles (Level 2).

Standardized urine sediment under a coverslip. Standardized centrifuged urine sediment should be investigated under a defined size coverslip with a known volume of specimen in the view field (Appendix, Annex 12.1.2). This is recommended as a routine visual procedure in *examination for kidney-related urine particles* for the following reasons:

(1) The sediment method detects particles indicating renal damage. With uncentrifuged

specimens, the investigator misses rare elements (most importantly casts and renal tubular epithelial cells) that are clinically significant but can be concentrated at low-speed centrifugation. To allow screening of a large volume, i.e. up to 10–20 μL under a coverslip, a low-power (e.g. ×100) magnification should always be used in parallel to high-power (usually ×400) optics. It is understood that centrifugation methods are never quantitative in counting RBC and WBC that are variably lost during centrifugation. The procedure is then prone to high uncertainty of certain particle counts despite standardization.

(2) Differentiation of important formed elements (= detection of renal damage) is easier through a thin fluid layer under a coverslip (40–50 μm) than through the thicker fluid layer of a chamber (usually ≥100 μm high). A thicker fluid layer may require more focusing levels to be examined.

Urine sediment counted in a chamber. Counting concentrated, centrifuged sediments in a chamber has been advocated by some investigators [168, 175] because of the large counting volume (3.2 μL), which results in a more precise count and possibly a higher sensitivity compared to uncentrifuged specimens counted in a chamber. The easier performance of the coverslip method on centrifuged sediment makes this a preferred routine method over chamber counting despite the lower precision. The reference intervals of chamber counts (the number of particles seen in the chamber) in health vary because of the different concentration factors used in different systems [167, 176].

Gram staining. Correctly performed, examination of urine using a microscope will detect bacteria which are either nutritionally fastidious and will not easily grow on standard media, or which will only grow anaerobically, but are nevertheless responsible for urinary tract infection in a minority of patients. Microbiology laboratories may benefit from their capability of interpreting Gram staining when trying to reduce the total work-flow. This staining has the added advantage of immediately ascribing a Gram status to the infecting organism. A specific model incorporating the results of Gram staining of uncentrifuged urine for diagnosis of urinary tract infection in young women has also been developed [177]. Uncentrifuged urine may be accepted because bacteria are not concentrated very much at low-speed centrifugation. Reporting at ordinal scale is usually sufficient. Details of Gram staining are described further under microbiology methods (Appendix, Annex 13.5.2).

Chamber counting of uncentrifuged specimens. In some microbiology laboratories, simple microscopy is used to improve the detection of urinary tract infection only [178, 179]. Chambers were advocated originally to provide more precise leukocyte counts than those obtained from sediment preparations, a finding of 10 or more leukocytes ×10⁶/L representing significant and abnormal levels of leukocyturia [180–182]. Because centrifugation results in a variable 20–80% loss of RBC and WBC [170], no sediment method can be considered as reference for quantitative urinary particle counting. Analytical sensitivity for bacteria will be poor at lower concentrations when compared with bacterial cultures. Performance figures for bacteriuria for techniques using uncentrifuged samples of urine are very variable, depending on the criteria used for positivity in culture [183]. They will depend also on operator experience, whether bacilli or cocci are present, and on the degree of interference by debris. Sensitivity at the 10^7 bacteria/L (10^4 bacteria/mL) level usually exceeds 80%.

The major disadvantage of chamber counting is that it tends to be time-consuming for routine laboratory practice. Without staining or phase contrast optics, differentiation of particles is not sufficient for renal elements. The advantage of precise, quantitative measurement of elements is outweighed by cost as a routine procedure, but chamber counting is useful as a comparison method in instrument evaluations.

Filtration of urine particles. Filtration of urine particles on supportive media allows recovery of essentially all particles existing in a defined volume of urine if remaining intact. This principle has been used to improve the sensitivity to detect urine casts using a 3-μm pore size, and starting from 20–100 mL of urine [184]. Another publication used filtering to examine

physiological concentrations of RBC in urine as detected by scanning electron microscopy [185]. There is also a commercial version of filtration which uses disposable syringes and special equipment [176]. The losses of material during filtration depend on the pore size and pressure applied on the particles. Staining of the preparations is needed for proper identification of particles, which may also cause lysis of some cells.

6.2.4. *Rapid microscopy methods (Level 1). Non-standardized urine sediment under a coverslip.*

Traditional urine sediment is still investigated in many laboratories by pouring supernatant after centrifugation, inserting a drop of sediment under a coverslip and investigating an unknown volume under a microscopic viewfield. Without staining and using bright-field technique some of the elements are not seen; with little knowledge of the differentiation of particles others are missed in classification. In repeated routine process, this procedure gives results associated with disease conditions in an arbitrary ordinal scale. Because of a wide uncertainty of results and reduced sensitivity in detecting essential particles, non-standardized sediment procedure is not recommended.

Microtitre tray method. Microbiology laboratories with a high throughput of samples may consider the microtitre tray method with an inverted microscope because it is rapid, cheap and occupies very little bench space (a basic inverted stage microscope fitted with a 96-well microtitre plate holder). A fixed volume of urine (usually $60 – 80 \ \mu L$) is placed in a well of a 96-well flat-bottom microtitre plate and allowed to settle. The well is then examined using an inverted microscope. The presence or absence of white cells, bacteria and red cells is noted on an ordinal scale. The technique requires operator training, as differentiating (e.g.) cocci from debris may be difficult. Disposable plastic 96-well plates may not be available to all laboratories; it is however perfectly possible to cold disinfect, wash and thoroughly dry plastic plates and re-use them (see detail in Appendix, Annex 13.5.3).

Test strip is used in many clinical environments to detect haematuria, pyuria or bacteriuria without microscopy [71].

6.3. *Instrumental particle analysis*

Mechanization of urine particle analysis commenced with the introduction of automated microscopes [186] and flow cytometry devices [187, 188] about 20 years later than for blood cell counting. New technology now offers the opportunity to replace the basic level of particle analysis (see Table VI). Renal damage is partially detected by identifying some casts

TABLE VII.   Clinical presentations for rapid bacteriuria detection.

(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, e.g., women in the antenatal clinic
(4)	Selecting specimens for extended investigation in the laboratory (Sections 7.3.6 and 8.2)

TABLE VIII.   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 patients with diabetes or anomalies of the urinary tract, recurrent stone disease, or immunocompromised state
(4)	Patients failing first line antimicrobial chemotherapy
(5)	Febrile patients with indwelling catheters
(6)	Clinical suspicion of urinary tract infection in men (symptomatic)
(7)	Clinical suspicion of urinary tract infection in pregnant women (symptomatic)
(8)	Suspicion of urinary tract infection in children and adolescents (symptomatic)

and small epithelial cells [69] and microcytic (small) erythrocytes [189]. Rational combination of automated and visual particle analysis with chemical measurement and bacteriological procedures is crucial in the new urinalysis workflow strategy.

7. MICROBIOLOGY EXAMINATIONS

7.1. Medical indications for microbiology investigation of urine

The aims of urine bacterial culture are (a) to identify aetiological agents of urinary tract infection, i.e. relevant pathogens but also mixed flora as a sign of contamination, (b) to estimate the concentration of bacteria, (c) to offer susceptibility testing for antimicrobial treatment, and (d) to follow the effects of antimicrobial treatment during the course of urinary tract infection. In clinical practice, however, it is not necessary to perform all examinations for every patient suspected of having urinary tract infection. A simple division of the patients into usual, uncomplicated cases suspected for lower urinary tract infection and special or problematic cases will improve the efficiency of clinical laboratory practice.

7.1.1. Clinical presentations requiring rapid examination for bacteriuria.
Rapid examinations are recommended in the situations listed in Table VII. In recurrent acute lower urinary tract infection from low-risk women (item 1), no laboratory examinations are usually necessary when the symptoms are clear-cut [5, 6, 190]. If symptoms remain unclear, rapid methods for detecting bacteriuria help in the differential diagnosis of patients with medical emergencies. Before classifying otherwise healthy women into this group, anatomic abnormalities in the urinary tract should be considered (Table VIII). Usually asymptomatic bacteriuria should not be sought nor treated to avoid enrichment of multi-resistant

TABLE IX.   The pathogenicity and frequency of micro-organisms in midstream urine.

Pathogenicity in the urinary tract	Frequency (percent of isolates)			
	A. Common (>10%)	B. Fairly common (1–10%)	C. Uncommon (0.1–1%)	D. Rare (<0.1%)
I. Primary pathogens	*E. coli*	*S. saprophyticus*		*E. coli* CO_2-dependent, *Salmonella* spp.[a] (*Leptospira*, mycobacteria)
II. Secondary pathogens		*Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *P. mirabilis*, *P. aeruginosa*	*Citrobacter* spp., *M. morganii*, *P. vulgaris*, *Serratia* spp., *S. aureus*	*Corynebacterium urealyticum*, *Haemophilus* spp.[b] Pneumococci[b]
III. Doubtful pathogens		GBS[c], Yeast, CNS (others)[d]	*Acinetobacter* spp., *Pseudomonas* spp., *Stenotrophomonas maltophilia*	A great number of reported cases have been published with exceptional cases of infections caused by other species
IV. Usually urethral or genital flora[e]		α streptococci, *Gardnerella vaginalis*, Lactobacilli, etc.	*Bifidobacterium* spp., "Diphtheroid" rods, etc.	

[a] Low concentrations are reported even if they are most likely caused by contamination during specimen collection.
[b] Most often isolated from children.
[c] GBS=group B streptococci (*S. agalactiae*).
[d] CNS=coagulase-negative staphylococci, urease-forming isolates or isolates found in patients with indwelling catheters have increased significance.
[e] No identification and susceptibility testing (only exceptionally, if especially indicated).

bacterial strains. However, screening of selected clinical populations, such as pregnant women, is warranted (Section 8.2.3).

7.1.2. Indications for urine bacterial culture with identification of species and susceptibility testing. Urine samples should be sent to the bacteriology laboratory for quantitative culture and susceptibility testing from the patient groups listed in Table VIII.

7.1.3. Indications for urine bacterial culture after completed treatment. Low-risk women who become asymptomatic after treatment for acute cystitis do not need follow-up with a post-treatment urine culture.

7.2. Microbes of the urinary tract

The presence of organisms in urine *per se* is not diagnostic of infection. Species such as *Escherichia coli* and *Staphylococcus saprophyticus* seem to be more aggressive than others and may therefore be identified as primary pathogens (see below). Other factors, such as whether urine is analysed as part of a screening programme, bladder incubation time, coexisting symptoms of infection, pregnancy and age, all influence the specificity attached to the presence of any concentration of bacteria in urine. In addition, the largest variable that is impossible to control accurately is the technique of the "mid-stream urine" at the time of obtaining the urine sample, which often is not perfect. Despite instructions, a proportion of samples will contain commensal contaminants which may be present in large enough numbers to make interpretation difficult. Diagnostic rules therefore depend on whether bacterial growth is pure or mixed. This emphasizes the importance of a laboratory system that insists on, and takes account of, clinical details for individual specimens. It is not possible to provide effective patient management if the results of urinary investigation are not interpreted in the light of clinical information.

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.

TABLE X. Examples of bacterial concentrations expressed as traditional colony-forming units and recommended colony-forming bacteria.

Conventional unit (CFU/mL)	Standardized unit (CFB/L)
1	10^3
10^3	10^6
10^5	10^8

7.2.1. Classification based on uropathogenicity. The classification of pathogens causing urinary tract infections is given in Table IX. Different groups of micro-organisms have been classified into 16 categories based on four degrees of pathogenicity (I–IV) and frequency in clinical populations (A–D) [7]. The cut-offs between the degree of uropathogenicity and species frequency should not be interpreted as strict and unchangeable. The table is merely an attempt to organize many years' experience in clinical microbiology [5, 6, 191, 192, 193]. Pathogenicity may be classified as follows:

I. *Primary pathogenic species:* Species that have the ability to cause urinary tract infection in *individuals with normal urinary tracts*. This group consists of *E. coli* and *S. saprophyticus*. Also found in this group are certain rare primary pathogens and other species which should be reported according to national regulations, e.g. *Salmonella* spp.

II. *Secondary pathogenic species*: Species that seldom cause primary infection in patients with normal urinary tracts, but often occur in *hospital-acquired urinary tract infection*. *Klebsiella* spp. (species), *Enterobacter* spp., *Proteus* spp., *Morganella morganii*, *S. aureus* and *Enterococcus* spp. are a few common examples.

III. *Doubtful pathogenic species*: Skin flora and other species. Those *sometimes* colonize in hospitalized patients and cause *hospital-acquired urinary tract infection*. The culture findings can only be considered relevant if suprapubic aspiration has been performed. Even if coagulase-negative staphylococci (CNS) occasionally cause urinary tract infection, findings are often a sign of contamination and have a low predictive value.

IV. *Urethral/genital flora*: Species belonging to *normal urogenital flora*. These often contaminate voided and drawn urine samples. They are

TABLE XI. Diagnostic performance of different cut-offs for "significant" coliform bacteriuria in women with acute voiding difficulties (160).

Mid-stream urine (CFB/L)	Sensitivity	Specificity	Predictive value	
			Positive	Negative
10^5	0.95	0.85	0.88	0.94
10^6	0.81	0.90	0.90	0.82
10^8	0.51	0.99	0.98	0.65

examined for antimicrobial susceptibility only in exceptional cases (when especially indicated). This group has been introduced to give more detail for clinical interpretation and to minimize false positives.

7.2.2. Background for limits of clinically significant bacteriuria.

7.2.2.1. Unit recommended for expressing bacterial concentrations. New technologies for direct particle counting and development in standardization of nomenclature ask for a redefinition of the unit used to report bacterial concentrations. The classical "colony-forming unit/millilitre" (**CFU/mL**) has the pitfalls that the word "unit" should be used as an abstraction rather than count of visible things such as bacteria, and millilitre is not the standardized volume to express concentrations. Therefore, bacterial particle concentrations are recommended to be expressed as **bacteria/litre (L)** in a way similar to cells in body fluids. When grown on agar, the living bacteria appear as colonies, corresponding to a standardized unit **colony-forming bacteria/litre (CFB/L)**. Equivalence is given in Table X.

7.2.2.2. Traditional Kass's criteria. Evaluating urine culture findings has long been dominated by Kass's criteria for significant bacteriuria. Kass found that 95% of women with pyelonephritis had $\geq 10^8$ CFB/L ($\geq 10^5$ CFU/mL) of one bacterial species in a clean-catch mid-stream urine, and that such a finding in two consecutive mid-stream urine specimens in asymptomatic women would, with 95% probability, give the same result in a third mid-stream urine specimen [194, 195]. Thus, in order to diagnose asymptomatic bacteriuria with reasonable accuracy, $\geq 10^8$ CFB/L of the same bacterial species was necessary in two mid-stream urine samples obtained within an interval of a few days. Kass also showed that

TABLE XII. Possible 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
Slow bacterial growth
Contaminated specimen

$< 10^7$ CFB/L indicated contamination during sample collection, whereas bacterial concentration in the interval of $10^7 - < 10^8$ CFB/L was difficult to interpret. Despite the fact that the criteria were developed for acute pyelonephritis and asymptomatic bacteriuria in women, they began to be used generally, even for symptomatic lower urinary tract infection. Only the discovery that *S. saprophyticus* was a common cause of seasonal, aggressive cystitis in younger women resulted in the cut-off of significant growth being lowered to $\geq 10^7$ CFB/L in these cases.

7.2.2.3. Significance of low bacterial concentration in urine. Stamm *et al.* examined 187 sexually active young women with dysuria and urinary urgency [160]. Cultures of mid-stream urine samples were compared to urine cultures obtained through suprapubic aspiration or urethral catheterization. "Coliform" (the term is not defined in Stamm's article and most likely refers to *Enterobacteriaceae* or lactose-positive *Enterobacteriaceae*) bacteria were isolated from bladder urine in 98 (52%) women, whereas non-coliform bacteria such as *S. saprophyticus*, *S. aureus* and enterococci were cultured in 26 (14%). The women who had "coliform" bacteria in bladder urine were further analysed regarding the number of CFB/L. If 10^8 CFB/L mid-stream urine was used as a cut-off for "significant" bacteriuria, the sensitivity was 51% and the negative pre-

dictive value was 65% (Table XI). Specificity was high. If, on the other hand, a cut-off of 10^5 CFB/L mid-stream urine was used, the sensitivity was 95% with a negative predictive value of 94%, whereas specificity declined from 99% to 85%. Among the 48 who had $<10^8$ CFB/L mid-stream urine of "coliform" bacteria, at least one additional bacterial species was isolated in 35 cases, i.e. there was mixed flora.

Thus, low cut-off of "coliform" bacteria in mid-stream urine more accurately predicted bladder infection in symptomatic women than in asymptomatic. The main differences between Kass's and Stamm's studies were the prevalence of infection in the patient populations, as well as the presence of symptoms (dysuria). Thus, the chosen cut-off for "significant" bacteriuria should be adjusted to the population under examination.

Many additional studies support the observation that low bacterial concentrations of *E. coli* in particular have diagnostic relevance, even in mixed flora [33, 196–205]. Findings of *E. coli* have been interpreted as the first phase in urethritis in an ascending infection. Causes of low bacterial concentrations in mid-stream urine are given in Table XII. Short bladder incubation time (see also Section 3.3.3) is a less important factor than the lowered cut-offs for significance in symptomatic individuals. On the basis of available information, the cut-offs could be set even lower than those proposed in Table XIII, but with the risk of a lower specificity, leading to a decreased predictive value of positive results for clinical urinary tract infection. The importance of specificity should be stressed in diagnostic practice.

7.2.2.4. Mixed cultures. Most acute uncomplicated urinary tract infections result from one bacterial species. The isolation of more than one organism from a single specimen of urine must always be interpreted in the light of (a) whether one organism is dominant, (b) how the sample was collected (chronic catheterization versus mid-stream specimen), (c) the presence of other features suggesting either true infection (presence of WBC) or contamination (presence of squamous epithelial cells), and (d) clinical signs, symptoms and history. True infection with two species (even three occasionally) may occur. Policies concerning how such mixed growths are subsequently investi-

gated and reported should vary according to the local case mix and should not interfere with the needs of specific patients.

7.2.3. Laboratory-related decision limits for "significant" bacteriuria. The following decision limits for diagnostic laboratory workup are proposed (Table XIII) [7]. They should guide the practical process. Consideration has been given to symptoms, bacteria category, number of species isolated, method of specimen collection and gender. One should note the uncertainty of quantitation when using routine loop cultures. A count of 3×10^6 CFB/L (3 colonies on a plate with a 1 μL loop) is more than 10^6 CFB/L, but reproducibility may be within a confidence interval of $1–10 \times 10^6$ CFB/L in routine work. Few laboratories report one colony (1 colony with a 1 μL loop $= 10^6$ CFB/L). It is obvious that the *symptoms and clinical status* of patients are important when evaluating culture results. This implies that considerably greater attention must be paid to the information given on the request and report forms (Appendix, Annex 10.1).

The *cut-offs for symptomatic urinary tract infection* caused by *primary pathogens* (*E. coli* and *S. saprophyticus*, Table IX) are set at $\geq 10^6$ CFB/L in mid-stream urine specimens. For *secondary pathogens* (group II), lower cut-offs of $\geq 10^7$ CFB/L mid-stream urine for women and $\geq 10^6$ CFB/L mid-stream urine for men are recommended. For men, the risk of contamination during specimen collection is much smaller, increasing the specificity of low bacterial concentrations. For *doubtful pathogenic bacteria* (group III) a cut-off of $\geq 10^8$ CFB/L in mid-stream urine is proposed for pure culture in symptomatic patients.

If symptoms are absent, the classical criteria apply for asymptomatic bacteriuria: $\geq 10^8$ CFB/L of the same bacterial species (in two consecutive mid-stream urine specimens, or, alternatively, a positive specific rapid examination and $\geq 10^8$ CFB/L mid-stream urine in one sample).

When *two species are present* in mid-stream urine, it is proposed that only these be regarded in symptomatic patients and that the cut-offs are set at $\geq 10^6$ CFB/L for primary pathogens and at $\geq 10^8$ CFB/L for secondary pathogens. When more than two species are isolated from mid-stream urine, only findings of primary

TABLE XIII.   Suggested limiting concentrations of bacteria colonies justifying identification and susceptibility testing in the laboratory.

Symptoms[a] and specimens	Inoculum, min volume	Species type[b] and number		Significant colony concentration	
				CFB/L	(CFU/mL)
Mid-stream urine specimen:					
Yes[a]	1 μL	I	1–2[c]	10^6	(10^3)
		II	1	10^7 (women)	(10^4)
		II	1	10^6 (men)	(10^3)
		II	2	10^8	(10^5)
		III	1	10^8	(10^5)
No[a]		I–III	1	10^8	(10^5)
Yes (Special)	10 μL[d]	I	1–3[c]	10^5	(10^2)
Suprapubic aspiration specimen					
Yes or no	100 μL[e]	I–IV	1–2	10^4	(10^1)
Specimen from cystoscopy or single urethral catheterisation:					
Yes or no	10 μL[d]	I–III	1–2	10^5	(10^2)
Specimen from indwelling catheter:					
Yes	1 μL	I–III	1–3[f]	10^7	$(10^4$
No	1 μL	I–III	1[f]	$10^{8, f}$	$(10^{5, f})$

[a] Yes = The patient has symptoms, No = No symptoms, or no information about symptoms.

[b] Suggestive category based on growth characteristics (see Table IX). Species of normal urogenital flora (IV) are examined for susceptibility only if especially indicated.

[c] Usually, only one species is identified if 2–5 similar colonies grow (as locally agreed) and antimicrobial susceptibility is examined. Occasionally, two species may be identified for specific patient populations. Three or more species are usually reported as ''mixed culture'' and considered as contaminants. Susceptibility testing of isolates from mid-stream urine specimens as well as other detailed strategic decisions need local clinical and microbiological consultation.

[d] In routine workup, a 1-μL loop is practical. However, in specific patient groups, such as in patients with certain urological diseases, or in a precise evaluation of patients with simple cystitis, a result at $\geq 10^5$ CFB/L $(10^2$ CFU/mL) and a statistically reliable culture result at 10^6 CFB/L $(10^3$ CFU/mL) may be clinically significant. This is obtained only by using a 10-μL loop. This sensitized culture procedure should be specially requested to avoid inadvertent extra work and costs caused by routine application of a 10-μL loop for all specimens.

[e] Suprapubic aspiration specimens should be cultured from a 100 μL inoculum to reach the highest sensitivity, since $>10^4$ CFB/L $(10^1$ CFU/mL) and a statistically reliable culture result at 10^5 CFB/L $(10^2$ CFU/mL) may be significant.

[f] Three species are isolated on special request only from symptomatic patients (suspicion of pyelonephritis or urosepsis). Susceptibility testing from catheter specimens is done only for *E. coli* and Gram-negative bacteria if they are present at concentration of 10^7 CFB/L $(10^4$ CFU/mL) per species, or more. For asymptomatic patients, a higher limit of significant growth (10^8 CFB/L) is suggested for Gram-negative bacteria.

pathogens are reported. When no primary pathogens grow, ''mixed culture'' is noted.

Lower cut-offs are proposed for samples obtained *via suprapubic aspiration, cystoscopy and single (in-and-out) urethral catheterization*. Samples from patients with indwelling catheters are handled depending on symptomatology: In symptomatic patients all isolates are considered for identification and susceptibility testing. In asymptomatic individuals the analysis is focused on Gram-negative rods.

Findings of bacteria belonging to the *normal flora of the urethra and genitalia* (group IV) are reported as specimen contamination. Further examination should be done only if especially indicated after individual evaluation.

The reduced cut-offs for ''significant'' bacteriuria necessitate cultures performed on larger volumes of urine. With inoculation of 10 μL, one colony would then correspond to 10^5 CFB/L. When culturing suprapubic aspiration specimens, a 100-μL inoculum is recommended to approach the sensitivity $\geq 10^4$ CFB/L. A statistically reliable positive result (using the limit of 10 colonies on a plate) corresponds to 10^6 CFB/L (10 μL inoculum) and 10^5 CFB/L (100 μL inoculum), respectively.

7.3. Bacterial cultures

The culture methodology is structured into three levels to satisfy different clinical and

bacteriological needs (see Section 5.1 for general definitions): Qualified comparison methods (Level 3; due to the lack of documented reference methods), quantitative field methods (Level 2) and ordinal scale or rapid methods (Level 1). Individual laboratories and their customer clinicians must decide, on the basis of local patient populations and resources, the way in which urine cultures should be organized locally.

7.3.1. Comparison method for bacterial culture (Level 3). A qualified comparison method (Level 3) is needed to establish traceability of local variations in technology. This method can also be chosen for individual patients or patient groups. The indication for more extensive culture is usually evaluation of results from routine methodology, or from other examinations suggestive for urinary tract infection, such as those from rapid examinations.

Inoculum: Since the cut-off for detection of bacteriuria is reduced, a greater culture inoculum is necessary. With a precisely pipetted 10-µL inoculum, a culture result of 10^6 CFB/L is equivalent to 10 colonies on the agar surface, whereas 10^5 CFB/L equals only 1 colony on a plate. At 10 colonies, a minimum imprecision $CV = 30\%$ $(SD = \sqrt{10})$ is due to the uneven distribution of bacteria in the urine. For more critical applications, such as evaluation of a particle analyser, a more accurate procedure is needed, e.g., by pipetting 100 µL volumes of serially diluted specimens (see Annex 13.1.).

Culture: The quantitative culture should be performed on CLED agar in aerobic, on blood agar in anaerobic, and haematin agar in CO_2 conditions for 48 h, as opposed to the 24-h incubation of CLED agar in the routine method. Comparisons with blood agar are particularly important to describe and follow the reliability of the routine culture method for accreditation of microbiological diagnostic procedures. The blood agar under anaerobic conditions is recommended in parallel for the following reasons: it serves as a quantitative reference, reveals false-negative results on CLED plates, improves the isolation of Gram-positive bacteria, assists with the identification of isolated species and helps in differentiating contaminants from uropathogenic species, thereby increasing diagnostic

specificity. Details of the comparison method for bacterial culture are explained in Appendix, Annex 13.1.

7.3.2. Routine cultures on plates. Common sense is obviously 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 traditions and costs of health care in different countries. The ideal in terms of patient preparation, specimen collection and transport, and finally in the analytical process may not be attainable. Previously suggested significance limits and volumes of inocula (Table XIII) serve as goals for optimal practice. Expert microbiology advice is necessary in locations possessing only minimal resources.

Inoculum: A 1-µL disposable loop inoculum is accepted for routine practice although it is less than optimal. On special request, higher volumes should be used as recommended. Statistical reliability starts at 10^7 CFB/L with a 1-µL inoculum, but this is also influenced by the accuracy of the fluid volume in the loop. The statistical unreliability of $2-3$ colonies growing on a plate is understood and considered when expressing the limits of significant growth in Table XIII, but accepted in routine practice.

Culture: Quantitative culture should be performed at least on a relatively non-selective agar plate, such as Cystine-Lactose Electrolyte Deficient (CLED) medium. Alternatives to CLED agar also exist [5]. In addition, culture on blood agar is recommended as an optimum approach ([6, 7]; see Appendix, Annex 13.2). Incubation for 24 h is sufficient though not optimal as routine. It can be less reliable when fastidious organisms cause infection or when mixed culture is obtained. Inoculation of four different specimens on a single plate ("quarter-plate technique") is prone to contamination and reading problems. It is, however, understood that it appears advantageous particularly in large laboratories with high throughput. Strategies to reduce the number of non-significant bacterial cultures are highly encouraged to improve the quality of those cultures that are clearly indicated.

The uncertainties of the routine process should be controlled by the comparison

method (Section 7.3.1). The addition of blood agar incubation in a CO_2 atmosphere for $1+1$ days will increase both sensitivity and specificity. Depending on the success of these adjustments, the routine process may be considered as a quantitative method (Level 2) or an ordinal scale method only (Level 1). Details of routine culture are included in Appendix, Annex 13.2.

7.3.3. Dipslide cultures. Dipslide cultures can be considered suitable for identifying negative specimens and those with significant growth of *E. coli* at ordinal scale level (Level 1). For reliable results, reading by laboratory professionals, or special training to clinical personnel is recommended.

The urine dipslide system has developed from devices originally designed over 30 years ago [206]. It presently consists of agar media coating both sides of an immersible plastic paddle that can be dipped in urine at point-of-care, and sent to the laboratory in a sealable universal container. Media usually consist of a relatively non-selective agar such as CLED on one side and a Gram-negative selective medium such as MacConkey on the other. There may also be a third section of agar that detects beta-glucuronidase activity by producing brown or black colonies [207]. These devices provide comparable results to those obtained using standard loop inoculation methods [208], and may be useful where delays between the point-of-care and the laboratory are either unavoidable, unpredictable, or both, and where no facilities for refrigeration are available. In two studies, the selective agar has been shown to have a high sensitivity (95.5%, 92%) and specificity (97.2%, 100%) for detecting *E. coli* in urine [207, 209]. These studies were performed in clinical microbiological laboratories – and did not evaluate the interpretation of dipslide cultures by primary health care personnel.

Problems with species identification: Estimating growth on CLED compared to MacConkey agar in order to differentiate Gram-positive from Gram-negative bacteria has far too many sources of error to be useful in practice. For example, enterococci and staphylococci can grow weakly on MacConkey, fungi grow on MacConkey and certain *Pseudomonas* species do not grow on MacConkey agar. In addition, the dipslide technique does not allow identification of *Staphylococcus saprophyticus*.

Problems with quantitation: Inoculum on a dipslide is not reproducible. Any colony count is therefore uncertain. At high bacterial concentrations, confluent growth necessitates a subculture for identification. Mixed flora may also be missed.

To reduce the risk of false interpretation, dipslide-culturing should be viewed as an ordinal scale method for bacteriuria, combined with the possibility of identifying *E. coli*. It may be possible to satisfactorily handle up to 90% of ambulatory urine cultures requested for suspected urinary tract infection with this simpler rapid procedure. Details of the method are described in the Appendix, Annex 13.3.

7.3.4. Enrichment cultures. Enrichment cultures from urine using bottled broth media are discouraged because no quantitative results are obtained. Specificity is poor due to inevitable overgrowth of commensal organisms.

7.3.5. Identification of species. A proposal for identifying species of urinary isolates in routine diagnostics is compiled in the Appendix, Annex 13.4. It is based on the practice where cultures are performed on CLED and blood agar. The tabular data suggest at least the minimum criteria for identification of each species.

7.3.6. Antimicrobial susceptibility testing. The goal of susceptibility testing 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 should usually be examined when isolated in clinically significant concentrations. To reduce the workload in laboratories, it is, however, sufficient to rely on the *predicted* antibiotic sensitivity of individual bacterial species when community-acquired lower urinary tract infection is suspected in low-risk female patients, and the information on local resistance in strains is available (then, even bacterial culture may be unnecessary). The antibiotic sensitivity patterns of individual species vary considerably according to location, patient case mix and background antibiotic usage.

Choice of method. Standardized disc diffusion is the frequently used method. Non-standardized disk diffusion, so-called "direct

antimicrobial susceptibility testing", is used in many laboratories to increase the speed of diagnostics. Comparisons have shown a 96% agreement with the standardized practice [210–212]. The method can be useful provided adequate exclusion criteria are in place. Trained personnel should be responsible for the method and it *should not be used* on mixed culture or on low bacterial concentrations ($< 10^8$ CFB/L) because the results are not clear-cut. Thus, rapid methods can assist by allowing laboratories to incorporate direct susceptibility testing into their work-flow only for those specimens with abnormalities likely to represent a true infection (Fig. 1).

Several alternative microdilution methods are commercially available for automated or semi-automated susceptibility testing. Some of these provide acceptable accuracy in comparison with the traditional reference methods for antimicrobial susceptibility testing; they also provide results within a working day or sooner.

Further specific instructions concerning the procedures for antimicrobial susceptibility testing are beyond the scope of this document. Guidelines exist that describe how such examinations should be performed, such as the British document [213], the NCCLS document [214] and reports of the Swedish Society of Medicine's Reference Group for Antibiotics (SRGA-M) [215], and German guidelines [216]. Local recommendations on the practice and minimum selection of antibiotics are advised to increase clinical effectiveness of antimicrobial susceptibility testing and to direct clinicians to the use of inexpensive and effective antibiotics least likely to generate resistance.

7.3.7. Detection of antimicrobials in urine. Antimicrobial drugs can lead to failure of bacterial growth despite the presence of large numbers of bacteria in the urine. If bacteria or leukocytes are detected by microscopy, confusion occurs if no subsequent growth is seen. We suggest the following as the most economic approach: *Request forms should be designed so that the presence and type of antibiotic therapy can be documented by the requesting physician at the time the specimen is sent* (see Appendix, Annex 10.1.1). This will eliminate the necessity to perform any examinations.

If clinical information on ingested antibiotics by the patient cannot be easily transferred, a surrogate method is to measure concentrations of the most common anti-microbial agents in urine. Laboratories may also choose to detect antimicrobial activity (AMA) by placing a drop of urine on a lawn of sensitive *E. coli*; a zone of growth inhibition will form in a positive case on the site where the urine was placed. Another option is *Bacillus subtilis* (ATCC 6633) grown on Mueller-Hinton agar [6]. A report stating that antimicrobial activity was detected is then given.
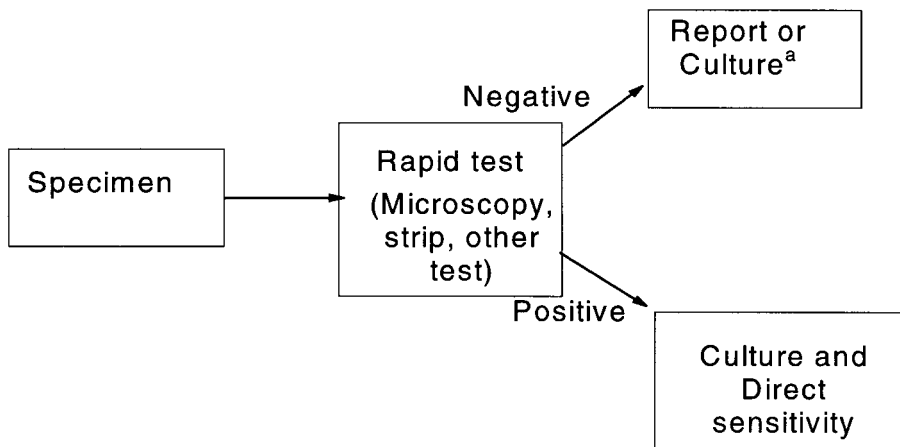
ᵃ Depends on the diagnostic sensitivity and specificity of screening = rapid test technique

FIG. 1. Application of direct susceptibility testing in microbiological investigation of urine specimens.

7.3.8. Automation of bacterial culture. For large microbiology laboratories, automated urine culture may be an option in addition to the methods used for rapid detection of potentially positive cultures. Several automated systems exist which satisfy the requirements of Level 2 cultures. Advances in technology mean that laboratories, especially large ones, will be tempted to assess analysers that can replace expensive manual work. Finally, it is recommended that any new automated technique be assessed against acceptable Level 3 comparison methods. Evaluation should preferably be three-way, where performance is also compared to the currently used method (usually not Level 3).

7.4. Bacterial detection by non-culture (rapid) 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 patients groups, such as pregnant women. Development of analytically sensitive and specific rapid procedures for detection of bacteria is encouraged both for adults and children [217].

Sensitivity view: A high performance high-throughput screening procedure with low false-negative rates (ideally $<10\%$) would identify true negative specimens and allow significant reduction in costly and unnecessary urine culture. Health care savings would then be achieved even in the face of an appreciable false-positive rate. The validation of method performance for detection of bacteria at concentrations less than $10^8/L$ ($<10^5/mL$) becomes very important.

Specificity view: Acutely ill patients need an examination with high specificity (preferably $>90\%$) to demonstrate the presence of bacteria reliably when the clinical presentation is not obvious from symptoms and signs. In these cases, a positive specific result from a rapid diagnostic procedure supports a correct immediate treatment decision, while the rest of the cases await results from bacterial cultures [218]. A typical question is how does a method correctly classify contaminants and debris as negatives.

Measurement principles. Multiple test strips including the nitrite field are described in Section 5.3.1. Several new technical developments have been suggested [63, 67, 70, 93, 132, 219–222]. These include enzymatic examinations based on adenosine triphosphatase/luciferase, catalase or glucose oxidase, filtration methods, advanced microscopy of Gram stained smears and automated flow cytometry. Some of these methods are well suited to point-of-care testing, but sensitivity and specificity of procedures must be investigated and quoted in relation to the patient population studied. Others require laboratory-based experience for their performance, interpretation, or both.

Urine particle analysis is discussed in Section 6. Bacterial analysis may theoretically adopt either direct particle counting (of living and dead bacteria) or automated rapid culturing of urine in selected media. Laboratory staff must remember that these analysers may detect particles and not growth. In microbiology evaluation, the arising new view is that some specimens may contain true, aggressive pathogens that do not grow on conventional agar plates. There may also be the issue concerning the presence of antibiotics in the urine that inhibit growth. Finally, it should be noted that automated techniques assigning Gram status to organisms were very helpful since they could predict effective antibiotic treatment.

8. STEPWISE STRATEGIES IN URINALYSIS

8.1. Examinations for general patient populations

Most of the costs arising from screening programmes result from the need for confirmation of positive findings. Screening of completely unselected individuals, i.e. at general epidemiological level, is discouraged except for research purposes. Selected asymptomatic individuals may be investigated if justified by cost/benefit analyses, e.g., pre-school children or pregnant women [88, 223]. Examinations for diseases in kidneys and urinary tract can be recommended for many clinical populations (=patients attending health care services in hospitals or at ambulatory clinics because of their symptoms or diseases), but not for all

patient groups. Use of urine test strips, as well as of other laboratory measurements, should always be associated with prognostic significance [224]. The outlined strategy has been derived from earlier recommendations [2, 123, 225, 226].

In addition to the minimum properties shown (Fig. 2), *acute* cases generally benefit from measurements of urinary *glucose, ketone bodies and pH* (urine stone formers). The strategy is only an average general approach and not sensitive to the individual needs of patients. These strategic proposals are designed to improve the general efficiency of urinalysis. Clinical decisions should be based on direct patient data; laboratory and imaging methods provide ancillary information only. The recommendations presented describe some approaches based on the diagnostic performance of the methods available. The term "first visit" refers to the initial diagnostic investigations; the follow-up investigations should be based on evaluation of detailed needs. Consultation of a nephrologist, clinical specialist for infectious diseases, internist, paediatrician or another specialist may be necessary to plan all medically required investigations. A multiple urine examination should be performed at point-of-care or in the laboratory as locally agreed.

8.1.1. Detection of urinary tract infection.
It is important to clearly differentiate high-risk patients from low-risk (recurrent female) cystitis patients when applying any diagnostic strategies for *symptomatic* patients suspected for urinary tract infection (see Section 7.1 for definitions and 8.2 for strategic proposals).

Acute cases may also benefit from a laboratory result within 0.5–2.0 h, while a highly specific result, such as that from bacterial culture, serves to finally classify cases even after 1–2 days.

Some *clinical patients* may have *general or vague symptoms* that were not associated with urinary tract infection initially. These cases then appear as unexpected findings. Exclusion of urinary tract infection at a cut-off of 10^8 CFB/L is possible from concentrated morning specimens with a sensitivity of 90% by rapid methods (see Sections 5.3.1 and 7.4). Urine microscopy should be performed to detect possible renal involvement if relevant. In cases of so-called "sterile pyuria" (no usual uropathogens grown

despite a standardized specimen) it may be appropriate to perform additional investigations to detect or isolate organisms with special growth requirements (*Chlamydiae*, gonococci, *Mycobacterium tuberculosis* and fungal infections) by *collecting another appropriate specimen and investigating it accordingly.*

General screening of *asymptomatic* individuals for bacteriuria should be avoided.

Bacterial culture is initiated only if the asymptomatic bacteriuria is going to be treated with high probability (Section 8.2.3 for detailed patient groups).

8.1.2. Detection of proteinuria.
Transient proteinuria is a fairly common finding among acutely ill patients [227] even if detected at the traditional 0.2 g/L albumin level. In these cases, an occasional positive result may lead to inappropriate investigations. Exclusion of transient proteinuria is accomplished by repeated measurement and review of anamnestic data.

Quantitative specific measurements are *generally* not part of a primary screening process for *general patient populations*; they are second-line investigations. Total albumin or protein quantitation can be requested – if not already the first-line method – as albumin or total protein/creatinine ratio (or albumin or protein/osmolality ratio), or as measurement of 24-h protein excretion rate. Orthostatic (postural) proteinuria can be identified by separate day and overnight collections. *Selected patient groups* do require a sensitive protein determination to detect renal damage already in the first line. In addition to albumin and a tubular marker protein, immunoglobulin light chain determinations may be important (see Section 8.3).

8.1.3. Detection of haematuria.
Exclusion of other coloured substances (red beets, porphyria, drugs; Table II, page 14) should always be a first thought. Particle analysis is needed to obtain a count of RBC excretion from a standardized specimen. Renal elements may be seen on urine microscopy. The RBC should be assessed for isomorphism, dysmorphism and the presence of acanthocytes if no proteinuria is present. This must be performed in a laboratory capable of distinguishing the different types of RBC morphology.
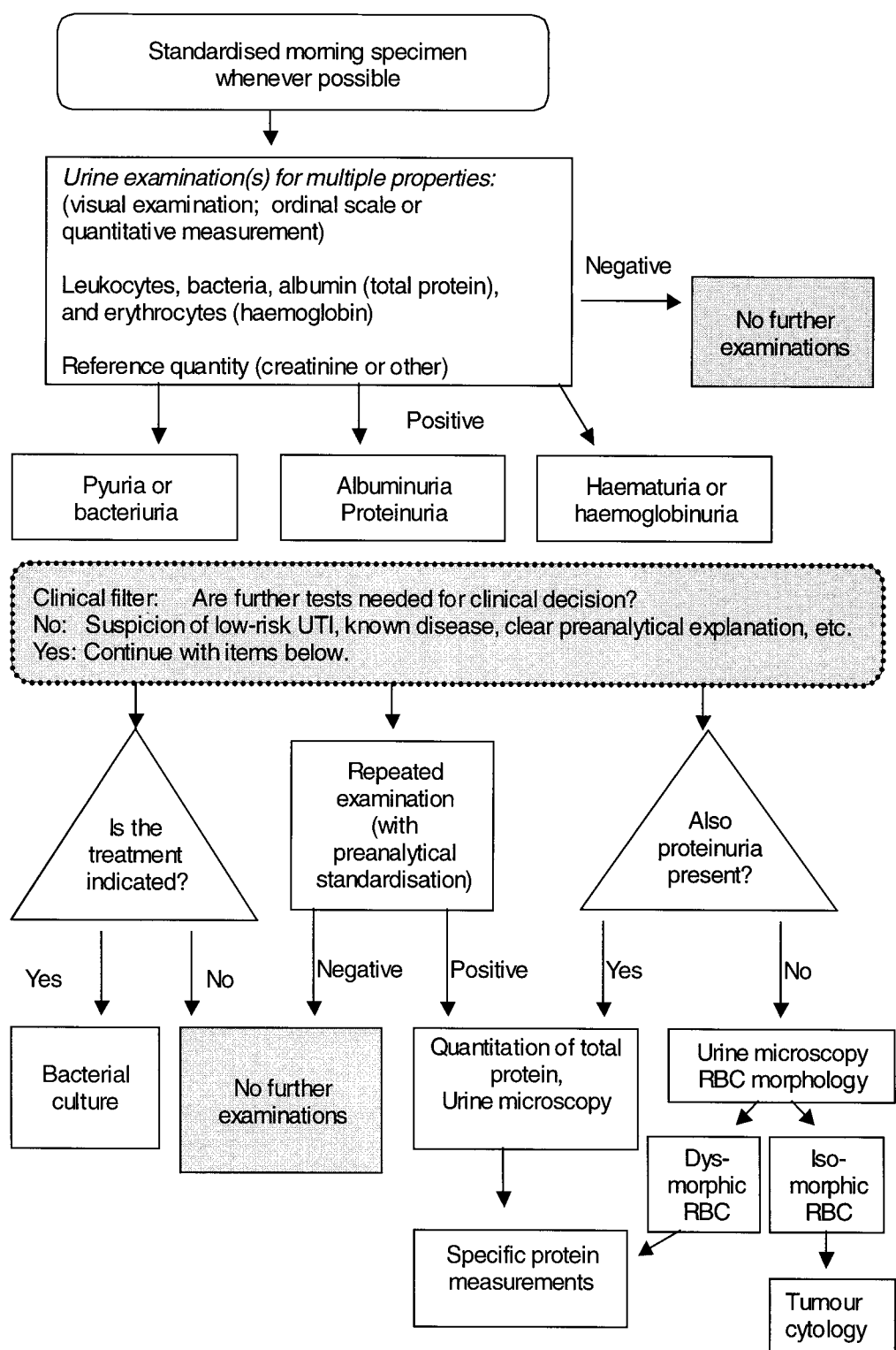
FIG. 2.   Urinalysis examinations for general patient populations.

An alternative approach is differentiation of the bleeding site based on urinary IgG/albumin and alpha-2 macroglobulin/albumin ratios as measured in a centralized laboratory [123].

Urine sediment examination only occasionally offers additional information after a test-strip measurement in unselected emergencies [71], but it helps in predicting renal damage if investigated carefully from a standardized specimen [22]. When the test-strip result is negative for erythrocytes, leukocytes and albumin/protein, sediment microscopy is not generally warranted [228, 229]. Automated particle counting possesses higher precision than visual urine sediment examination, but it may not detect all significant renal elements [69].

A clinical (urological and/or nephrological) consultation is usually needed to search for possible local or systemic diseases causing haematuria. The presence of a haemorrhagic diathesis should not be forgotten. Urothelial and renal *cancers* often present as unexpected haematuria. On the contrary, prostatic cancer rarely presents with haematuria or proteinuria. Detection and follow-up of prostate cancer is undertaken by measurements of serum PSA (prostate-specific antigen), currently as total and free PSA molecules. Screening for bladder tumour cell antigens in urine is under development. Experienced cytopathologists must interpret urothelial tumour cell cytology.

8.2. Detection of urinary tract infection, specific populations

The suggested sieving strategy to reduce the number of bacterial cultures in patients suspected for urinary tract infections (UTI) is illustrated in Fig. 3. Cultures of low-risk patients are not needed [207, 219] with given precautions.

This strategy can be difficult to follow exactly because of problems with specificity, such as contamination and false-positive reactions, and sensitivity, because rapid detection methods for uropathogenic bacteria are not perfect to date. Despite this, such strategies should help in reducing traditional cultures markedly, but should remain sensitive to needs of problematic or specific cases. For example, it may be acceptable to use a highly specific rapid examination for identifying positive patients and for excluding them from further investigations for urinary tract infection, whilst it is understood that such sieving strategies must not be applied in high-risk cases.

8.2.1. Symptomatic low-risk patients. Low- and high-risk patients with respect to urinary tract infection were defined in Section 7.1. Low-risk patients with suspicion of lower urinary tract infection can be examined as follows: No investigations are needed if the diagnosis is clear from the symptomatology. Empirical treatment can be justified with known local epidemiology of community-acquired infection. If the symptoms are not clear, a specific examination (specificity $> 90 - 95\%$ for uropathogens) allows a rapid confirmation of bacteriuria and justifies treatment. Symptomatic low-risk cases remaining negative with the specific rapid examination (during emergency hours) should be treated or investigated by a culture method after obtaining a standardized morning specimen.

Follow-up of low-risk patients should be organized as follows: if no symptoms remain after treatment, no further examination is needed. If symptoms persist, bacterial culture with antibiotic susceptibility testing is warranted.

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. This gives valuable information on relapsing infections often due to reduced antibiotic susceptibility of the species. National and regional efforts to create and maintain these data are encouraged.

8.2.2. Symptomatic high-risk patients. Specimens from *symptomatic high-risk patients* should always be cultured for uropathogenic bacteria both to confirm the diagnosis and to ensure treatment (with the help of antimicrobial susceptibility testing). Urgency of micturition may prevent sufficient bladder incubation time, leading frequently to false-negative results – even with culture methods. Significant growth may be as low as $10^5 - 10^6$ CFB/L (corresponding to $10^2 - 10^3$ CFU/mL), occasionally needing a culture with a 10-μL (or even 100-μL) inoculum to reach this sensitivity
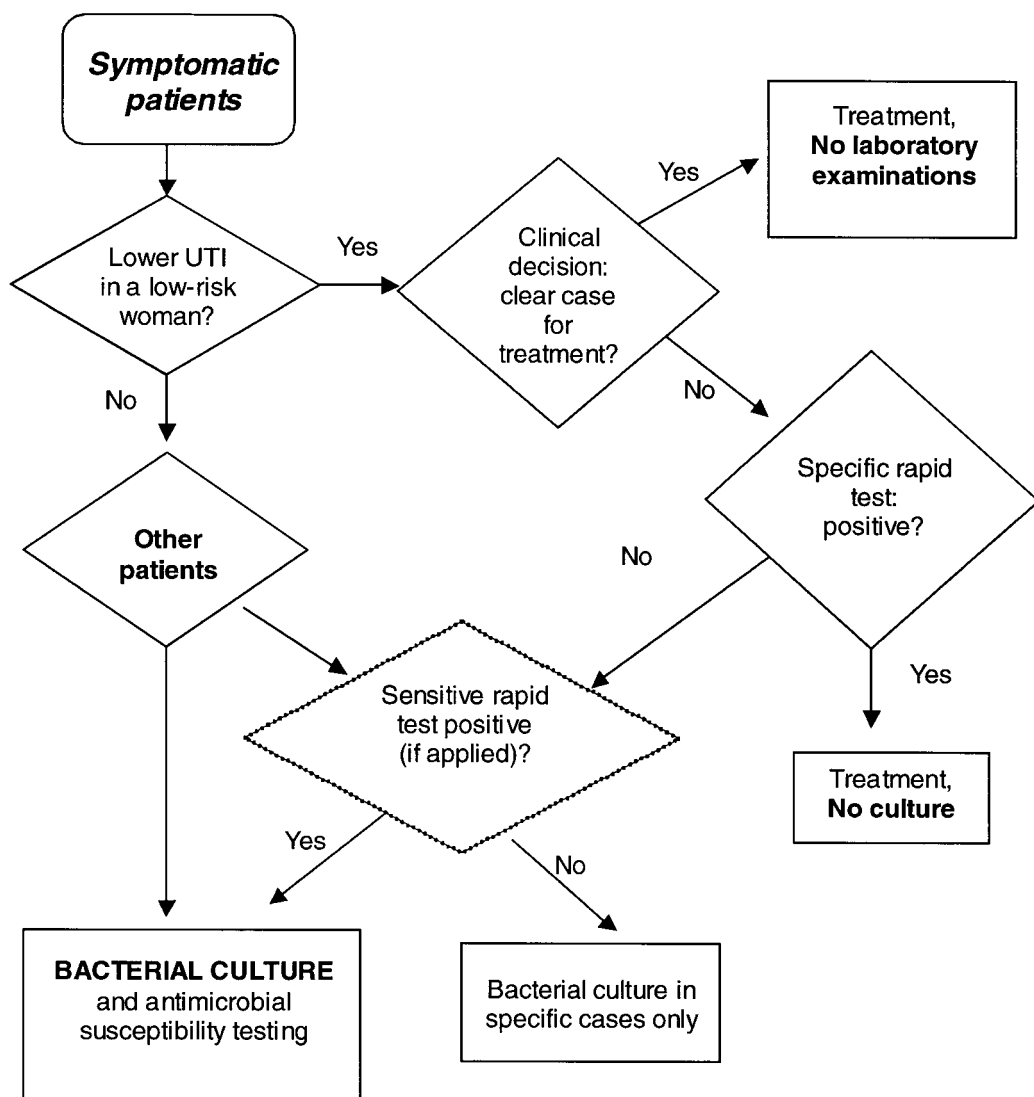
FIG. 3.   A sieving strategy to reduce requests for bacterial cultures.

reliably. The specificity of low-count findings should be considered. Local microbiological consultation is recommended to establish rational routine processes.

8.2.3. Asymptomatic bacteriuria. The need for investigation of *asymptomatic* individuals is questionable. Liberal treatment of patients with asymptomatic bacteriuria by using antimicrobials results in development of multiresistant strains that replace the less dangerous original species.

Exceptions to be treated may include pregnant women (a prevalence of about 5% for asymptomatic bacteriuria), some patients undergoing urogenital surgery (if the indwelling catheter is going to be used postoperatively) or receiving organ transplant, and many immunocompromised individuals. During pregnancy, bacteriuria is treated to prevent symptomatic infection and premature birth [230, 231]. A rapid method, reaching analytical sensitivity $>90–95\%$ at 10^8 CFB/L (equal to 10^5 CFU/mL) when compared to bacterial culture is

recommended, with confirmatory culture of positive cases.

8.3. Sensitive detection of renal disease – specific populations

Table XIV lists examinations used to detect renal disease in patient populations with high-risk for renal damage. The minimum and optimum lists refer to economical possibilities by different health care service providers.

Primary renal disease may present with symptoms. Haematuria and albuminuria, sometimes even leukocyturia may be demonstrated by sieve measurements; in addition, quantitative total protein excretion (measured as a 24-h excretion rate, or protein/creatinine ratio of morning urine) is usually elevated. In tubular disease, defect in urine concentrating capacity may be demonstrated (measured by relative density or osmolality). Albuminuria is a major feature in most glomerular diseases. Monitoring for side effects from clearly tubulotoxic drugs, such as aminoglycoside antibiotics, many cytotoxic drugs and some non-steroidal anti-inflammatory drugs is possible with measurements of urinary α_1-microglobulin, retinol-binding protein (RBP) and other markers (see Section 5.4.1).

Measurements of GFR are important in renal disease. Urine creatinine measurement is also needed for calculation of creatinine clearance. Positive findings from these investigations should be followed by more specific diagnostic procedures. In central hospitals, definitive

TABLE XIV. Measurements for sensitive detection of renal disease in urine.

Minimum measurements
Urine albumin (20–30 mg/L) and total protein (quantitative, sensitivity 50 mg/L)
Urine microscopy
Optimum quantitative measurements additionally
Urine albumin (sensitivity 2–10 mg/L)
Urine α_1-microglobulin (sensitivity 2–10 mg/L), retinol-binding protein, or another tubular marker protein
Urine creatinine
(or another reference quantity for volume rate)
Optional urinary proteins in special cases
Ig, κ and λ light chains, α_2-macroglobulin
Other diagnostic examinations (based on clinical presentation)

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diagnosis is often based on renal imaging and biopsy.

Secondary renal diseases are more common than primary due to the high prevalence of diabetes mellitus, hypertension and systemic rheumatic diseases. For specified asymptomatic patient populations, sensitive screening programmes should be established when improvement in life expectancy or cost containment is anticipated. Currently, such a screening programme is established for incipient diabetic nephropathy by sensitive measurements of albuminuria. Renal involvement in hypertension should be screened at least by measurement of total protein excretion (24-h collection or total protein/creatinine ratio) if sensitive albuminuria measurements are beyond existing resources. Renal disease secondary to multiple myeloma may be detected by finding immunoglobulin light and occasional heavy chains in urine in association with glomerular or tubular proteinuria.

The role of urine particle morphology is to confirm the presence of renal damage (casts, renal tubular cells, dysmorphic red cells) because non-renal proteinuria due to, e.g., fever, epileptic seizure or cardiac insufficiency is also frequent. Occasionally, renal elements may occur in urine due to secondary damage caused by abdominal or retroperitoneal (surgical or gynaecological) disease, or hypotension due to cardiogenic shock. The analyses of both urine proteins and formed elements complement each other in detection and follow-up of renal damage. The former are more sensitive but less specific, while the latter are more specific but less sensitive markers of renal damage.

9. QUALITY ASSURANCE

9.1. General principles

Urinalysis faces many challenges when its quality is being defined formally. While traceability and description of uncertainties are well established in clinical chemistry and haematology, these concepts are still premature to the semi-quantitative or ordinal scale examinations used in urinalysis. For morphological analysis, systematic peer reviews are being used in cytopathology to reach the highest possible agreement between observers [232–234]. In

microbiology, error in the identity or in a count obtained from cultured colonies may be difficult to set into an acceptable statistical framework. Quality assurance practices must also encompass point-of-care testing [235 – 237]. Finally, the increasing costs of health care tend to challenge the acceptable minimum requirements [238]. These guidelines are sensitive to such pressures and are designed to propose practical solutions.

Publications already exist at a general level [239 – 241] and specifically for quantitative serum chemistry [242]. A Scandinavian group has advised on assessment of analytical quality specifications from a theoretical point of view [243]. In urinalysis, the healthy concentrations or excretion rates of many substances or particles are traditionally reported as ''negative'' rather than numerically. The performance goals at detection limits must take into account the large biological variations that occur at low concentrations characteristic for the upper level of the reference range in health.

Adherence to the international vocabulary of metrological terms (VIM) is encouraged [126, 244 – 246], although this was not done thoroughly in these guidelines owing to their only gradual acceptance in routine work. Substance-based SI units (e.g. mmol/L) should replace conventional mass-based (e.g. mg/dl) units in expressing results. The volume unit litre (L) has been used throughout this document, including bacterial colony counts (particle concentrations), as an example of this standardization. The term accuracy now encompasses both precision and trueness of measurements; and, inversely, inaccurate measurements may be erroneous because of systematic effects (bias) or random effects (imprecision).

9.1.1. Quality systems. The concept and practice of Total Quality Management (TQM) is now commonplace in many clinical laboratories. Laboratory practice can be divided into three stages (pre-analytical, analytical and post-analytical) all of which must be quality assured in order to obtain reliable chemical and microbiological results for patient care [247].

The essential components of quality systems may be created nationally with reference to published national guidelines. Organization of Good Laboratory Practice (GLP) should address systematically all facets of laboratory activity. Accreditation based on such international standards as EN45001 and ISO/IEC Guide 25, or the later ISO17025, should be considered for all facets of urinalysis as well. These guidelines encourage local establishment of quality systems even in small European laboratories working under the supervision of specialized laboratories. Quality systems need regional co-operation and support.

9.1.2. Quality manual. General principles of good laboratory practice apply to all clinical laboratories. The quality system is described in the quality manual of a laboratory. The following contents were suggested by a Scandinavian group [248]. As an example, some specific views are reminded for microbiological investigations as performed in general laboratories under the supervision and support of a local microbiology laboratory.

9.1.2.1. Description and identity of laboratory – In most European countries, national governments have designated, by decree, certain microbiology laboratories to investigate infections of epidemiological importance. National regulations may also apply to other microbiological examinations, such as cultures from urine.

9.1.2.2. Quality policy. (Modified from the EN45001 suggestion for a policy statement)

''The aim of the laboratory is to provide clinically useful information through laboratory examinations of samples from patients, taking into account the allocated resources. The quality policy is implemented by the following means:

Proper specimen collection, labelling and transport

Reliable analytical work

Turnaround time within specified limits

Results reported in a clear format and supplemented with relevant explanatory information (either together with the result or in a separate laboratory handbook)

Appropriate communication is maintained with clinical customers to ensure correct integration of laboratory information into evaluation of patients''.

9.1.2.3. Staff and education. Training and certification of competence: The necessary educational requirements for certification of specialist competence should be documented.

Training and certification should be carried out in consultation with the microbiology laboratory. Competence should be updated regularly (e.g., yearly) and after long absences (e.g., absences for longer than 6–12 months).

9.1.2.4. Management and quality assurance. The documentation and procedures describing the quality system in detail must only be changed with the authority of the Head of the laboratory or persons nominated to authorize such changes. These documents must form a laboratory manual (with separate appendices), relevant sections of which are issued to staff. When changes are made, all working copies of the modified document must be updated. The old copies must be stored (one archival copy) or destroyed (at the working sites).

Continuous quality improvement is a result of professional external and internal audits, in addition to development of analytical techniques. Audits should be arranged on a regular basis, e.g., once a year.

The laboratory manager must have access to advisory documents issued by relevant bodies including health notices, safety information bulletins, health equipment information, health building notes and health equipment notes.

9.1.2.5. Records, maintenance and archiving. Final patient records and quality assurance data should be archived according to national or institutional regulations, typically 3–15 years, to allow later investigation for clinical, epidemiological and, occasionally, for legal purposes. Laboratories should also keep records on their maintenance.

9.1.2.6. Facilities. Careful planning is needed in siting a microbiology laboratory to ensure stable environments (temperature, atmosphere, humidity, etc.), minimum contamination risks between specimens and safety of personnel.

Facilities must be available which are designed to be safe for the processing and containment of specific pathogens.

9.1.2.7. Equipment and material. In both manual and automated laboratory work the quality of equipment should be maintained and its performance monitored daily. In urine culture, this includes, e.g., incubators with their required temperatures and atmospheres, and culture plates.

9.1.2.8. Safety and environment. Regular handling of biologically hazardous material needs special attention in routine procedures. The details of safety management should be organized and explained.

The facility must comply with current safety legislation.

9.1.2.9. Research and development. The quality manual should state how a laboratory is developing its routine programmes. Possible participation in basic research should be quoted as well.

9.1.2.10. Measurement procedures. Reference materials and methods produced by manufacturers must be clearly described to allow audit and traceability of measurements.

Detailed operating procedures for any investigation must be in place, as must instructions for use of equipment.

Level of identification of uropathogenic species must be discussed with the local microbiology laboratory: a small laboratory may decide to report only negative culture results or to undertake a preliminary typing of positive cultures in agreement with the supporting microbiology laboratory.

Reliable microbiological results are often dependent on detailed adherence to specific procedures. Procedures and interpretation charts should be followed strictly. The medically responsible physician or other specialized clinical microbiologist, who should be available, must have approved these.

Both commercial kits and media developed in the laboratory should be verified in a traceable manner (standardized procedures and strains obtained from international collections).

It is important that the number of examinations is large enough to maintain the level of skill (approximate minimum is about 20 urine cultures/week).

9.1.2.11. Request protocols, sample collection and handling of laboratory samples. Instructions for the receipt and recording of specimens must be clear and unambiguous.

Detailed clinical information, including current antimicrobial treatment as well as the real time and anatomical localization of the obtained specimen (sample), is crucial for interpretation of bacterial cultures. In urine cultures, the collection procedure is of similar importance (see Annex 10.1 for detail).

9.1.2.12. Verification of results. Data transfer to individual patient records may be vulnerable in manual work with plates. Procedures for specimen identification, verification of results and reporting on paper or by electronic data processing systems should be explained.

9.1.2.13. Analytical control. A method must be set in place for monitoring results, with the ability to recognize those where failures have arisen due to laboratory or human error.

External quality assessment (EQA): laboratories should participate in external quality assessment programmes. The purpose of EQA is to assess how well a laboratory fulfils the analytical quality specifications of bacterial culture (Section 9.5.2).

Current results of EQA must be displayed for all to see if they wish and a file of all results for the past few years must be held and available for inspection.

Internal quality control (IQC) by recommended strains should confirm the daily process.

IQC of even dipslide cultures should be organized with the microbiology laboratory.

Organized submissions of sample cultures (dipslides and plates) to the supporting microbiology laboratory for verification are also very useful (see Section 9.5.2 and Annex 13 for detail).

9.1.2.14. Report. The procedures for issuing reports must be clear and unambiguous.

Delay in reporting results from bacterial cultures should be avoided and must satisfy the clinical need for treating the patient (see Section 9.5.3).

Reasons for any delay in reporting results beyond the agreed limits must be identified, as must the likely clinical impact of such delay. Corrective measures must then be applied.

9.1.2.15. Co-operation with the clinicians, ward staff and patients. Laboratory handbooks should explain detailed procedures for specimen collection and transport of microbiological investigations, including those for urine collection. Interpretation of usual culture results, antimicrobial susceptibility testing and rapid microbiological methods should also be included in the handbook.

Physicians with specialist competence in clinical bacteriology should be available for clinical consultation.

9.2. Test strips and equivalent rapid examinations

9.2.1. Trueness of measurements. The ordinal scale (semi-quantitative) measurements are usually expressed as categorized data. 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. Reference methods, according to a strict definition, are currently unavailable.

It is recommended that evaluation data be classified within two limits obtained from the comparison method: a detection limit (L_D; from the point where the ordinal scale method starts to give positive results) and a confirmation limit (L_C; from the point where all ordinal scale results should be positive). These delineate the "grey zone". From experience with test-strip technology, it is recommended that the ratio between the concentrations $L_C/L_D = 5$ (see Appendix, Annexes 11.1.2 and 11.1.3 for detail). Optimal trueness of measurements is suggested to be a **FP rate <10% at L_D and a FN <5% at L_C** (compared with the most accurate, closely related method) (Tables XXIII & XXIV). In many situations, or with a less optimal comparison method, a minimum performance is acceptable (see Appendix, Annex 11.1.1 for principles of measurements). With many comparisons, such as in leukocyte and erythrocyte detection, the different principles of measurement (enzyme activity versus chamber counting) must be understood for correct interpretation. The same applies to comparisons between bacterial culture and rapid chemical examinations, such as the nitrite or similar examinations.

For more than two ordinal scale categories, agreement should be calculated using κ statis-

tics, thus subtracting random agreement from observed data [249, 250] (see Appendix, Annex 11.1.4). The simple κ coefficient is used when there are only two or three classes. For multiple (4 – 5) classes, the agreement should be calculated based on weighted κ coefficients. In this case the sum of expected disagreement exceeds 100% because of the squared weighting factors. Thus, the goal must be tighter (Table XV). It is possible to artificially increase the κ value by subdividing the results into six or more classes, but then the goal for a minimum performance should be even higher (0.75 – 0.8). The quality specifications are based on common sense: any clinical laboratory examination should classify more than half of the non-random cases correctly. Arbitrarily, this is equivalent to κ >0.6, although optimally the value should be >0.8 if achievable.

9.2.2. Precision. Success in reproducing ordinal scale measurements over time is expressed as the fraction of correct results within binomial confidence intervals. It is recommended that specimens with an original probability of close to 100% for the measured category be used, since the follow-up is then most precise due to a narrow confidence interval (see Appendix, Annex 11.1.5.) As an option, despite the final ordinal scale output of patient results, a valid continuous signal, e.g. a reflectance value obtained from the measuring device, can be followed, allowing a normal calculation of performance limits. The low positive range (1+) is more important than the high positive range (3+) in rapid examinations.

For good control, reproducibility (day-to-day variation) should remain within the binomial confidence limits in internal quality control. Stable control solutions are already available for test-strip reading. Daily implementation should then be the rule to allow judgements on the levels of assays within a reasonable time

TABLE XV. Analytical quality specifications expressed as Kappa coefficients.

	Optimum	Minimum
κ coefficient (simple) (2 – 3 classes)	>0.8	>0.6
κ coefficient (weighted) (4 – 5 classes)	>0.9	>0.7

span. Dilutions of control solutions (with buffer or pooled human negative urine) help in following performance at concentrations in the low positive range, where few stable control solutions are available. However, since there is a matrix problem in test-strip reading, stable low positive control solutions would be better than daily dilution of high positive control solutions with aqueous buffers.

9.3 Quantitative chemical measurements

These guidelines acknowledge the efforts of the IFCC in creating reference materials for clinical chemistry [251]. For most urinary constituents, genuine reference materials and reference measurement procedures are awaited. Co-operation between manufacturers in preparing calibrators for their quantitative technologies is greatly appreciated in achieving comparable concentrations in clinical laboratory practice. Recommendations exist for analytical quality specifications for routine quantitative serum chemistry [242]. These should be implemented in both internal quality control [252] and external quality assessment [253]. The formulae suggested to describe the maximum allowable total analytical error for *serum* chemistry are quoted in Table XVI. For reference methods serving serum chemistry, a maximum of half these errors is proposed [254].

For urine chemistry, these proposals should be adjusted to reflect the fact that changes in pathological states may be in a logarithmic scale compared to the low or almost negative concentrations seen in health. Even if chemical analytes are excreted in urine in large quantities, they often occur in skewed distributions. Since the same measurement is often used for both monitoring and diagnostic testing, a combination of quality criteria should be considered.

9.3.1. Calibration of the measurements. Calibration of urinary total protein is preferably performed using the CRM 470 (albumin) standard (see Appendix, Annex 11.2.3). Glucose measurements can be calibrated with the SRM909 material obtained from the National Institute of Standards and Technology [255]. There is also a reference method for urine glucose measurements approved by CDC/FDA/AACC/NRSCL [256]. Calibration of

TABLE XVI. Analytical quality specifications for quantitative routine *serum* chemistry.

MONITORING (precision is important): $TE_{Mon} \leq \frac{1}{2}s_i$, where $TE_{Mon} =$ total error in monitoring, and $s_i =$ within-subject biological standard deviation
DIAGNOSTIC TESTING (trueness/total accuracy is important): $TE_{DT} \leq \frac{1}{4}s_c$, where $TE_{DT} =$ total error in diagnostic testing, and $s_c = \sqrt{(s_i^2 + s_g^2)} =$ composite biological standard deviation; $s_g =$ between-subject biological standard deviation

creatinine measurement can be performed using the SRM914a serum calibrator from the same source [257].

9.3.2. Analytical quality specifications. Analytical quality specifications for **monitoring** are derived from within-subject biological variation of urine constituents. In the case of proteinuria, the average coefficient of variation of one subject ($Cv_{within-subject}$) is $40–50\%$ at the upper reference limit in health [258, 259]. For creatinine, it is about 20% [109, 260], giving an average CV for albumin/creatinine ratio of about 40%, and maximally 80% [258]. This within-subject variation results in an estimate of maximally allowable imprecision ≤ 20 $(-40)\%$. It would allow discrimination between twofold and threefold changes in excretion rates (analyte/creatinine ratios) in multiple monitoring of the same patient, by using the equation for critical difference $D_C = \alpha \times CV$, where $\alpha = 2.77$ at a statistical probability $p < 0.05$.

In discrimination between health and disease (**diagnostic testing**), the average $Cv_{between-subject}$ is about 25% for total protein, 75% for albumin and about $25–30\%$ for creatinine in 24-h output [259, 260]. When calculated as ratio to creatinine concentration in single-voided urine, about 70% for total protein/creatinine and 40% for albumin/creatinine ratio have been reported [109]. The composite biological standard deviation (coefficient of variation) is then about $50–90\%$ for proteinuria, depending on the analyte and method of calculating the excretion rate. The estimate of maximally allowable relative deviation (bias) would be then $\leq 12–25\%$. The validity of these figures must be judged against the low level of physiological proteinuria compared to the levels of clinical albuminuria or proteinuria that are up to 100-fold (10,000%) higher in nephrotic syndrome (albumin/creatinine ratio from 1 to 100 g/mol; total protein from 0.1 up to 10 g/day).

Analytical performance should allow detection of microalbuminuria cases and monitoring of diseases based on these figures. As an approximation, the following performance specifications are proposed for urine proteins (Table XVII).

In addition to total protein, analytical quality specifications for maximum allowable imprecision and deviation (bias) have been calculated and reported for calcium, creatinine, P-amylase, phosphate, potassium, sodium, urate and urea based on 24-h collections of urine [259].

9.4. Particle analysis

9.4.1 Statistical uncertainty. Counting at low particle concentrations needs special attention because of statistical uncertainty. Particle counting follows the Poisson distribution [261]:

$$s = \sqrt{m}; \text{ and}$$
$$CV_m(\%) = 100\%/\sqrt{m} = 100\%/\sqrt{T/n}$$
$$= CV_T(\%) \times \sqrt{n}, \text{ and}$$
$$CV_T = 100\%/\sqrt{T} = CV_m/\sqrt{n}$$

where $T =$ total amount of counted cells and $n =$ number of counted unit volumes (traditionally squares in a chamber or high-power fields under a coverslip); $m =$ mean count $= T/n$, $s =$ standard deviation, $CV_m =$ coefficient of variation of the mean count, $CV_T =$ coefficient of variation of the total count.

At low particle concentrations, imprecision of the total count becomes critical: If only 1 µL is counted at a concentration of 3×10^6 particles/L, the result has a theoretical $CV_T = 60\%$ with ideal procedures. Five microlitres of the same suspension gives $CV_T = 26\%$ and counting only 10 µL gives $CV_T = 18\%$, permitting the calculation of an estimate for the mean value.

9.4.2 Suggested analytical quality specifications. **Quantitative counting:** Urine particle counting may benefit from comparison with the quality specifications described for haematological analysers with their already proven clinical accuracy [262]. In urinary tract infections, a

TABLE XVII. Analytical specifications for trueness (expressed as relative deviation) and reproducibility (expressed as imprecision) in quantitative *urine* chemistry, with special reference to proteinuria.

Property	Performance level	
	Optimum	Minimum
Trueness, maximum deviation from the target value	10%	25%
Reproducibility, maximum imprecision $CV_{day-to-day}$	20%	50%

10-fold difference (from 10 to 100×10^6 WBC/L) exists between non-infected and infected urines (see Section 6.2.3). Variation in daily volume rate (diuresis) also affects particle concentrations easily by a factor of 2 (100%).

For trueness, an optimum performance should have a relative deviation (bias) $<30\%$. This is important in particular for evaluation purposes. A minimum performance with a relative deviation <50–100% can be accepted for most routine applications, and in particular at particle concentrations below 3×10^6/L urine. To achieve these figures, one has to count several microlitres of urine from each sample. In the future, these specifications may be replaced by more accurate calculations.

The volumes of counting chambers or other similar devices should have an error $<10\%$ to be negligible. The manufacturer should specify the volume accuracy for the device.

Routine sediment examination (ordinal scale specifications): The generally used standardized sediment technique essentially provides ordinal scale analysis of urine particles. This can be controlled against counts of uncentrifuged urine within a chamber or by an instrument. In practice, delays in investigation after micturition, losses during centrifugation, suction of supernatant and inadequate review of the full coverslip area appear to contribute most to inaccuracy of results, despite a standardized procedure. Awareness of the many possibilities for systematic error helps in designing the most accurate procedure for routine sediment analysis. Categorized performance criteria are tentatively suggested below as an experienced opinion (Table XVIII) that can be replaced by more accurate specifications in the future.

Identification of clinically significant urine particles should be internally reviewed (peer review by staff members) and externally eval-uated (EQA schemes). Each site should document its personnel training.

9.5. Microbiology examinations

9.5.1. Good microbiological laboratory practice. Good Laboratory Practice should be implemented in all clinical laboratories, including chemistry, microbiology and general laboratories. Currently, this is often described in a local quality manual. Some details of such a quality manual were suggested in Section 9.1.2 using an example of microbiological investigations as performed in general laboratories under the supervision of a local microbiology laboratory.

9.5.2. Analytical quality specifications for bacterial culture. These specifications relate to the microbiological analysis of urine in a Level 2 facility. The performance at Level 1 may require further adjustments, e.g., at points-of-care (see Section 7.3 for specifications at this level). The given specifications do not necessarily fulfil the requirements for the Level 3 specifications (designed as benchmarks for comparative trials and quality control). Variation of exact techniques and technology is permissible and indeed necessary at Level 2,

TABLE XVIII. Maximum allowable false-negative rates in urine microscopy.

Particle type	Particle concentration ($\times 10^6$/L)	Maximum allowable false-negative rates (%)
RBC	10	20
	100	5
WBC	20	10
	200	5
Bacteria	10	20
	100	5
Casts	10	10
	50	5

because of cost and work-flow restrictions, the rapid performance of emerging technology and individual patient case-mix.

9.5.2.1. Acceptable false-negative rates for detection by routine culture. Maximum allowable false-negative rates are suggested for detection and identification of established primary, secondary and doubtful pathogens, either in pure culture or as part of a mixture, as defined by organisms in the common, fairly common and uncommon groups (as defined in Table IX) in Table XIX. These are based on expert opinion.

9.5.2.2. Acceptable routine level of identification. Laboratories working at Level 2 should be able to isolate – by culture from clinical samples – urine organisms at any concentration above those set out in Table XIII, and to identify the species to the given minimum level (Fig. 4).

This is not an exhaustive list of organisms recognized as causing urinary tract infection. Instead, this level of identification of species is based on a combination of the prevalence and pathological significance of their isolation, as well as the relation between the organism group and both antibacterial sensitivity and possible resistance acquisition. The list is therefore driven by clinical relevance rather than by microbiological

TABLE XIX. Maximum allowable false-negative culture results of uropathogens.

Colony concentration	Allowable false negative rate
10^8/L or more	2%
$10^7 - 10^8$/L	5%
$10^4 - 10^7$/L	10%

identification for its own sake. It also represents that which is practically achievable, rather than a "wish list" which would require more resources and time. Individual laboratories must decide whether their particular case-mix requires additional investigation beyond the suggested level. As an example, isolation of yeast from urine is more relevant in large centres with significant transplantation, intensive care and immuno-suppressed patient activity; the division into *albicans* and non-*albicans* species relates to predicted sensitivity to certain antifungal agents. Finally, there is a wide choice of agar media that can be used for isolating these organisms. A reduction in the number of media routinely used for urines (such as CLED only) *may* be acceptable. Suggestions for readily performable examinations to achieve such identification are given in Appendix, Annex 13.4. Many other methods may be equally appropriate and can be found in microbiology textbooks.
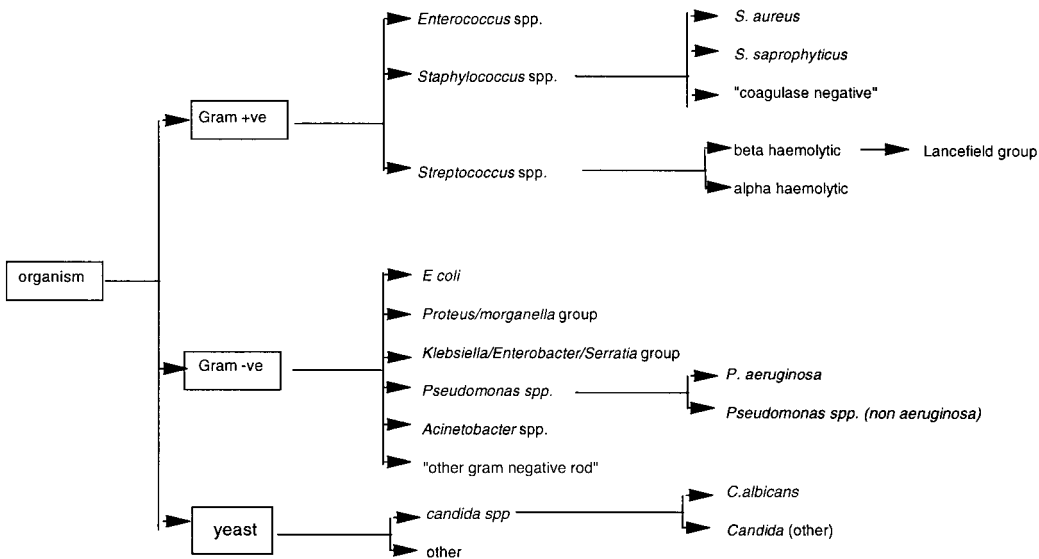
FIG. 4.   Minimum level of bacterial identification in clinical urine specimens.

9.5.3. Acceptable turnaround times from specimen receipt. The requirement for Level 2 relates to the importance of speed in terms of overall cost of patient treatment episodes. It should be viewed in the context of complicated and potentially serious urinary tract infections.

The following time limits are suggested for relevant uropathogens following receipt of the specimen (Table XX). These time limits must incorporate a laboratory information system that allows the clinician easy access to the result, either by telephone or, preferably, by computer interface. The higher minimum percentage required reflects the clinical priority of antibiotic sensitivity over organism identification. It is more important to ensure correct choice of antimicrobial agent than it is to know the name of the organism responsible for infection.

9.5.4. Microscopy in the microbiology laboratory. All laboratories wishing to operate at Level 2 must have facilities for microscopy. It is recognized that microscopy is labour-intensive and significantly impacts on overall cost. Results from particle analysis (visual or automated) and test-strip examination by general or chemical laboratories or at point-of-care units may partially replace the need for microscopy in the microbiology laboratory. However, these results should only be used to assist in the microbiological evaluation of cultures. Only experienced evaluators, usually in microbiology laboratories, however, should perform Gram staining. For detailed quality specifications of microscopy, see Section 9.4.

9.6. Urinalysis device performance evaluation

Improvements in laboratory technology are welcome and lead to better control of disease.

TABLE XX. Acceptable turnaround times in bacterial culture.

Identification to Level 2 in pure culture	90% within 24 h
Identification to Level 2 in mixed culture	90% within 48 h
Available antibiotic sensitivity of relevant organism	98% within 48 h

Point-of-care units and small laboratories are encouraged to consult with colleagues in the larger laboratories before proceeding with the evaluation of new instruments. Earlier European recommendations exist for procedures in instrument evaluation [263]. When replacing existing techniques with new systems at least the following issues must be considered.

Analytical performance.
Evaluation versus established reference or best comparison methods
Calibration and traceability of devices
Level of identification (particles, microbes), intended clinical use
A statistically significant sample of patients and healthy reference individuals
A detailed, pre-planned protocol
Analytical sensitivity and specificity of results (false-positive and false-negative rates and predictive values at different prevalences)
Reliability of quantitation if applicable = linearity of measurement.

Practical performance.
Ease of use and robustness
Ability to self-check and recognize and/or diagnose faulty performance
Instrument throughput
Ease of interfacing with laboratory computers.

Clinical and economic impact.
Cost/benefit assessment including all costs (reagents, manpower, running and maintenance and indirect costs)
Impact on patient care (if medical decision-making is altered, impact on outcomes).

Regulations. Any medical device intended for *in vitro* diagnostics should be compatible with the European directive for such devices [32].

Generally, a minimum sensitivity of 80–90%, depending on the patient population and application, is considered adequate, while a specificity of 90–95% should be maintained in diagnostic reports. When the instrument or examination is used for screening (exclusion of disease), a sensitivity of 95% with a specificity of at least 90% should be the aims when trying to reduce the total cost of confirmatory examinations.

APPENDIX (DETAIL OF
MEASUREMENTS AND PROCEDURES)
   ANNEX 10. TRANSMISSION OF INFOR-
MATION AND DETAIL OF PRE- AND
POST-ANALYTICAL STAGE

*10.1. Essential information in requests and
reports*

10.1.1. Specimen identification and patient data.
The following boxes structure clinical back-
ground information for laboratory processes.

*10.1.2. Requesting urinalysis examinations, siev-
ing principle.* Stepwise strategies (Section 8)
should be translated into practical requesting
routines. Considerable savings result if a sieve
principle is applied for **manual work**, e.g. *visual
microscopy* is performed for specimens positive
with a sieving examination (usually defined
fields of a multiple strip or an automated par-
ticle count) only. In selected cases, however,
sensitive protein measurements and even visual
microscopy should be requested independently
of sieving examinations to detect the presence
of renal damage. Detailed requests help focus
laboratory activities correctly.

   Bacterial culture should *mostly* be requested
independently of results from a rapid exami-
nation (multiple strip or automated counter) for
symptomatic high-risk patients to avoid false-
negative cases (this principle may change with
improved sensitivity of sieving examinations
within a laboratory process). For *low-risk
symptomatic patients* (females with symptoms

Patient identification Last name, first name Personal ID code (recommended if available) Date of birth (if not included in the personal ID code) Requesting unit (where patient is being treated) Return address (to whom report should be sent) Responsible physician/nurse (to be contacted if consultation is needed) Concurrent antimicrobial therapy (bacterial culture requested) Additional clinical information (signs, symptoms, tentative diagnosis). If no information is given, a minimum level of investigation should be applied as agreed locally on the basis of patient populations served.

Specimen details Specimen identification (ID) code (barcode, if used) Date and time of voiding (final real time) Collection method (mid-stream urine, single catheter urine, indwelling catheter urine, suprapubic aspiration of urine, bag specimen of urine; other) Success in patient preparation and collection (coded): qualified specimen or defective collection (such as untimed collection, urgency, difficulties in technique, etc.; classified by health care personnel when known) Results from rapid examinations (if performed at point-of-care; such as test strip)

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of repeated lower urinary tract infection and no
background risk factor), a positive rapid
examination may be sufficient to indicate
antimicrobial treatment *without a need for
culture*. *Patients with no clear-cut urinary tract
symptoms* (medically justified screening or
exclusion of urinary tract infection) may also
be able to collect standardized morning urine,
from which a rapid examination is able to
exclude the presence of bacteriuria at 10^8 CFB/
L level with a sensitivity of $80-90\%$.

   The box below should be part of a request to
communicate the need for additional proce-
dures if the sieving examination(s) is (are)
performed in the laboratory. The preferred
approach depends on the condition of each
patient or patient group.

Conditional request for visual microscopy ... Detection of albumin/protein, leukocytes or erythrocytes should be followed by visual microscopy **Conditional request for bacterial culture ...** Detection of leukocytes or bacteria by a rapid examination should be followed by a bacterial culture (*treatment* of possible bacteriuria is anticipated) **No additional examinations with positive results ...** (Mark if applicable; may be used if the rapid examination is sufficient for a clinical decision, such as in low-risk urinary tract infection or acute cases)

10.1.3. Reports. Reporting results from ordinal scale examinations. Report format: Date, time, technique/instrument and operator ID should be traceable.

Test strip results: In order that the clinician is aware of the uncertainty of the measurement, ordinal scale reporting is recommended (negative, 1+, 2+ or 3+).

It is recommended that the range of arbitrary concentrations corresponding to the assigned categories is always made available for the clinical interpreter either with the report or, more practically, in the laboratory handbook. A range is more informative than a single arbitrary concentration, e.g., for protein 1+ (0.2–1.0 g/L) is preferred over 1+(0.3 g/L). To avoid continual confusion, it would be of great help if all manufacturers were to adopt the same arbitrary categories for rapid examinations of the same type. As a minimum, specified analytical sensitivity limits for positivity should be identical.

Reporting results from particle analysis. The method should be stated in the report or in the laboratory handbook (standardized sediment; standardized chamber counting; automated analysis).

Morphological detail should be reported at a basic or advanced level (see Section 6.1). Units and quantities must be defined: particle counts/high power field (HPF, ×400 usually) are used for sediments examined under coverslips; counting chambers have defined volumes (e.g. 1 μL). Even with HPF units, a calculation of particles/litre (L) of original urine must be provided for the clinician. Recommended unit for publications is particles/L.

Particle counts should be given as averages per unit volume (HPF or L), not as ranges seen in different viewfields. Only micro-organisms and clumps of cells are uncountable by visual methods and may be reported in ordinal scale from "negative" to "3+".

Reporting microbiological results Gram staining
Oil immersion field (OIF) is used for Gram staining. The report may be formatted in ordinal scale from "negative" to "3+" if quantities are used.

Bacterial cultures. Quantity of growth (recommended unit for particles: 10^x colony-forming particles, CFB/L). The species or genera grown (depending on the methods of identification). Antimicrobial susceptibility results as locally agreed.

The bacterial culture report or laboratory handbook should state the method of culture, since the level of precision varies accordingly (from dipslide to accurate cultures with 1–100 μL inocula; grown for 24–48 h).

10.2. Role of computers in transmission of information

The need for increased efficiency goes in parallel with automation and computerized data transmission. Specific needs of urinalysis usually create additional work to satisfy the transmission of medically relevant information. Details of requests and reports are compiled in Section 10.1. In addition, the following issues should be considered.

Standardization. National coding of laboratory examinations is highly recommended. Additional codes are needed to Supplement urinalysis requests (Section 10.1). Morphological and microbiological reports need special coding, but also space for free-form reporting (occasional written notes on individual results). Units of quantities and formats of reports must be standardized.

Request specifications. Conditional requests (Section 10.1.2) and selections of correct procedures (such as shown in Table XIII for different options for bacterial culture) need decision rules in the on-line programmes when computerized; rapid results must be transmitted to other working sites to organize work-lists for microscopy or bacterial culture. Algorithms applied in these decisions may be simplified from the given guidelines as agreed locally.

Evaluation and reporting. Serial work-flow should be easy and accessible when working with microscopes or reading culture plates. Computer-assisted interpretation and graphic display of daily results are encouraged where possible.

Internal quality control (IQC). Internal quality control data should be accessible for responsi-

ble operators in both individual and cumulative form; this means collection of separate files. IQC data are expected to be stored for long periods (up to 15 years) in most quality systems.

Connection to laboratory and hospital information systems. A computerized urinalysis laboratory should have a standardized interface with the general laboratory information system and hospital administrative system, including patients' medical records. Medical information on patients will be increasingly available with electronic patient records. Access to crucial parts of this information should be permitted based on laboratory user ID and patient IDs of investigated samples, as agreed locally.

Orientation towards a real time process. It is not possible to know the precise time or success of qualified urine collection in advance; sometimes even the method of collection may change. Thus, the possible preliminary computerized request must be completed after obtaining the specimen for such post-collection detail.

Computer transmissions should have a negligible effect on turnaround times of emergency reports. Comparison with an earlier result of the same patient should be possible.

Safety of data: Back-up procedures must be organized to allow replacement when erroneous transmissions occur or files become corrupted in repeated use.

Quality control data should be easily accessible for the reviewer of patients' results.

10.3. Collection of urine specimens

Nurses or laboratory personnel usually instruct patients how to obtain an adequate urine specimen. Health care personnel must first understand the minimum requirements of standardized specimen collection. 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, is necessary. Each institution is encouraged to modify the text given below to make their local practice as efficient as possible. Pictures showing the basic procedures for females, males and children should be used and can be freely copied; these may be the only

means of understanding by individuals unfamiliar with the native language (illustrations enclosed). Professional "hands on" assistance is often needed for small children and elderly people.

10.3.1. Collection of mid-stream urine specimen (patient instructions)

Females: Wash your hands with soap and water or a towelette. Dry-wipe them. Have the clean collection container beside 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 a sterile towelette) without using any disinfectants. Dry-wipe. When urinating, let the first portion pass into the toilet (bedpan). Collect the mid-portion in 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 locally. If any problems occur, please consult the clinical attendant (see illustrations 1a and 2a, pages 91 and 93).

Males: Wash your hands with soap and water or a towelette. Dry-wipe them. Have the clean collection container beside you. Avoid touching the inside with your fingers. Uncover the urethral opening by withdrawing the foreskin if necessary. Wash the end of your penis, including 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 in 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 locally. If any problems occur, please consult the clinical attendant (see illustrations 1b and 2b, pages 92 and 94).

Children (capable of controlled micturition): After appropriate explanation, reasonably ade-

quate mid-stream specimens can be collected from children old enough to sit on a potty chair. This can be achieved by placing the collection container in the potty chair (see illustration 3, page 95). 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 locally. If any problems occur, please consult the clinical attendant.

10.3.2. Collection of sequential urine specimens for diagnosis of chronic bacterial prostatitis and related disorders (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 [27]. The results are better if the patient has not ejaculated at least for 3 days before the collection of specimen. 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). The examination starts when you want to void.
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, including 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 an additional 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.

10.3.3. Collection of suprapubic aspiration specimen. From infants and toddlers able to control their urination, a container inserted into a potty chair helps get a mid-stream specimen (see illustration 3, page 95). If not, suprapubic aspiration (or catheter specimen) should be attempted when the diagnosis or exclusion of urinary tract infection is crucial and a bag specimen does not work (see illustration 4, page 96).

10.3.4. Timed collection of urine. A 24-h urine sample is the most common example of a timed collection. Patient instructions must be provided. *Different preservatives* to be used in timed collections are listed in Table XXI. The table was compiled for analytes requested from outpatients. Based on preservatives listed in the NCCLS Urinalysis Guidelines [3], new analytes have been added. Moreover, technical development in measurements and changes in the body fluid have been taken into account (serum measurements replacing urine assays), as well as European experience in preservation of the analytes [39, 264].

Patient instructions: 24-h collection of urine. As part of your medical examination you have been asked to collect a timed urine sample because the doctor wants to know the exact amount of (the examined substance) excreted into your urine. You have been asked to collect for a 24-h period. If you are not in hospital, select a day when you expect to be able use the toilet where you keep the collection container throughout the continuous collection period. READ THESE INSTRUCTIONS CAREFULLY BEFORE YOU START THE COLLECTION.

TABLE XXI. Preservatives for single and timed urine collections (maximum documented stable time is expressed, 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 is used.

Measurand	Room temp (20°C)	Refrigerated (4–6°C)	Frozen - 20°C	HCl 6 mol/L	Boric Acid	Na$_2$CO$_3$	Notes
Albumin	7 d	1 mo	6 mo*				*Depends on the procedure
Alpha-1 microglobulin (Protein HC)	7 d	1 mo	6 mo*				*Depends on the procedure
Alpha-2 macroglobulin	7 d	7 d					
Aluminium	3 d	7 d	1 y				Special container
Amino acids			+	+			
5-Aminolevulinate, (or delta-)	1 d	4 d	1 mo	No		+	A
Bacteria	No	1 d			1–2 d*		*Combined preservatives also available
Calcium	2 d	4 d	3 w	+		No	
Catecholamines	4 d	4 d	20 d	+			
Chloride				No			
Citrate			4 w*	1 d*			* if pH < 1.7
Collagen type I, N-terminal telopeptide			+				
Cortisol			+				
Creatinine	2 d	5 d	6 mo				
Cystine		3 mo*	1 y*	7 d*			* add HCl
Glucose	<2 h	2 h	8 h		+		Azide
Glycosaminoglycans		+					
Homovanillic acid		+	+	+			
Human chorionic gonadotropin (pregnancy examination)		+					
Hydroxyproline				+	+		
5-Hydroxyindoleacetic acid	2 h	2 d*	2 d*	+	+		*add acid
Immunofixation and electrophoresis of urine proteins	7 d	1 mo	6 mo				
Immunoglobulins (intact), quantitative	7 d	1 mo	No*				*cryoproteins may not redissolve
Immunoglobulin, kappa and lambda light chains	7 d	1 mo	6 mo				
Iron	3 d	7 d	Years				
Lead							L
Lysergic acid diethylamide	1 m	1 m	2 m				
Magnesium	3 d	3 d	1 y				
Metanephrines				+			
Multiple strip		4 h*/1 d	No				*Erythrocyte and leukocyte detection most sensitive
Myoglobin	12 d*	12 d*	>12 d*				*at pH > 7
N-acetyl-beta-glucosaminidase	1 d	7 d	1 m				
Oligosaccharides		+					
Osmolality	3 h	7 d	3 mo				
Oxalate			4 mo*	*+			E, *if acidified

TABLE XXI. (*continued*)

Measurand	Room temp (20°C)	Refrigerated (4–6°C)	Frozen - 20°C	HCl 6 mol/L	Boric Acid	Na$_2$CO$_3$	Notes
Particles except bacteria (sediment)	1–2 h	1–8 h	No	No			pH < 7 and Osmol >300 mOsm/kgH$_2$0 favourable
Phosphate (inorganic)				2 d*			*if acidified
Porphyrins	4 d*	7 d*	1 mo*			+	* at pH 6-7, S
Potassium	2 mo	2 mo	1 y				
Protein, total	1 d	7 d	1 mo		+		Depends on the procedure
Pyridinium crosslinks (of collagen)	6 w		10 y				Depends on the procedure
Relative volumic mass (relative density, old: specific gravity)		+					
Sodium	45 d	45 d	1 y				
Steroid profiling		+	+				
Test strip, see Multiple strip							
Tetrahydrocannabinol-9-carboxylic acid	10 w	1 y	1 y				Albumin stabilizes
Urea	2 d	7 d	1 mo		+		
Urate	4 d*			No	No		*pH>8
Urobilinogen						+	S
Vanillylmandelic acid		7 d*	years*	7 d*			*at pH 3–5

A=acetic acid is not obligatory, E=EDTA addition in laboratory is helpful, L=lead-free container, S=protect from sunlight; No=not recommended. *=explained in Notes for each line.

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.

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 in the container.

Exactly 24 hours after starting the collection, empty your bladder and add this to the collection container. 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. Store and transport the container to the laboratory as advised.

If only a portion of the 24-hour specimen was requested, mix the complete collection thoroughly before pouring a small sample into the small container you have been given. Dry-wipe the small container and place the label provided on to it. Check or write your name, personal identification number and detailed collection date and times on the label.

If any questions arise, please contact your clinical attendant.

ANNEX 11. DETAIL OF CHEMISTRY EXAMINATIONS

11.1. Detail of multiple test strips

11.1.1. Analytical principles. See Table XXII.

11.1.2. Calculation of trueness in ordinal scale: grey zone. The trueness of ordinal scale measurements is expressed by defining detection limits (L_D) and confirmation limits (L_C) from comparison measurements. They delineate a grey zone. 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 ratio between concentrations L_C/L_D is recommended to be = 5.

TABLE XXII.   Detection principles and their limitations for multiple strips (modified from references 14 and 15).

Measurand	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)*	Nitrite detected with Griess's test (azo dye)	No vegetables in diet, short bladder incubation time, vitamin C, Gram-positive bacteria	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)	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 between 5 and 9	Formaldehyde lowers pH	
Relative volumic mass (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 with copper complex	EDTA	Haemoglobin or myoglobin above 50 mg/L
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

*Bacteria are detected on the basis of nitrate reductase present in most Gram-negative uropathogenic rods, such as *E. coli* (Griess's test). Nitrate reductase is lacking from some common uropathogens, i.e. Gram-positive bacteria, such as *Staphylococcus saprophyticus* and *Enterococcus* spp.

Example: Leukocyte detection by esterase activity. Acute urinary tract infections are associated with urinary leukocyte counts $\geq 100 - 200$ WBC $\times 10^6$/L, while at the level of < 10 WBC $\times 10^6$/L no association exists [180, 265]. What is the performance of a test-strip leukocyte esterase method detecting pyuria? Test-strip results are compared with chamber counts of WBC from freshly voided (< 2 h) urines with the following (imaginary) data (Table XXIII):

The following fractions describe the trueness of measurements:

1. The fraction of false positives at the detection limit $(L_D) = FP_D = b/(a + b)$ (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 $(L_C) = FN_C = e/(e + f)$ (in the example: $5/45 = 0.11$ or 11%).

Quality specifications for trueness:

If not otherwise clinically justified, the trueness of a test-strip field may be judged as shown in Table XXIV (when used for an average patient population)

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 (random variation, but also 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.

11.1.3. Detection and confirmation limits for different test-strip fields. The following detection limits (L_D) and confirmation limits (L_C) are proposed for general test strips (Table XXV). They are created by multiplying the approximate healthy upper reference limits of concentrations in morning urine by a factor of 2, to avoid false-positive results due to intra-individual (biological) variation.

To allow sensitive detection of albuminuria (microalbuminuria range), the following quality specifications are given to sensitive rapid albumin measurements (Table XXVI). Albumin concentrations (mg/L) are not expressed in substance-based units (mol/L) to be comparable with total protein concentrations, such as in Table XXV.

11.1.4. Concordance analysis (κ coefficient). κ coefficients [249] should be calculated when comparing agreement between two or more ordinal scale categories, such as test-strip results obtained with two different measurement procedures, or when comparing agreement with four or more ordinal scale results to those measured with a quantitative comparison method (procedure).

The principle is to subtract agreement expected by chance alone. If formal significance testing is needed, the p value from McNemar's test can be presented. Principles of the calculations of simple and weighted coefficients have

TABLE XXIII. 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:		L_D	L_C	

TABLE XXIV. Analytical quality 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	$< 20\%$	$< 50\%$	$< 10\%$

TABLE XXV.   Suggested detection and confirmation limits for multiple test strips.

Property	Comparison method	Detection limit (L_D)	Confirmation limit (L_C)
Leukocytes ($\times 10^6$/L)	Chamber counting[a]	20	100
Erythrocytes ($\times 10^6$/L)	Chamber counting[a]	10	50
Albumin (protein) (g/L)	Immunochemical (or dye binding for total protein)	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 volumic mass	Refractometry	± 0.005[b]	N/A[b]
Creatinine (mmol/L)	Enzymatic; (kinetic Jaffé no more recommended)	± 4[d]	N/A[b]
Urobilinogen (µmol/L)	Not commonly available	20[c]	100[c]
Bilirubin (µmol/L)	Bilirubin solution	10	50

[a] Microscopic chamber counting of fresh (less than 2 h) uncentrifuged specimens.
[b] N/A = detection and confirmation limits not applicable; an arbitrary class width is given.
[c] Commonly available comparison methods are lacking. Manufacturers should document their evaluation.
[d] Manufacturer reports an arbitrary scale of 0.9, 4.4, 8.8, 17.7 and 26.5 mmol/L.

TABLE XXVI.   Analytical quality specifications suggested for sensitive albumin (rapid) examinations.

Property	Comparison method	Detection limit (L_D)	Confirmation limit (L_C)
Albumin (sensitive; mg/L)	Immunochemical	10	50
Albumin (sensitive): Creatinine ratio (g/mol)	Immunochemical, ratio to quantitative creatinine method	3	15

been published earlier [250]. A more detailed explanation is given elsewhere [266].

$$\kappa = (P(o) - P(e))/(1 - P(e))$$

$$= 1 - (Q(o)/Q(e))$$

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 2×2 table, a sensitivity of 90% and specificity of 90% against the comparison method result in a $\kappa = 0.8$. $\kappa = 0.8$ 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%). κ coefficient varies between – 1 (complete disagreement) and + 1 (complete agreement).

11.1.5. Estimation of repeatability. Ordinal scale data are amenable to binomial statistics. Table XXVII gives selected 95% binomial confidence limits to the observed proportion of results for those readers who do not have statistical tables or computer programmes at their bench. It shows that a specimen that lies within a certain concentration category (e.g. 1+) at a probability of 95% or more is a better control than another with a lower (even as low as 50%) original probability, because the higher original probability has a narrower confidence interval.

11.1.6.   Qualified procedures for test-strip reading

11.1.6.1. Visual reading. Qualified test strip measurement is summarised in a tabular form both for visual reading (11.1.6.1) and instrumental reading (11.1.6.2).

Item	Standard	Method of checking
Identification of specimen	Label the specimen	Compare label with the working list if analysing several specimens at once
Homogeneous specimen	Mix immediately before dipping	Even colour
Temperature of the specimen	$+20°C$	Allow to stand for 15–30 min before analysis
Quality of strips	Date still acceptable	Expiration date
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	Observation by trainer
Timing	Use a timer showing seconds	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, organized with local supporting laboratory	Reports available
Storage of strips	No physical problems associated with storage	Outlook of the strips (bent, wet, etc.), closed vials
Reporting	Use the predefined units. Fill in the patient record or working list immediately	Train before actual patient analysis

11.1.6.2. Reflectometric reading

Item	Standard	Method of checking
Identification of specimen	Label the specimen	Compare label with the working list if analysing several specimens at once
Homogeneous specimen	Mix immediately before dipping	Even colour
Temperature of the specimen	$+20°C$	Allow to stand for 15–30 min before analysis
Quality of strips	Date still acceptable	Expiration date
Protocol for instrumental measurement	Protocol written locally after training	Written protocol available
Internal quality control	Control solutions measured daily	Follow-up charts maintained
External quality control	Participation expected, organized 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	Methods described mainly by the manufacturer. *In vitro* medical devices directive by the European Council	Documentation by the manufacturer, changes of strip lots recorded

TABLE XXVII.   Binomial 95% confidence intervals of proportions.

No. of results[b]	Proportion of results falling into the same concentration category[a]							
	50%	60%	70%	75%	80%	85%	90%	95%
20	27–73%	36–81%	46–88%	51–91%	56–94%	62–97%	68–99%	75–100%
50	36–65%	45–74%	55–82%	62–87%	66–90%	73–94%	78–97%	86–99%
100	40–60%	50–70%	60–79%	65–83%	71–87%	77–91%	82–95%	89–98%
1000	47–53%	57–63%	67–73%	72–78%	78–83%	83–87%	88–92%	94–96%

[a] Obtained proportion of results within the most frequent category when first estimating the level of a quality control solution.
[b] No. of test strip measurements from a patient or control specimen used for the estimate.

11.2. Detail of quantitative urine measurements

11.2.1. Health-associated reference intervals of urine proteins.
The health-associated upper reference limits shown for excreted urine proteins derive from references 109 and 111 (Table XXVIII). These point estimates have wide confidence intervals owing to skewed distributions of values as shown in the latter reference.

11.2.2. Diagnostic classifications based on proteinuria.
Sensitive detection and differentiation of prerenal, glomerular, tubular and postrenal proteinuria is now available with a low detection limit by means of specific protein measurements [267, 268]. Algorithms have also been developed to classify patients based on these measurements using two-dimensional reference areas [269] (Fig. 5).

Other diagnostic classifications have been created from different protein to protein concentration ratios in urine [80, 123, 270, 271] (Table XXIX).

11.2.3. Detail of quantitative measurement procedures
Total protein
Principles of measurement
Benzethonium chloride [272] and trichloroacetic acid precipitation [273], dye binding methods with Brome-phenol blue [274], Coomassie brilliant blue [275], Ponceau red [276] and pyrogallol-red [277], nephelometry and turbidimetry have been suggested. All these methods can be automated, in contrast to the biuret examination [278]. 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 with a reference sample of diluted serum. The use of albumin solution is preferable and more reproducible, however. CRM 470 standard is available as a primary calibrator [279].

TABLE XXVIII.   Upper 95% reference limits (URL) for protein-creatinine ratios in urines from healthy individuals. SI units are favoured over conventional units.

Protein	Type of specimen	Upper 95% reference limit (g/mol 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
	Random	5.3	47
IgG	First morning	0.7	6
	Random	1.0	9
Protein HC	First morning	0.5	4
(α_1-microglobulin)	Random	0.7	6
κ-immuno-reactivity	First morning	0.4	4
	Random	0.7	6
λ-immuno-reactivity	First morning	Below detection limit	Below detection limit
	Random	0.7	6

[a] Turbidometric trichloroacetic acid precipitation method (see below).
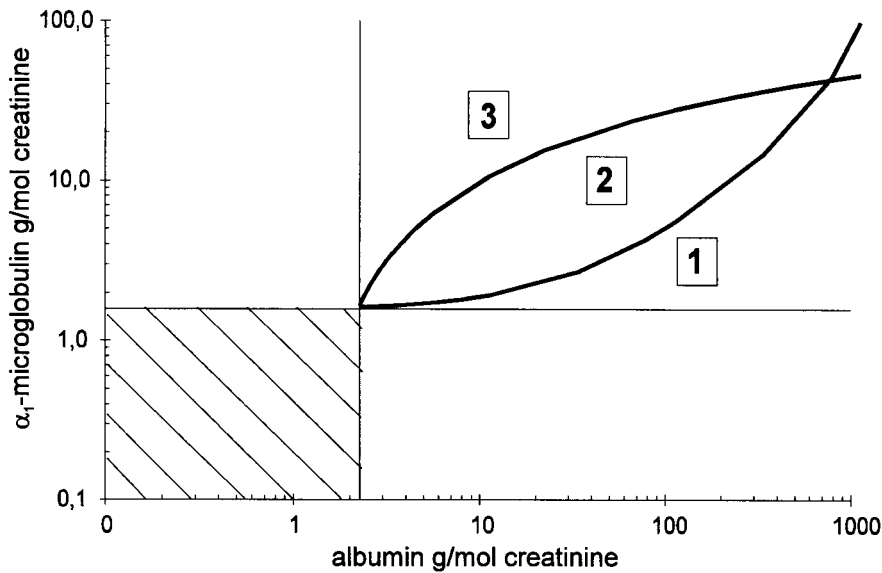
FIG. 5. Differentiation of (1) primary glomerulopathies, (2) secondary glomerulopathies and (3) tubulo-interstitial nephropathies by specific protein measurements. The shaded area represents the health-associated values.

TABLE XXIX. Concentration ratios of proteins used for differentiation of proteinuria.

Concentration ratio	Decision limit	Suggested pathological condition
α_2-macroglobulin/albumin	<0.02	Renal haematuria
	>0.02	Postrenal haematuria
IgG/albumin	<0.03	Selective glomerular proteinuria
	>0.03	Non-selective glomerular proteinuria
α_1-microglobulin/albumin	>0.1	Mixed proteinuria
	<0.1	Glomerular proteinuria
CRP (serum)/CRP (urine)	>1.0	Bacterial infection
	<1.0	Rejection of renal transplant
(Albumin+IgG+α_1-microglobulin)/total protein)	>0.6	Renal proteinuria
	<0.6	Suspicion of Bence-Jones proteinuria
Immunoglobulin light chain κ/λ ratio	<1	Monoclonal lambda chain
	1–5.2	Polyclonal light chains
	>5.2	Monoclonal kappa chain

Upper limits of reference intervals in healthy individuals. Because these are method-dependent, several intervals are cited:

Pyrogallol red method: <180 mg/day [280], for pregnant women <260 mg/day [281]
Biuret method: <150 mg/day [278]
Turbidimetric method: <75 mg/day [273]
Turbidimetric method: Protein/creatinine <8 g/mol (second morning urine [109]); (or <70 mg/g in conventional units)

Albumin
Principles of measurement

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

Calibration. CRM 470 standard is the primary reference material [279]. Professional societies and major diagnostic companies have agreed to create secondary standards and join consensus interim reference ranges based on the CRM 470 [282].

Prediction limit for incipient diabetic nephropathy. An albumin excretion rate of >20 µg/min (traditionally cited unit), which corresponds to an approximate albumin/creatinine >4 g/mol (30 mg/g in conventional units), will predict incipient diabetic nephropathy [10].

Interpretation. Albumin/creatinine ratios decrease slightly with age [283]. The albumin/creatinine ratio is also slightly higher in women than in men because of lower creatinine excretion in women. Upper normal limit in pregnancy is 30 mg/day (24-h collection) [281]. The average intra-individual coefficient of variation of albumin excretion from day to day is approximately $20-30\%$ [109], and even larger for diabetic patients [258]. Diagnostic decisions should therefore not be based on a single measurement, especially if the result is equivocal.

α_1-microglobulin (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 from day to day is 20% on average [109]. 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 to 99.8%. Increased concentrations in the urine are found in tubulo-interstitial dysfunction or in nephropathies.

Creatinine
Principles of measurement
Methods based on the Jaffé reaction [284] should be replaced with more specific enzymatic methods [285]. Reference measurement procedure is based on isotope dilution mass spectrometry [286].

Calibration. A known reference material for serum creatinine is SRM 914a [257]. Weighed out creatinine solution may serve as an approximate estimate.

Reference interval from healthy individuals and interpretation. Creatinine in urine: $7-20$ mmol/day $(0.8-2.2$ g/day). 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.

Osmolality
Principles of measurement
Osmometry follows directly the definition of osmolality: it is based on either of the two colligative properties, a decrease in freezing point or an increase in the evaporation point of solutions.

Interpretation. 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 H_2O. Maximal diuresis may result in an osmolality as low as 50 mOsm/kg H_2O [127]. The concentrated morning urine after an overnight restriction of fluid intake reaches an osmolality of at least 700 mOsm/kg H_2O in healthy individuals. In chronic renal failure, the urine remains isotonic within the range $300-350$ mOsm/kg H_2O.

Relative volumic mass (old terms: relative density; specific gravity).
Calibration. Relative volumic mass measurement can be calibrated in practice by measuring volumic masses (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 [127]. It is to be noted that there are marked differences in the accuracy of these methods [287].

Reference interval and interpretation. Relative volumic mass is primarily a function of glu-

cose, phosphate and carbonate. For human urine, the values are within the interval 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 [127].

Conductivity. Conductometry of urine (measured as a current flow between two electrodes) has recently also become readily available [128, 132]. Since the amount of charge in urine (the ionic strength) is related to urine concentration, the conductivity is also related to water excretion. Diet-dependent intake affects the excretion of salt from healthy individuals as well as from patients. Data on reliability in clinical practice are accumulating.

11.3. Detail of pregnancy examinations

Specimen

The concentrated morning urine usually contains the highest concentrations of hCG and is therefore the best specimen. If the urine specimen is too dilute, false-negative results may occur in early pregnancy. *Stability and storage*: If the examination is performed within 48 h after collection, the specimen can be kept in the refrigerator (2 – 8°C). If examination is delayed for more than 48 h, the specimen should be frozen.

Examinations

Different commercial systems for rapid and sensitive detection of human chorionic gonadotropin (hCG) are available. Specific monoclonal antibodies to the intact hCG molecule (both alpha-chain and beta-chain) should be used.

Laboratories or point-of-care units should consider carefully what is the best sensitivity limit for their practice, and whether urine or blood specimens should be used. Nominal sensitivity may be chosen between 25 and 500 IU/L in different clinical situations (emergency diagnostics of extrauterine pregnancies, antenatal clinic detecting normal pregnancies, X-ray department excluding pregnancies). The rapid test selected should be evaluated against a quantitative hCG method with the help of local laboratory.

Interpretation

Ordinal scale reporting with two categories is sufficient for a rapid examination (either positive or negative result). A weak positive result indicates a slightly elevated hCG concentration but below 25 IU/L (or below 200 – 500 IU/L if used in some ambulatory units). It is then good laboratory practice to ask for a new sample and retest it after an additional 2 days. Pharmacies selling kits for home testing often apply the highest sensitivity of 25 IU/L.

ANNEX 12. DETAIL OF PARTICLE ANALYSIS

12.1. Visual microscopy methods

12.1.1. Chamber counting of uncentrifuged urine for evaluation purposes (Level 3). Chamber counting of uncentrifuged urine is recommended for comparisons of automated particle counting with visual methods, because counts obtained from a chamber are more precise than those obtained under coverslip on a microscope slide. If counted without centrifugation, the loss of particles during centrifugation does not interfere with the results. In routine methods, cells and other particles in a volume of 1 μL are counted. For evaluation of particles other than WBC or RBC, this is not statistically sufficient. For precise evaluation, at least 100 cells should be counted to reach a $CV = 10\%$, and 400 cells should be counted to reach a $CV = 5\%$, based on the Poisson distribution (see Section 9.4.1). Health-associated reference intervals of many urine particles are, however, below 2 particles/μL ($< 2 \times 10^6$/L).

Variation of the counting volume should be less than 5% in a chamber used for reference counting (compared to less than 10% in routine chambers).

Staining of specimens and/or phase contrast microscopy is needed for accurate identification of the various urine particles. For evaluation of test-strip readers, leukocyte (granulocyte) and erythrocyte counts are needed only. An example of a reliable comparison method to validate an automated particle analyser was recently published which used both phase contrast optics and supravital staining but only 1 μL volumes for practical reasons [69]. Consequently, counts

below 10 (-30) particles $\times 10^6$/L were statistically unreliable.

12.1.2. Standardized urine sediment examination (Level 2).

Fixation and drying of the specimen, to permit careful cytological examination [41], is generally considered too time-consuming for basic urinalysis. Wet sample preparation with or without preservatives remains a pragmatic compromise for routine laboratories.

Centrifugation. The exact concentration factor is important to relate the findings to the original volume of urine. Optimum selection of the factor depends on the size of the specimen tubes used and the suction equipment to remove the supernatant; the range is usually 10 to 25-fold concentration. Centrifugation should be at $400 \times g$ for 5 min (adjust rpm values according to the radius of the rotor in the centrifuge; see the equation below). A refrigerated centrifuge is an advantage when working with larger series of non-preserved specimens or when undertaking parallel tasks. On the other hand, precipitation of crystals is increased at low temperatures.

$$RCF = 1.118 \times 10^{-5} \times r \times RPM^2$$

where r = the radius of the centrifuge from the centre of the spindle to the bottom of the tube (cm)

$$RPM = 6000 \times \sqrt{1/r}, \text{ if } RCF = 400 \times g$$

Staining and optics: Phase contrast microscopy is recommended. Compared with bright-field microscopy, the phase contrast technique allows better detection, especially of bacteria, RBC and hyaline casts. With bright-field microscopy, supravital stains are obligatory for proper differentiation. In routine use, a colour contrast with blue and red (e.g. Sternheimer's Alcian blue – pyronin B staining [171, 288]) is better than the older violet-coloured gentian violet – saffranin O (Sternheimer-Malbin) method [289]. Publications on the successful use of toluidine blue also exist [172]. Stained preparations can also be routinely reviewed by phase-contrast optics to benefit from both techniques. Filters for polarizing light are helpful for correct identification of crystals and, in some cases, also of lipids.

For special purposes, other staining procedures should be used. For detection of eosinophilic granulocytes, Hansel's stain is recommended [145]. In the proper cytological examination of urine to detect cancer cells, Papanicolaou staining with a fixation procedure is a worldwide practice. This is beyond the scope of the general laboratory.

Quantitation. A high-quality binocular microscope should be equipped with objectives for low $(10 \times$ to $16 \times)$ and high $(40 \times)$ magnification. Oculars are traditionally from $10 \times$ to $12.5 \times$ magnification. The volume applied under a coverslip defines the average height of the fluid layer under a coverslip of defined size. The diameter of the viewfield is equal to the ocular viewfield number (usually from 18 to 22 mm; given by the manufacturer) divided by objective magnification. It can be measured with special tools as well (metric scales are available as microscope slides).

Example: 10 mL of urine is concentrated into 0.5 mL = 20-fold concentration; 0.05 mL stain is added. Then 13 µL is applied under a 18×18 mm coverslip. Thus the fluid layer = $13/(18 \times 18) = 0.040$ mm thick. If it is investigated under 10×40 magnification with ocular viewfield number 22 (diameter of the viewfield = 22 mm/40 = 0.55 mm), the volume of a high-power field (HPF) is then:

$$0.040 \times \pi \times (0.55/2)^2 = 0.00950 \text{ mm}^3 \text{ or } \mu L$$

Because of the 20-fold concentration and 10% dilution because of the dye, one HPF corresponds to 0.173 µL of original urine volume.

In this case, n RBC/HPF correspond to n RBC/0.173 µL = 5.8 $\times$ n RBC $\times 10^6$/L.

E.g. 4 RBC/HPF equals to 23 RBC $\times 10^6$/L.

Reporting. An average particle count rather than a range should be reported. It is practical to report it as particles/high power field (HPF). It is, however, recommended that the number concentration of particles be calculated also to the internationally comparable units of volume (L), as shown above. Precise counting may have an upper limit for practical reasons, equalling about $200-300$ particles $\times 10^6$/L. For numerous microbes, ordinal scale reports, such as "none", "few", "moderate" and "abundant" are acceptable.

The details for the standardized sediment examination are summarized in Table XXX.

Visual microscopy procedure for routine. Cool down the specimen $(+4^{\circ}C)$ to allow a delay

of up to 4 h if not preserved or specifically investigated for crystals (precipitation of urates will follow after cooling). At $+20°C$ the delay of microscopy should not exceed 1 h after voiding.

Always use a defined volume of urine (usually $5-15$ mL). Centrifuge the specimen at $400 \times g$ at $+4°C$ (if not preserved) for 5 min. Remove the supernatant with an adjusted vacuum tool; do not decant (an inaccurate method). Aim at the decided concentration factor, e.g. 20 (12 mL $\rightarrow$ 0.6 mL or 10 mL $\rightarrow$ 0.5 mL).

After suction, add 10% of the volume (60 μL or 50 μL) of supravital stain if your laboratory is using a stain. Mix gently. Use a cooling plate if the specimens have to wait before investigation.

After gentle resuspension, pipette a known volume of sediment on to a microscope slide. Add the coverslip horizontally to maximize even distribution. Examples: 13 μL is an appropriate volume for an 18×18 mm coverslip, while $35-50$ μL may be used for a 24×32 mm coverslip. The smaller volumes and coverslips have economic advantages in bigger laboratories.

Investigate the sample first under low-power $(10 \times$ or $16 \times)$ magnification to see the usually uneven distribution of particles on the slide, as well as to note the presence of rare elements, such as casts and epithelial cells. Then count the number of different particles per high-power $(40 \times)$ objective field, reporting the average number observed in at least 10 fields selected from all areas of the coverslip (the

TABLE XXX. Summary of standardized urinary sediment examination.

Item	Standard	Method of checking
Delay	Investigate within 4 h from micturition (if stored at $+4°C$), or within 30 min at $+20°C$ to allow evaluation of all cells; otherwise use preservatives after evaluation	Documented times of collection
Original volume of urine	$5-12$ mL	Line marked on the tube
Centrifugation	$400 \times g$ for 5 min, preferably at $+4°C$ if delays occur	Check with the Supplier of the centrifuge
Removal of supernatant	Suction to a defined final concentration factor	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 and high-power magnification $(\times 400)$	Consult local supplier
Volume 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 (particles/$400 \times$ magnified high-power field)	Calculate the equivalence
Reproducible process	Written operating procedures	Training of personnel, blind peer reviews
Internal quality control	Training courses organized locally Double-check specimens weekly	Two independent investigations for the same specimen
External quality assessment	Participation in an EQA programme	Documents of results available
Calibration	Traceability of measured quantities	Evaluation against uncentrifuged specimens

total number of fields to be counted depends on the concentration of particles: lower numbers require more fields to reach statistical reliability).

12.1.3. Morphologic criteria for urinary sediment findings (Level 2). Recent atlases showing detailed morphology of urinary elements for reference purposes must be consulted until enough experience is accumulated [15, 41, 56, 288, 290 – 292].

12.1.3.1. Detailed characteristics. **Erythrocytes**. The size and haemoglobin content of erythrocytes can vary according to the osmolality of urine (the lower the osmolality the larger the cell and the lower the haemoglobin content). "Ghost erythrocytes" that have lost their haemoglobin content can be missed if bright field microscopy is used alone. At low osmolality values, erythrocytes can even lyse. Erythrocytes usually have a diameter of 4 – 7 μm. The appearance can vary according to the source of haematuria: isomorphic erythrocytes usually indicate a post-renal bleeding, while dysmorphic erythrocytes indicate glomerular disease (see below).

Dysmorphic red cells. Phase-contrast microscopy is mandatory. Report the total number of erythrocytes/HPF and the percentage of dysmorphic red cells. Fassett *et al.* (1982) originally proposed that a fraction of 80% dysmorphic RBC or higher indicates renal bleeding [147]. A fraction of 80% isomorphic (normal) RBC indicates post-renal bleeding. In the middle, there are mixed cases. It is recommended that the fraction of acanthocytes be reported as well, because they are readily identified by the blebs on the cell surface and a presence of 5% or more acanthocytes is indicative for renal bleeding (see also Section 6.1). The morphological appearances of dysmorphic red cells, especially acanthocytes, can be studied from the available publications. The correct evaluation of abnormal RBC morphology may depend on pH and osmolality of the urine, which is why morning specimens are recommended if possible [151]; acanthocyte formation does not occur *in vitro* with the same frequency as other dysmorphic shapes [150]. Special training of the investigator is needed.

Leukocytes. Polymorphonuclear **granulocytes** are the most frequent leukocytes found in urine.

Their multilobular nucleus and cytoplasmic granules make them readily identifiable. In dye-binding methods, living granulocytes do not always allow dyes to enter the cell. In Sternheimer staining, nuclei and inclusions usually stain bright blue, while cytoplasm remains reddish or brownish. Granulocytes may aggregate to form clumps. They also lyse easily when the osmolality is low or when the pH is high [89]. **Eosinophils** need special stains for their detection (Hansel's stain [145]).

Macrophages (histiocytes) are often seen in association with inflammation. They show thin pink granular cytoplasm, often filled with red blood cell remnants and other vacuoles, and bluish nuclei with unevenly distributed chromatin on Sternheimer staining.

Lymphocytes have smooth nuclei that almost fill the cell. In Sternheimer staining, they are usually dark blue. Cytoplasm is scarce and without granules.

Tubular epithelial cells. Different types of tubular cells line the different segments of the renal tubules. As a consequence, several types of tubular cells can be found in the urine, which differ in size and shape. Tubular cells are mononucleated with granular cytoplasm and are larger than leukocytes. Most nuclei are round to ovoid. They have an average diameter of about 13 μm, and probably come from the proximal segments of the tubules. Less frequently, rectangular, polygonal or even columnar cells can be seen, which originate from distal tubules or collecting ducts. In Sternheimer staining, tubular cells usually have dense, granular red cytoplasm that may contain lipid droplets in patients with severe proteinuria, and a blue or purple nucleus. A practical way to learn the morphology of tubular cells is to look for these cells within casts: epithelial cells within casts are by definition tubular epithelial cells.

Transitional epithelial (urothelial) cells. Division of urothelial cells into superficial and deeper cells has been described recently [293].

Superficial urothelial cells are usually round to oval mononucleated cells with a mean diameter of about 30 μm, and a pale halo around the nucleus. Occasionally they can be bi- or multinucleated. These cells are a frequent finding in patients with urinary tract infection and urological disorders.

Deep urothelial cells are smaller than superficial cells (mean diameter about 17 μm). They

may exhibit various shapes, but they have mostly a club-like or an ovoid appearance, a central or peripheral nucleus and thin granular cytoplasm. Usually they are found in association with urothelial carcinoma, ureteric stones or hydronephrosis. These cells stain darker than superficial urothelial cells. Atypical forms of urothelial cells can also be found in association with the rapid techniques of cellular preparation used in routine urinalysis [172, 288]. Their interpretation should be undertaken in specialized cytological laboratories.

Squamous epithelial cells. Squamous epithelial cells are the commonest cells of the urine. They have a polygonal shape, a central nucleus and an average diameter of about 55 μm. Squamous cells derive from urethra and vagina, and are usually markers of contamination of urine during specimen collection.

Casts. Casts are elongated elements with a cylindrical shape that varies due to bending, wrinkling and irregular edges. The main types of casts are described below.

Hyaline casts. These have a matrix with low refractive index and are best identified by phase contrast microscope. They are found in both renal parenchymal diseases and in normal subjects.

Granular casts. These can contain either fine or coarse granules. They are not usually found in normal subjects and when present suggest the presence of renal disease.

Waxy casts. These are usually large, with clear-cut edges or indented borders, and are refractile. Waxy casts have a homogeneous appearance, just like wax. They are found in patients with renal insufficiency or failure.

Fatty casts. These contain lipid particles. Fatty casts are typical of patients with heavy proteinuria associated with lipoproteinuria.

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

– *Erythrocyte casts* (which always indicate bleeding from the renal parenchyma)

– *Leukocyte (usually Granulocyte) casts* (which may indicate acute pyelonephritis, acute interstitial nephritis or proliferative glomerulonephritides)

– *Tubular epithelial cell casts* (which are found for example in patients with acute tubular necrosis, acute interstitial nephritis, acute cellular rejection of grafted kidney and glomerular disorders).

Owing to degenerative phenomena, it is often difficult to say whether the cells within the casts are tubular rather than leukocytes. In such cases the correct definition should be "cellular cast" only.

Haemoglobin and myoglobin casts. These are brownish in colour with a granular surface. More frequently, haemoglobin casts derive from erythrocyte casts. Therefore, they too 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 crush syndrome.

Bilirubin casts stain yellow-brown due to water-soluble (conjugated) bilirubin excreted into urine. Urinary bilirubin may be used in differentiation of icteric patients if serum measurements are lacking.

Bacterial and yeast casts. These are rare. However, they may be seen in immuno-compromised patients with bacterial or fungal infection affecting the kidneys.

Lipids are usually seen as isolated or clumped droplets (either free or within cells and casts), or as oval fat bodies (round-shaped particles containing packed lipid droplets), fatty casts or cholesterol crystals (see below). Lipids are identified because of their refractility and their ability to polarize light.

Crystals. *Uric acid*. Lozenges, barrels or rosettes with a typical amber colour and birefringence under polarized light.

Calcium oxalate dihydrate. Typically bipyramidal. They can appear also in aggregates. Only large crystals show birefringence.

Calcium oxalate monohydrate. Ovoid, dumbbell or biconcave discs, always brightly birefringent.

Calcium phosphate. Prisms, needles or rosettes that polarize light. When occurring in plates, calcium phosphate is not birefringent.

Triple 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 polarize light while phosphates do not.

Cystine. Thin, hexagonal, birefringent plates with irregular sides. They can be isolated, heaped upon one another, or in clumps and rosettes. They can be seen at low pH (<6) and usually after an overnight incubation at +4°C.

Leucine. Oily-looking spheres with concentric striations.

Tyrosine. Thin needles, often aggregated in bundles or rosettes.

Cholesterol. Transparent thin plates with sharp edges and corners. These are associated with heavy proteinuria.

Crystals of drugs. Sulphadiazine (crystals with the appearance of "sheaves of wheat"), triamterene, acyclovir (birefringent and needle-shaped crystals), indinavir (plate or star-like crystals [294] and vitamin C [56, 163].

Microbes. Bacteria may be seen on routine microscopy; rods are particularly visible with phase contrast microscopy. Cocci may be confused with salt precipitation.

Fungi. Cells of *Candida* spp. appear as ovoid or roundish elements not absorbing stain. They also appear as hyphi. Budding is the most typical morphological feature. On most occasions they are due to contamination from the vagina, although they may represent true infection in the chronically debilitated or immunosuppressed patient.

Protozoa. Trichomonas vaginalis, when alive, is easily identified owing to the motility of the flagella and the rapid and irregular movements of the body. When dead it is similar to leukocytes. In most cases it is in the urine as a consequence of genital contamination.

Parasites. The diagnosis of parasitic infestation by *Schistosoma haematobium* relies on the observation of the eggs in the urine. These measure about 140×50 μm and 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.

Details of urinary particles are tabulated for easier reference in routine practice in Tables XXXI and XXXII.

12.2. Automated particle analysis

Manufacturers of urine particle analysers should describe in detail the differentiation capability of their instrument, including sensitivity and specificity data against a manual comparison method, such as chamber counting, sediment morphology or bacterial culture. General as well as specific patient populations should be targeted in the evaluations to establish the optimal intended diagnostic use for a given instrument. Based on the technical principles used, advice on specimen collection and storage is essential to obtain reliable results and avoid artefacts. Lists of known interferences should be made generally available as soon as they are discovered during evaluations and in clinical practice.

Customers should work out standard operating procedures with the help of manufacturers. These should include descriptions of regular working, combinations of different analyses from the same urine, quality assessment protocols and measures to be taken in the event of instrument alarms or error messages. Specimens not amenable to automated analysis should be listed, as well as the standardized alternative manual methods.

12.3. Health-associated upper reference limits of particles in morning urine

Healthy reference intervals depend heavily on preanalytical as well as analytical standardization and delay of examination. Table XXXIII cites some of the 98% and 95% upper reference limits found in the earlier literature [170, 185, 295–297]. Comprehensive detail necessary to evaluate the results has not been published. The figures are concentrations rather than excretion rates, as originally proposed by Addis [298]; lysis of cells unfortunately diminishes the validity of excretion rates based on 12-h collections. Adjustment to volume rate (diuresis) should be considered.

Rough estimates for arbitrary (non-parametric) 95% upper reference limits in morning urine without centrifugation: leukocytes and erythrocytes $<10 \times 10^6/L$.

NOTE 1. Owing to the procedural uncertainties these upper limits are given as arbitrary inequality figures only. Female specimens are more vulnerable than male specimens for preanalytical errors, as shown by the Stansfeld & Webb study. In addition, erythrocytes may be difficult to see without phase contrast optics and proper $\times 400$ magnification [295]. For best clinical comparability, concentrated first (or low-diuresis second) morning urine should be collected as a washed mid-stream collection and investigated according to an identical procedure that is applied to the patients as well. About

TABLE XXXI. Differentiation of urine cells by phase contrast optics or supravital staining.

Cell type	Nucleus	Cytoplasm	Other
Granulocyte	Multilobular or rod-shaped, does not stain always, bright blue when stains	granular, degenerates easily	occurs often in clumps, round shape
Eosinophilic granulocytes		Granular	Use Hansel's staining for identification
Macrophage	Bluish, chromatin uneven	"Thin", granular, varying size of red cells inside	Phagocytosed pieces of granules; round, tuberous and dendritic shapes occur
Lymphocyte	Fills the cell almost entirely, (dark) blue clear	Smooth, scarce broken cell membrane thin rim	
Squamous epithelial cell	Degenerated, small, round, (polygonal) central, even free in urine	Pale, large, slightly granular adhering bacteria	Often in clumps, polygonal shapes, may be folded
Transitional epithelial cell, superficial	Oval or round, small, chromatin finely granular, nucleolus is often visible >1 nuclei may occur	Large, with clear perinuclear halo finely granular pink	Round or oval shape, also in clumps (catheter?)
Transitional epithelial cell, deep	Well-defined, large, evident nucleoli, central or eccentric	Many granules may be dark red atypical forms	Various shapes, occasionally atypical forms
Renal tubular epithelial cell	Homogeneous, clear, blue/violet, nucleolus may be evident	Granular, often dark red, "thick", degenerative granules or fat often inside	Look at cells within the casts; round, or oval, also in clumps

   Prostatic epithelial cells cannot be differentiated from transitional epithelial cells by this method; prostatic particles may be seen occasionally.
   The intensity of staining varies and is dependent on the length of exposure to the stain as well as unknown factors related to the specimen. The ratio of blue to red is also affected by the batch of stain used in preparing the stain mixture.

TABLE XXXII. Differentiation of other urine particles (phase contrast or supravital staining).

Particle type	Features
Microbes:	
Bacteria	
rods	Dark, often in chains
cocci	Dark, isolated, in pairs, chains or clusters
Yeasts	Nucleus often visible, budding; do not stain well; also as branching hyphi (pseudomyceliae)
Casts:	
Cellular casts	Cells (erythrocytes, leukocytes, tubular cells) packed and/or plunged in the cast matrix
Microbial casts	Granular appearance; rods may appear clearly discernible
Hyaline cast	Low refractive index, may occur as darkly blue, compact or fibrillar, occasionally convoluted
Granular cast	Granules of various size and colour, usually red
Waxy cast	Refractile, with hard and indented edges; colour red rather than blue
Fatty cast	Lipid droplets can be isolated, in clumps, or packed; occasionally protruding cholesterol spicules
Pigmented cast	Haemoglobin and myoglobin (red-brown), bilirubin (yellow-orange-brown)

TABLE XXXIII. Health-associated upper reference limits (URL) of urine leukocyte (WBC) and erythrocyte (RBC) concentrations in uncentrifuged specimens.

Population	Procedure details	Sample size	Estimated URL (particle: percentile, value $\times 10^6$/L)	Reference
Boys, 0–14 years, in-patients	[a]Mid-stream specimen	359 patients/ 1142 specimens	WBC: 98%, 10 95%, 5	295
Girls, 0–14 years, in-patients	[a]Mid-stream specimen	273 patients/ 1167 specimens	WBC: 98%, 90 [a] 95%, 50 [a]	295
	[a]Single-catheter specimen	100	WBC: 98%, 10 [a]	295
Boys and girls, 2–16 years	Mid-stream specimen, volumic mass ≤ 1.005 deleted	228 boys and 160 girls	WBC: 95%, 4 RBC: 95%, 13	296
Males, voluntary individuals	Mid-stream specimen, Morning urine	75	WBC: 98%, 9 95%, 5–8 RBC: 98%, 4 95%, 3	170
Adults	Not specified	714 females+ 293 males = 1007	RBC: 95%, 8	297
Adults	[b]Scanning electron microscopy	27	RBC: 95%, 2	185

[a] Stansfeld and Webb (295) noticed the problem of collection of mid-stream urine specimens from girls. After collecting single in-and-out catheter urine specimens, upper reference limits comparable to those of boys were obtained.

[b] Pollock *et al.* (185) used filtration of known volume of urine and scanning electron microscopy to establish the true value for RBC concentration in first morning urine. Györy *et al.* (299) have also published health-associated upper 95% reference limits from 1720 urine sediments counted in chamber: The limits were 1 RBC and 3 WBC $\times 10^6$/L (299). To avoid confusion, the figures obtained after centrifugation were not listed in the table.

half of the erythrocytes and leukocytes are lost during centrifugation (when preparing urine sediments).

NOTE 2. With statistically reliable total numbers of individuals, selection criteria of reference individuals or calculations may have been defective, leading to non-standardized results. Because of the skewed distributions, parametric calculations may be misleading if the skewness is not corrected properly. Exact percentile estimates are in any case imprecise, and a marked grey zone should be considered (expressed as a confidence interval of the percentile estimate).

NOTE 3. Concentrations of erythrocytes and leukocytes in urines of newborn individuals are most likely higher than those given above.

NOTE 4. Diagnostic decision limits differ from the given upper healthy reference limits. For example, the probability of infection in a non-standardized specimen increases when the amount of granulocytes (leukocytes) rises from 10 to 100 $\times 10^6$/L [180, 265].

Automated methods show higher precision at lower cell counts due to the higher cell numbers counted. It seems likely that new more precise health-associated reference intervals will be created based on re-assessed procedures and new technology [132, 175, 300].

ANNEX 13. DETAIL OF MICROBIOLOGY EXAMINATIONS

13.1. Suggested comparison method for bacterial culture of urine (Level 3)

Substrates.
CLED (Cysteine-Lactose Electrolyte-Deficient) agar plate
Blood agar
Haematin agar
For more accurate identification of yeasts, special methods are required.
Control strains, see Table XXXV.

Inoculation. **Alternative A**:
Ten microlitres of urine is inoculated with a micropipette in the middle of a haematin agar

TABLE XXXIV. Quantitative interpretation of growth.

Volume of inoculum	No. of colonies on plate	Colony count in urine (CFB/L)	Colony count in urine (CFU/mL)
1 μL	1	10^6	10^3
	10	10^7	10^4
	100	10^8	10^5
10 μL	1	10^5	10^2
	10	10^6	10^3
	100	10^7	10^4
100 μL	1	10^4	10^1
	10	10^5	10^2
	100	10^6	10^3

plate and a blood agar plate. In addition, 1 μL is inoculated on to CLED agar with a plastic or platinum loop. When performing cultures on suprapubic aspiration urine, a large 100-μL amount of inoculum is recommended on blood and haematin agar. Different amounts of inoculum lead to different interpretations of quantitative growth for each colony count observed (Table XXXIV).

The inoculum is spread with a platinum or plastic loop by streaking back and forth through the drop a few times (Phase 1; Fig. 6). Finally, several streaks are made **very close** to each other and perpendicular to the first line (Phase 2; Fig. 6). Quantitation is performed on plates inoculated by a micropipette. Plates not used for quantitation can be divided for two different specimens.

Alternative B:

When a more accurate quantitative result than that obtained by the method in alternative A is necessary, the following procedure is recommended. The sample is diluted 1:10 with a neutral buffer; 100 μL of each dilution is inoculated by means of a Drygalski spatulum and a rotating platform (1 or 10 μL may not be precise enough volumes for normal pipettes). The colonies on the plates from at least three dilutions are counted. The original concentration is calculated as the mean concentration from the dilutions. (Scheme 1)

In order to raise the accuracy of the estimate for mean bacterial concentration the number of replicates can be increased, e.g., for disinfectant examinations the same sample should be analysed in 10–20 replicates. The counts follow Poisson distribution.

Incubation times. The following incubation temperatures, atmospheres and times are recommended for best identification of species and quantitative comparisons:

CLED agar   35–37°C, aerobic   1+1 day
Blood agar   35–37°C, anaerobic 2 days
Haematin agar 35–37°C, 5% CO_2 1+1 day

Phase 1
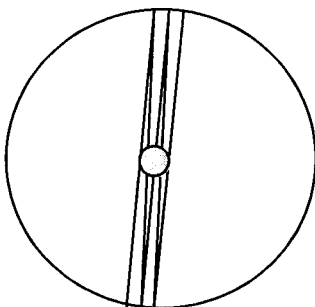
Phase 2
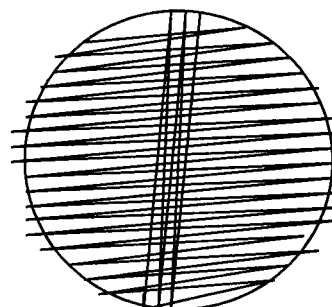
FIG. 6. Inoculation of a culture plate. (limitations of computer graphics must be understood in the interpretation of these drawings).

13.2. Routine loop cultures

The external work in quality assurance has shown that there is great variation in the methodology of performing conventional urine cultures despite earlier attempts at standardization. Greater demands for faster and less expensive service also justify the description that a minimum diagnostic performance be followed. The lowest statistically reliable count to be reported with this method is 10^7 CFB/L (10^4 CFU/mL).

Substrate.
CLED agar (for control strains see Table XXXV)
Blood agar

Inoculation. One microlitre is inoculated with a plastic or platinum loop on blood and CLED agar. The inoculum is spread as described above.

Incubation and interpretation
CLED agar 35–37°C, aerobic 1+1 day
Blood agar 35–37°C, CO_2 1+1 day

Clearly positive and negative results (10^8 CFB/L of primary pathogens or no growth) can be reported after incubation for 1 day. Colony morphology develops significantly after an additional day of growth (which can be carried out in a 5% CO_2 atmosphere for CLED agar if blood agar is not used). This facilitates identification of mixed flora that may contain more resistant species or variants. An increase of 8–10% in the frequency of isolation has been reported after 2 days compared to a 1-day culture [301, 302].

As an alternative to CLED-agar, there are several new chromogenic media on the market. These allow direct presumptive identification of *E. coli, Proteus/Morganella/Providencia* and

Process					
	Native urine				
Diluting	▨ → 1:10 → ▢ → 1:10 → ▢ → 1:10 → ▢ → 1:10 → ▢				
Plating	Plate 100μL	Plate 100μL	Plate 100μL	Plate 100μL	Plate 100μL
Growth	↓	↓	↓	↓	↓
Reading (CFB)	Lawn	10^4	750	95	5
Inoculated original volume (μL)	100	10	1	0.1	0.01
Calculations (CFB/L)	not possible	not possible	7.5×10^8	9.5×10^8	5×10^8
Final result: **Mean of single results**			7.3×10^8 **CFB/L**		
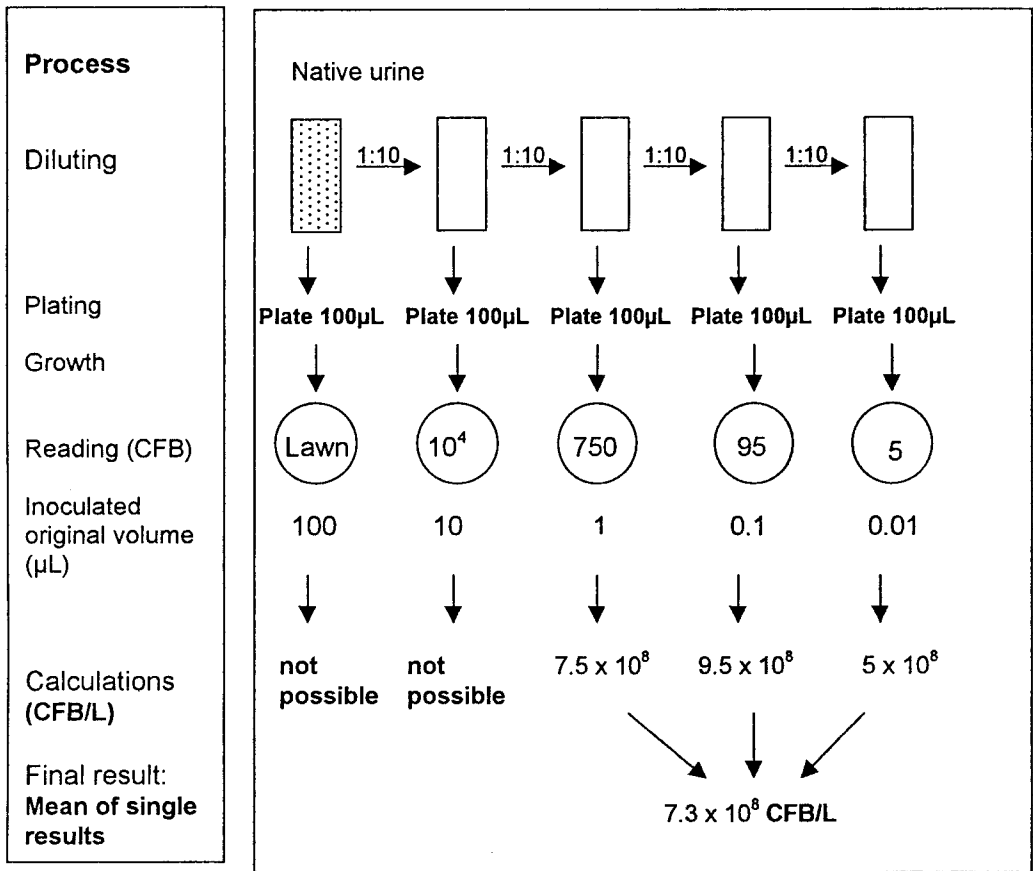
Scheme 1. Serial dilution for quantitative bacteria counting.

TABLE XXXV.   Control strains for urine bacterial culture.

Medium	Species	ATCC no.	Incubation	Expected reaction
Blood agar	*E. coli*	25922	35°C, aerobic 1 day	Growth
Blood agar	*S. aureus*	25923	35°C, aerobic 1 day	Growth
Blood agar	*S. pneumoniae*	6305	35°C, aerobic 1 day	Growth, alpha haemolysis
Blood agar	*S. pyogenes*	19615	35°C, aerobic 1 day	Growth, beta haemolysis
Blood agar anaerobic	*C. perfringens*	13124	35°C, anaerobic 1 day	Growth, haemolysis
Blood agar anaerobic	*P. anaerobius*	27337	35°C, anaerobic 1 day	Growth
CLED agar	*E. coli*	25922	35°C, aerobic 1 day	Growth, yellow
CLED agar	*E. faecalis*	29212	35°C, aerobic 1 day	Growth, yellow
CLED agar	*P. mirabilis*	29245	35°C, aerobic 1 day	Growth, blue, no swarming
CLED agar	*S. aureus*	25923	35°C, aerobic 1 day	Growth, yellow

enterococci. *S. saprophyticus* can also be identified using certain products. Aerobic incubation at 35–37°C is recommended for all products [5, 6, 303–307].

Urine samples showing the presence of yeast on microscopy can be inoculated on chromogenic yeast culture medium such as Chrom Agar, which allows a direct presumptive identification of *Candida albicans C. tropicalis* and *C. krusei*.

13.3. Dipslide cultures

Equipment

Incubator 35–37°C (note: thermometer for temperature control)

Comparison chart for interpretation

Dipslides

Procedure

1. Check the expiry date of the dipslide.
2. Label the dipslide. Open the dipslide without touching the agar surfaces. Verify that the agar surfaces are even and shiny and that the corners and edges are not dried or shrunken. Any condensation in the tube must be poured out.
3. Dip the slide in the urine specimen once, moistening 75% of the agar surfaces. If the amount of urine is inadequate, the agar surfaces can be moistened by pouring the urine over them once.
4. Let the excess urine drip off. Place the lower edge of the dipslide on clean, absorbent paper.
5. Screw the dipslide back together. Make sure that the lid is tightly closed.

6. Incubate the dipslide at 35–37°C overnight (for 18–24 h).
7. If interpretation cannot be made on the following morning, for example because of a weekend, proceed as follows: Place the dipslide as usual in the incubator and take it out when leaving. Store it at +20°C and interpret it on Monday morning. Culturing at +20°C for 2–3 days is comparable to culturing at 37°C for 24 h.
8. Store the original urine specimen in the refrigerator. If the dipslide is sent to the bacteriological laboratory for susceptibility testing or evaluation, the analysis will be more reliable if the original specimen can be sent as well. Urine specimens should be under refrigeration when transported.

Interpretation of results

Colony concentration. The bacterial concentration is interpreted on the green side (CLED agar) of the dipslide, since this does not contain antibacterial agents. Bacterial growth may consist of large or very small colonies. High bacterial concentration appears as merging colonies. Interpretation is simplified if only 75% of the dipslide is immersed. By doing so, a clean surface will be left for comparison. A magnifying glass (12×) also helps in the interpretation because small bacteria colonies are differentiated better, for example, from crystals.

Note: Do not forget to state the volume unit of the concentration (L or mL as used).

Mixed flora. Note different appearances of bacterial colonies; for example, different sizes, colours or shapes. The presence of several

| Alternative | Evaluation of growth | |
	Arbitrary quantity as read against the control chart	Interpretation
1	$<10^6$ CFB/L ($<10^3$ CFU/mL)	No growth
2	$<10^7$ CFB/L ($<10^4$ CFU/mL) not *E. coli*	Negative
3	$>10^6$ CFB/L ($>10^3$ CFU/mL) *E. coli*	Positive
4	$>10^7$ CFB/L ($>10^4$ CFU/mL) not *E. coli*	Urine and dipslide are sent to microbiology laboratory for further evaluation
5	Mixed flora (no concentration given)	Negative
6	Mixed flora with *E. coli* (no concentration given)	New examination recommended

bacterial species is often a sign of contamination during collection. Use a magnifying glass!

Growth of E. coli. On the special agar, *E. coli* appears as brown or black colonies. *E. coli* is usually lactose fermenting ($>90\%$). Even non-lactose fermenting strains that appear as black or brown growth on special agar for *E. coli* should be classified as *E. coli*.

Evaluation. Alternatives 1, 2 and 5 should be considered negative. Within alternatives 4 and 6 there is a possibility for both positive and negative findings. Urine specimen and dipslide should be sent to a microbiology laboratory for further evaluation and, if necessary, for identification and susceptibility testing. Alternative 3 should be considered a positive finding. *References* are given at the end of these guidelines [207, 209].

13.4. Detail of identification of uropathogenic species (Level 2 methodology)

A proposal for identifying species of urinary isolates in routine diagnostics is compiled below, based on cultures on CLED and blood agar. The aim is to state the minimum criteria for positive results and additional useful criteria when diagnosing urinary tract infection.

Non-fastidious micro-organisms

A. Gram-positive bacteria
Staphylococci Gram-positive, catalase-positive cocci in clusters. Routinely divided into only three groups:
- *S. saprophyticus*
- *S. aureus*
- Other coagulase-negative staphylococci (CNS).

1. S. saprophyticus: **Minimum criteria**: Typical colony morphology, catalase-positive, DNase-negative,* Novobiocin resistant, zone <16 mm with a 5 µg disc.

- Typical colony morphology: porcelain to ivory coloured colonies on blood agar, yellowish on CLED.
- Coagulase-negative.**
- Often sensitive to ampicillin, cephalosporin antibiotics and trimethoprim, resistant to mecillinam *in vitro*.
- Strains with variant patterns of resistance should be further typed before identified as *S. saprophyticus*.

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*The DNase examination is recommended for screening of urine isolates; the latex agglutination examination is less appropriate because *S. saprophyticus* often causes agglutination. Certain coagulase-negative staphylococci form DNase. In practice, a colony is subcultured to blood agar with a novobiocin disk and to DNA agar.
**Tube coagulase examination with rabbit plasma is the reference method and should be used if DNase or the agglutination examination give inconclusive results.

2. S. aureus: **Minimum criteria**: Typical colony morphology, catalase-positive, DNase-positive or positive *S. aureus* agglutination examination.

- Colony morphology: larger colonies and more uniform in size than coagulase-negative staphylococci, often yellow pigment on CLED- and blood agar.
- Coagulase-positive (reference method).**

3. Additional coagulase-negative staphylococci (CNS): Staphylococci that do not fulfil the criteria under numbers 1 or 2. White or yellow colonies on CLED and blood.

Enterococcus spp. and Streptococci Gram-positive cocci in chains, catalase-negative.

Enterococcus spp **Minimum criteria**: Typical colony morphology, bile esculin positive, positive group D agglutination examination.

- Grows in broth with 6.5% NaCl.
- Grey-white colonies on blood agar can have no haemolysis, alpha-haemolysis or beta-haemolysis, usually yellow colonies on CLED.
- Arabinose fermentation can be used to differentiate *Enterococcus faecium* (positive) from *Enterococcus faecalis* (negative).
- Reduced sensitivity for cephalosporin antibiotics (useful in separating enterococci from GBS (see below) if CAMP examination is the routine procedure).

Group B streptococci (GBS). **Minimum criteria**: Typical colony morphology, positive group B agglutination examination or positive CAMP examination.

- Small, uncoloured colonies on CLED, can sometimes be seen after 2-day incubation. On blood: blue/grey-white colonies with a narrow zone of β-haemolysis, sometimes α-haemolysis or no haemolysis.
- Sensitive to cephalosporin antibiotics.

Other β-haemolytic streptococci. **Minimum criteria**: Typical colony morphology, β-haemolysis, positive agglutination examination for each group, respectively.

- Often poor growth on CLED, β-haemolysis on blood.

α-streptococci. **Minimum criteria**: Typical colony morphology, α-haemolysis, Gram-positive cocci in chains.

- Small or no colonies on CLED, α-hemolysis on blood.
- Optochin ≤14 mm.

Lactobacillus spp. **Minimum criteria**: Typical colony morphology, Gram-positive rods with typical appearance, catalase-negative.

- Small or no colonies on CLED, sometimes lactose-positive.
- Often α-hemolysis on blood.
- Microscopy: Gram-positive (sometimes Gram-labile) long or short, regular rods.

Bacillus spp. **Minimum criteria**: Typical colony morphology, Gram-positive rods.

- Often large, dry colonies with haemolysis on blood; can sometimes be confused with *Enterobacteriaceae* or pseudomonas.
- Catalase-positive, positive/negative oxidase.
- Microscopy: regular, large, Gram-positive or Gram-labile rods, sometimes with visible spores.

Corynebacterium urealyticum. **Minimum criteria**: Typical colony appearance, Gram-positive rod, catalase-positive, quick urease-positive reaction. When in doubt verify the diagnosis with for example the API Coryne database.

- Slow growth, small shiny colonies on blood agar.
- Often very resistant to antibiotics.

"Diphtheroid" rods. **Minimum criteria**: Typical colony morphology, Gram-positive rods with typical appearance

- Often weak growth on CLED.
- Microscopy: Gram-positive rods, often conical, in "V" formations and palisades.
- Catalase-positive.

Corynebacterium urealyticum can be clinically relevant. Perform an urease examination.

Note! The above criteria do not exclude *Listeria*.

B. Gram-negative bacteria.
Enterobacteriaceae. The outline below is an example of simple species identification of urine isolates.

Basic criteria: Gram-negative rod, glucose-fermentative, Kovacs oxidase-negative, good

growth on MacConkey agar. For details and extensive species identification, see handbooks for *Enterobacteriaceae*.

Minimum criteria: "4 – examination": Lactose, Voges – Proskauer (VP), ONPG, indole examination with p-dimethylaminocinnamaldehyde (DMACA); alternatively, tube examination with p-dimetylaminobenzaldelyde according to Kovacs or Ehrlich.

A. Lactose-positive growth on CLED
1. Colony appearance suggests *E. coli*:
● Perform indole examination from blood agar.
● Positive indole = *E. coli*, negative indole = Gram-negative rod *Enterobacteriaceae*: perform VP.
positive VP, negative indole = *Klebsiella/Enterobacter*.
negative VP, negative indole = Gram-negative rod *Enterobacteriaceae*.
2. Colony appearance suggests *Klebsiella/Enterobacter:*
● Perform VP, indole examination.
● positive VP, positive/negative indole = *Klebsiella/Enterobacter*.
● negative VP, negative indole = Gram-negative rod *Enterobacteriaceae*.
● negative VP, positive indole = *E. coli*.
3. Positive beta-glucuronidase = *E. coli*.

B. Lactose-negative growth on CLED:
1. Swarming on blood agar:
● Perform indole only.
● Negative indole = *Proteus mirabilis*.
● Positive indole = *Proteus vulgaris*.
2. Additional lactose-negative:
● negative VP, positive ONPG, positive indole = *E. coli*.
● negative VP and ONPG, perform extended biochemistry.
● other combinations should be identified for species.
3. Positive beta-glucuronidase = *E. coli*.

Non-glucose fermenting Gram-negative rods. **Basic criteria:** Good growth on MacConkey agar, Kovacs oxidase-positive (see the exception below), non-glucose fermentative (negative KIA/TSIA).

Genus/species diagnostics need only be routinely performed for *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobac-*

ter. Other isolates can be reported as "Gram-negative rod, non-*Enterobacteriaceae*".

Pseudomonas aeruginosa. Minimum criteria*:
1. Characteristic odour and appearance (green-brown pigment, strawberry or "fruity" odour) can be directly reported; Kovacs oxidase-positive.
2. ADH-positive, growth at 42°C.

When non-glucose fermenting Gram-negative rods are examined for decarboxylase, it is appropriate to have both positive and negative controls because of the difficulty of interpreting the examination results. *P. aeruginosa* is ADH-positive and LDC-negative.

Stenotrophomonas maltophilia. **Minimum criteria**: Typical colony appearance after 2 days, non-fermentative, oxidase-negative, DNase-positive.
● Characteristic appearance, ammonia odour when grown on blood agar.
● Microscopy: Gram-negative, narrow rods.

Acinetobacter. **Minimum criteria**: Typical colony appearance, non-fermentative, oxidase-negative.
● DNase-negative, microscopy, Gram-negative coccoid rod.

Yeast. **Minimum criteria**: Typical colony appearance, microscopy: fungi.
● Convex, dry colonies with even edges (after longer incubation times, sometimes star-shaped), yeast odour in heavy growth.
● Microscopy: Gram-positive, large, oval cells, sometimes with budding.
● Positive serum examination = *C. albicans*.
● Negative serum examination = unspecified yeast.
Further typing is justified when *Cryptococcus neoformans* or other clinically relevant yeast infections are suspected.

Methods used in identification. The recommended methods are given in Table XXXVI.

13.5. Microscopy methods in microbiology

13.5.1. Slide centrifugation technique (Level 3). The reference describes the method in more detail [173]. The disadvantage of this robust

and consistent method is that it is labour-intensive and therefore recommended for evaluation purposes only.

Procedure:

1. 200 μL of well-mixed specimen is pipetted into a slide centrifuge chamber and centrifuged at $2000 \times g$ for 5 min.
2. Beginning from the centre of the spot, 12 consecutive fields are scanned under oil immersion from each specimen (see suggested pattern in Fig. 7).
3. Results are graded as shown in Table XXXVII.

13.5.2. Gram-staining procedure (Level 2).
Procedure of Gram-staining
Fixation

Resuspend urine thoroughly, or use a sample from bacterial culture. Take 1 drop of the urine
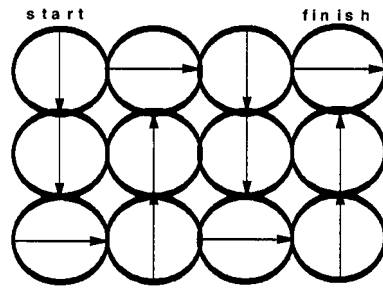
FIG. 7. Suggested pattern for examination of 12 oil-imersion fields.

and smear across a slide to an area of approximately 1 cm $\times$ 2 cm. Allow slide to air dry. Fix in a flame. Alternatively, fix by applying some drops of methanol on microscope slide and leave for 2 min. Then let the excess methanol evaporate and allow the specimen to dry again.

TABLE XXXVI.   Identification methods for uropathogenic bacteria.[1].

Examination	ASM 5th Ed	ASM 6th Ed	ASM 7th Ed
CAMP test	250	303	287
DNase test	1243		
Bile esculin	1230	303	298, 1690
Gram-staining	1306		1677
KIA (Kligler Iron Agar)	1255		
NaCl-broth	1273	308 – 11	298
TSIA (Triple Sugar Iron Agar)	1279		
Indole test, tube, Kovacs[2]	1295		1668
Indole test, tube, Ehrlich[2]			1668
Indole spot test[2]	1294		1668
Catalase test	1290		1666
Coagulase test, tube	1292	288 – 9	1666
Optochin test	249, 1222	303	288
Oxidase test, Kovacs	1295		1670
Serum test	620	727 – 8	1189
Voges-Proskauer test	1302		1672

[1] Page numbers refer to the American Society for Microbiology's Manual of Clinical Microbiology, 5th edition, 1991 (308), 6th edition, 1995 (309) and 7th edition, 1999 (310).
[2] For the indole examination, L-tryptophan must be added to the blood agar (appropriate amount is 10 ml 1% L-tryptophan mixture to one litre blood agar base). The indole examination should be performed with DMACA reagent (p-dimethylamino-cinnamaldehyde) since this is the most sensitive method. For a discussion about the indole examination in tube, see also: GI Barrow and RKA Feltham's *Manual for the Identification of Medical Bacteria* (Cambridge: Cambridge University Press, 1990: 36).

TABLE XXXVII.   Suggested grading system for bacteria detected by the slide centrifuge method.

Grade	Negat	Few	Moderate	Abundant
Observation	No bacteria in any field	Same morphological type in <6 fields	Same morphological type in ≥6 fields	>100 organisms in all 12 fields

Reagents
Crystal violet, stock solution 100 g in 750 mL 96% ethanol (Inflammable!).

Ammonium oxalate 1% (di-ammonium oxalate monohydrate).

Using solution:	100 mL	crystal violet stock solution
	500 mL	ammonium oxalate solution
	400 mL	water (laboratory grade)

Filter when prepared, store at +20°C.

Lugol's using solution:	
Iodine crystals, purissimum	1 g
KI (potassium iodide, pro analyse)	2 g
Water	300 mL

Dissolve iodine crystals and potassium iodide in water. Do not autoclave. Store in a dark bottle at +20°C.

Destaining solution:	
Acetone, purissimum	20 mL
Ethanol (96%)	80 mL

Store at +20°C. Very inflammable!!

Counter stain solution:		
Carbol fuchsin,	Basic fuchsin	8 g
stock solution:	Ethanol 90%	92 g
	Phenol 90%	50 g
	Water	ad 1000 g

Will keep for 3 years.

Using solution:	Stock solution	10 mL
	Water	90 mL

Store at +20°C.

Staining.
Apply crystal violet to the specimen. Leave for 30 sec.

Pour the stain away, rinse with Lugol's solution, and apply Lugol's solution for 60 sec.

Destain with acetone-ethanol until all stain is removed.

Counter stain with carbol fuchsin for 15 sec. Rinse carefully under tap water.

Dry in an upright position or use a commercial drying device.

Examination. Examine under oil with a ×100 objective (magnification ×1000 with ×10 oculars).

Gram-positive organisms look blue or violet, Gram-negative stain red. Interpretation of Gram characteristics needs experience, and is not recommended for occasional observers.

Examine a minimum of five fields and record the average presence of elements per field in accordance with Table XXXVIII.

13.5.3. Microtitre tray method (Level 1). A fixed volume of urine is placed in a well of a 96 well flat-bottom microtitre plate and allowed to settle for 5 min. The well is then examined using an inverted microscope. The presence or absence of white cells, bacteria and red cells is noted on an ordinal scale. The technique requires operator training, as differentiating (e.g.) cocci from debris may be difficult. Disposable plastic 96 well plates may not be available to all laboratories; however, it is perfectly possible to cold disinfect, wash and thoroughly dry plastic plates and re-use them. The method can also be used to detect the presence of yeasts, which may have pathogenic significance, as well for detecting pathogens such as *S. haematobium*, whether this is suspected or not.

NOTE: This method is used for detecting clear-cut infected urines in order to facilitate workup in a microbiological laboratory. It is NOT intended as a proper urine particle analysis.

Procedure

1. Gently invert the primary urine container. Pipette 60 μL of urine into a well, noting the coordinates of the well.

TABLE XXXVIII.   Grades of bacterial concentrations in Gram staining.

Grade	Negat	1+	2+	3+
Bacteria/OIF*	Any seen	1/OIF	1 to 5/OIF	>5/OIF

* OIF = Oil Immersion Field.

TABLE XXXIX. Suggested system for grading urine in microtitre tray method.

Grade	Negat	1+	2+	3+
Leukocytes/HPF	1 to 10	11 to 50	50 – 200	> 200
Red cells/HPF	1 to 10	11 to 50	50 – 200	> 200
% HPF obscured by bacteria	< 10%	10 – 30%	30 – 60%	> 60% – confluent

2. Allow the formed elements to settle for a minimum of 5 min.
3. Place the plate on the microscope and examine the bottom of the well using a × 20 objective and medium light intensity to maximize detection of bacteria.
4. Note the presence of leukocytes and red cells in one representative field after establishing that elements are evenly distributed across the well bottom.
5. Note the presence of bacteria, and estimate the High Power Field (HPF) area that is covered by them in accordance with Table XXXIX.

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INDEX

Collection of mid-stream urine, females

1

Wash your hands.

2

Spread your labia.

3

**Wash your outer genital
organs with water.**

4

**Dry-wipe with a paper
towel downwards.**

5

**Let the first
portion
pass into
the toilet.**

6 — **7**

**Allow any
excess urine
to pass again
into the toilet.**

½ dl

**Collect about ½ dl urine
into the container without
breaking the stream.
Avoid touching the inside.**

8

**Leave the specimen
at the reception
desk.**

Fig.1a

TAUH Department of Clinical Chemistry / T.Heittola

Collection of mid-stream urine, males

1

Wash your hands.

2

Withdraw your foreskin.

3

Wash the end of your penis.

4

Dry-wipe with a paper towel.

5

Let the first portion pass into the toilet.

6 — **7**

Allow any excess urine to pass again into the toilet.

½ dl

Collect about ½ dl urine into the container without breaking the stream. Avoid touching the inside.

8
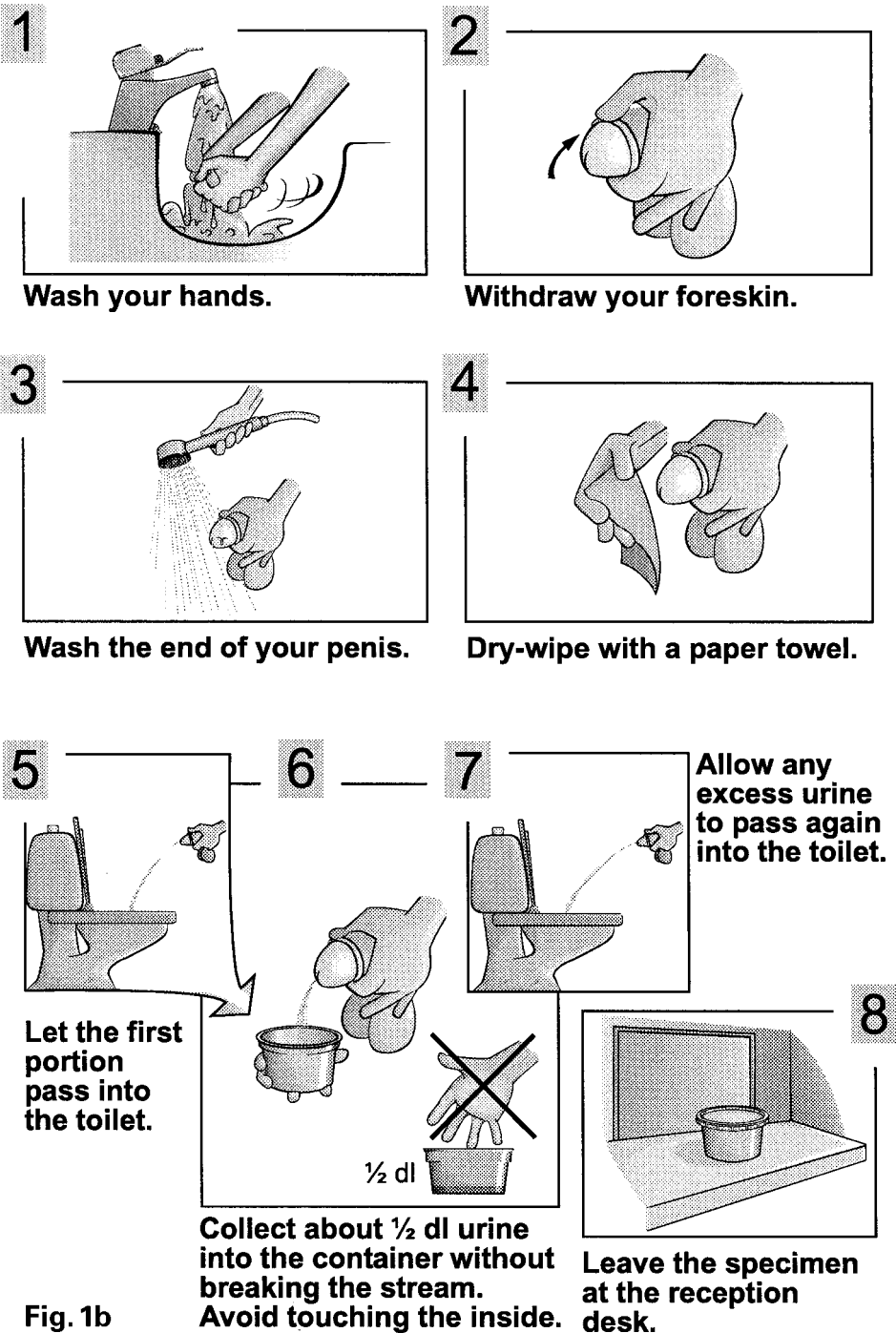
Leave the specimen at the reception desk.

Fig. 1b

TAUH Department of Clinical Chemistry / T.Heittola

FIG. 1.   Collection of mid-stream urine by females (1a) and males (1b) by washing genital organs with a shower. (Published with the permission of Tampere University Hospital.).

Collection of mid-stream urine, females

1

Wash your hands.

2

Spread your labia.

3

Wash your outer genital organs with 3-4 clean wet towelettes wiping downwards only once with each of them.

4

Dry-wipe with a paper towel downwards.

5

Let the first portion pass into the toilet.

6

7

Allow any excess urine to pass again into the toilet.

50 ml

Collect about 50 ml urine into the container without breaking the stream. Avoid touching the inside.

8

Leave the specimen at the reception desk.

Fig. 2a

TAUH Department of Clinical Chemistry / T.Heittola

Collection of mid-stream urine, males

1

Wash your hands.

2

Withdraw your foreskin.

3

Wash the end of your penis with a clean wet towelette.

4

Dry-wipe with a paper towel.

5

Let the first portion pass into the toilet.

6

7

Allow any excess urine to pass again into the toilet.

50 ml

Collect about 50 ml urine into the container without breaking the stream. Avoid touching the inside.

8

Leave the specimen at the reception desk.

Fig. 2b

TAUH Department of Clinical Chemistry / T.Heittola

FIG. 2.   Collection of mid-stream urine by females (2a) and males (2b) by washing genital organs with a towelette. (Published with the permission of Tampere University Hospital.).
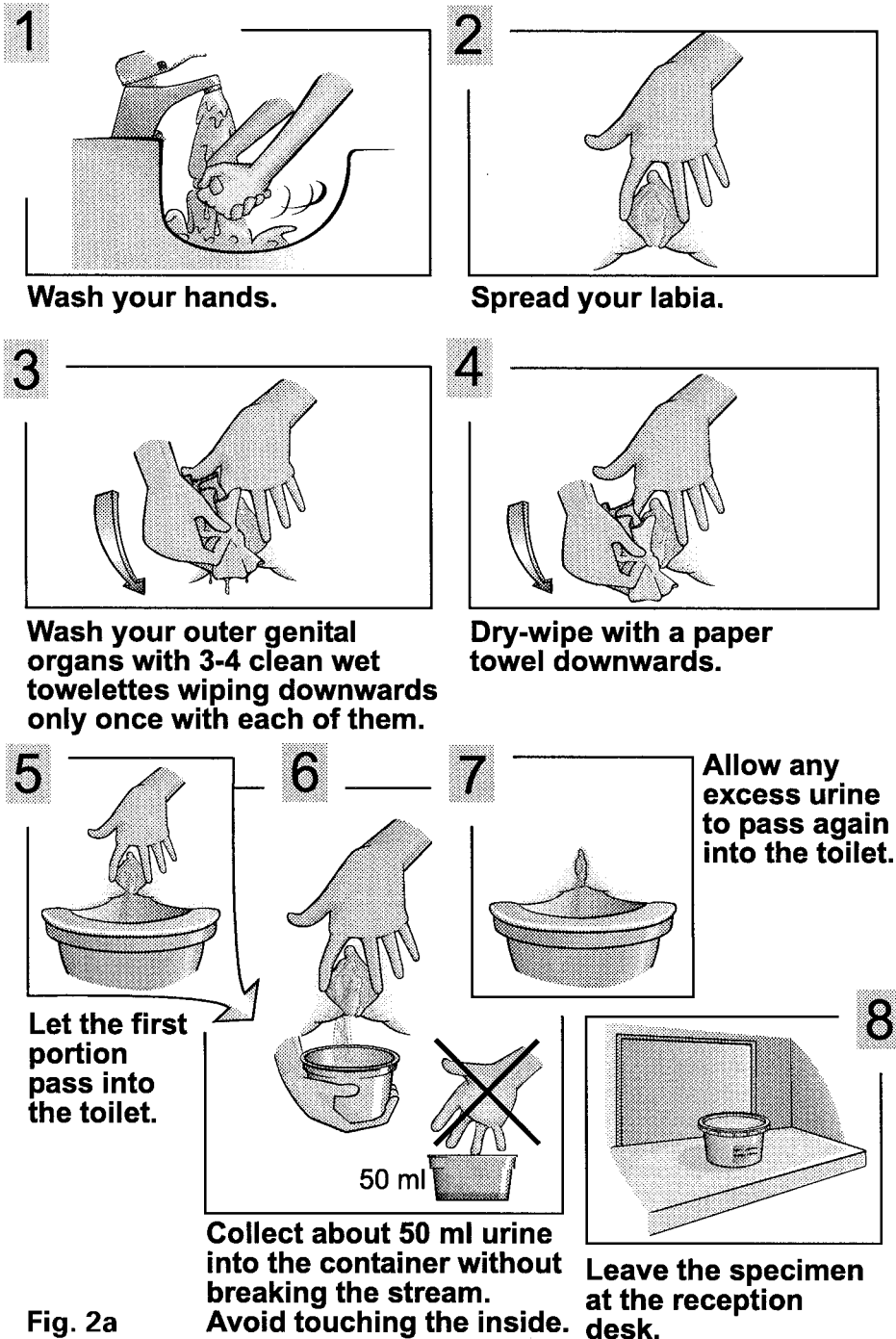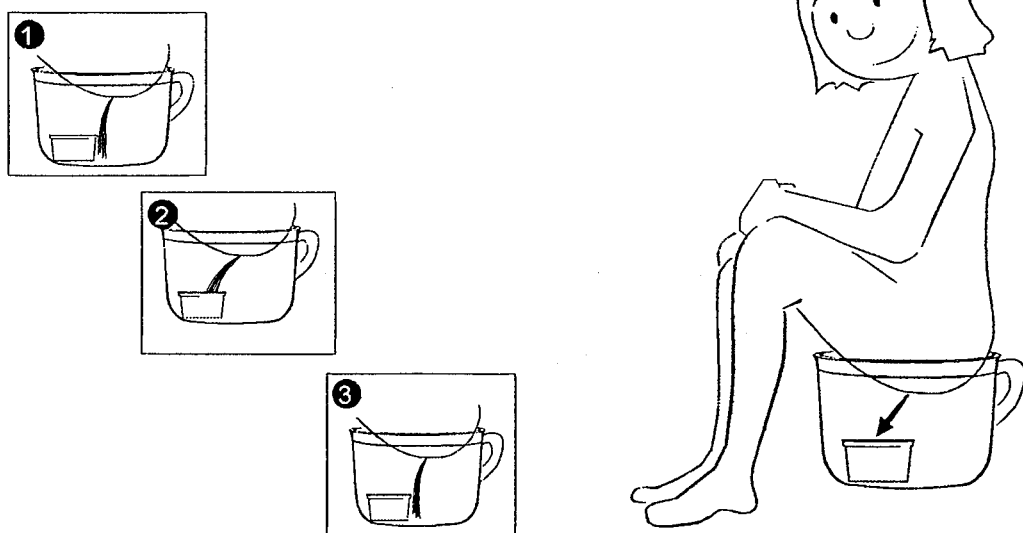
FIG. 3. Collection of mid-stream urine specimen when using a potty chair. (Published with the permission of Tampere University Hospital.).

Good method to hold the baby during collection

View from above
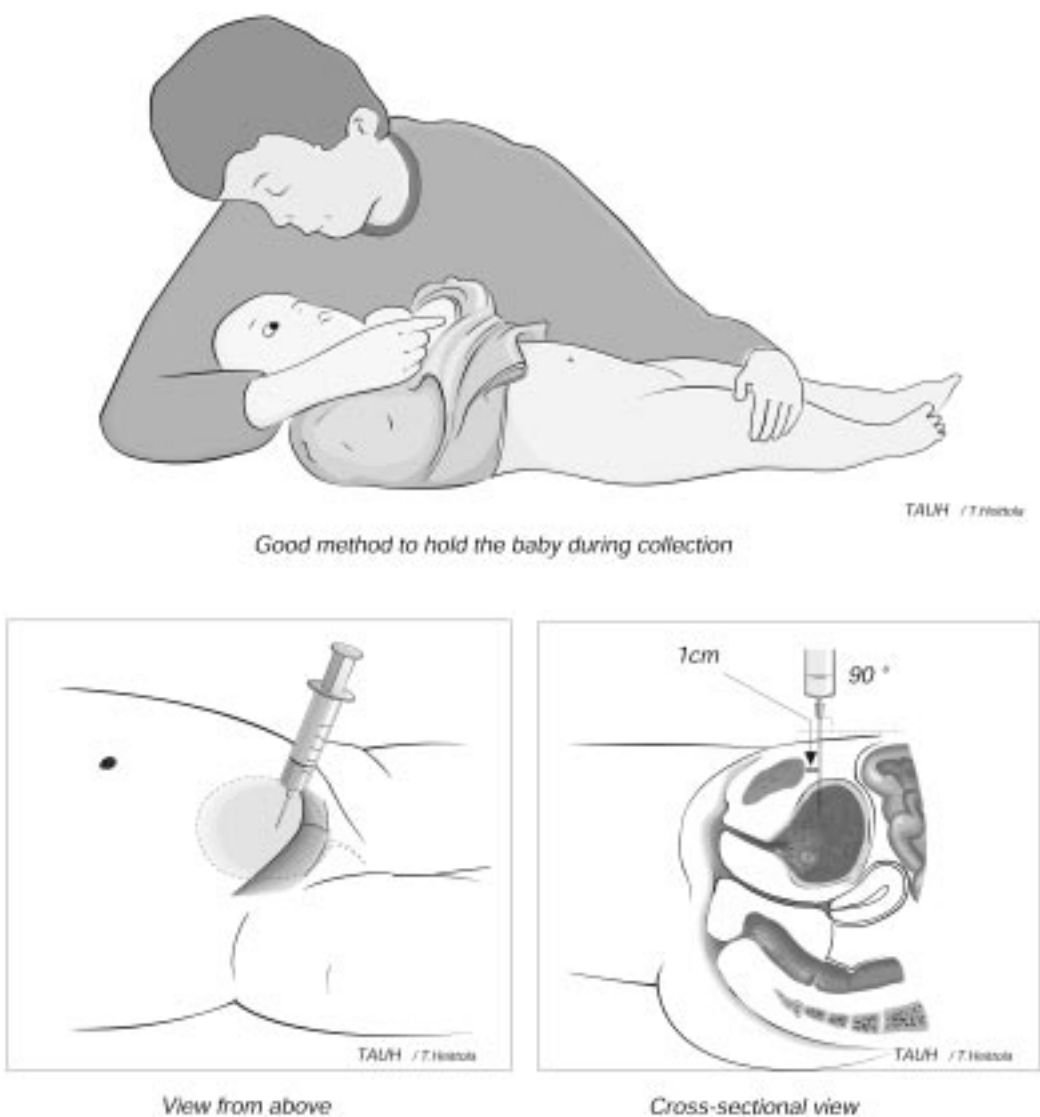

Cross-sectional view

FIG. 4. Specimen collection by suprapubic aspiration. (Published with the permission of Tampere University Hospital.) 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. 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 lidocain or prilocain 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, 0.5–2 mL is usually sufficient for inoculation.