EFLM WEBINAR

The EFLM Update of the European Urinalysis Guidelines

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Chair, EFLM TfG Urinalysis

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The members of the EFLM TfG Urinalysis have no personal conficts of interest interfering with this project

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From the ECLM to EFLM in the URINALYSIS GUIDELINES

EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

The document to be updated

ECLM. European Urinalysis Guidelines. (Kouri T, Fogazzi G, Gant V, Hallander H, Hofmann W, Guder W, editors). Scand J Clin Lab Invest 2000; 60 (Suppl 231): 1-96.

Sponsors from the IVD diagnostic industry

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Contents being Updated

Format of *GRADE*: RECOMMENDATIONS BASED ON EVIDENCE

Gyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schünemann HJ, for the GRADE Working Group. **GRADE:** an emerging consensus on rating quality of evidence and strength of recommendations. Brit Med J 2008; 336:924-6.

1. **Section: Medical needs and Preanalytics** (55 pages)
   Medical needs, Patient preparation and Specimen collection
   Hierarchy of Measurement Procedures
2. Section: Chemistry (56 pages)
3. Section: Particles (30 pages)
4. Section: Bacteriology (67 pages)

Total 207 pages

Provisional Recommendations: Medical Needs and Requisition

Recommendations (1-2) **1 = Strong, 2 = Weak recommendation**	Gyatt GH, et al, for the GRADE Working Group. Brit Med J 2008; 336:924-6.	**Level of Evidence** *
INDICATIONS: Urinalysis tests should be requested based on assessment of risk to severe disease. The specific tests should be planned between laboratories and clinics, to balance benefits against resource. (1)		B
Asymptomatic bacteriuria shall not generally be sought to avoid unnecessary antimicrobials and multiresistant strains of uropathogens. (1)		A
STRATEGY: General screening for low-risk and routine patients is to be separated from targeted diagnostics for high-risk patients. (1)		B
Requisition and reporting of urinalysis tests using electronic interfaces is encouraged, with local diagnostic algorithms. Electronic transfer improves exchange of systematic information between clinicians and laboratories. (1)		B

*Levels of Evidence: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts

TARGETED WORKLOAD: Bacteriuria screening

(1) Low-risk patients: non-pregnant females with typical symptoms of cystitis diagnosed with a validated ACSS questionnaire (Acute Cystitis Symptoms Score, from: http://www.acss.world/downloads.html)

(NO LABORATORY TESTS) (*note the exceptions, such as Chlamydia, yeast and Trichomonas diseases*)

(2) Other routine symptomatic patients: WORKLOAD OPTIMISATION

Mid-stream samples may be screened for bacteriuria by using WBC and Bacteria detection

by rapid examination (strip test), to detect bacteriuria at 10^5 CFU/mL (= 10^8 CFB/L) in emergencies, noting the false negative cases with symptomatic UTI

by automated counting to **rule out** bacteriuria at 10^4 CFU/mL (= 10^7 CFB/L) in the laboratories at a sensitivity of 90-95 % (considering clinical symptoms, way of urine collection, and leukocyturia)

Workflow to be organised locally/regionally based on patient populations, laboratory organisation & resource.

(3) High-risk patients: Sensitive cultures of special specimens and examination procedures

Provisional Recommendations: Patient Preparation

Recommendations (1-2) 1 = Strong, 2 = Weak recommendation	Level of Evidence *
PATIENT Interaction to reduce Contamination: Invite patients to become subjects in decision-making on their disease. This would encourage them to learn how to collect a mid-stream urine (MSU) specimen in a best achievable way, in order to minimise contamination. *Quality specification*: desirable rate < 10%, maximum rate < 15% of MSU specimens against 10^4 CFU/mL (or 10^7 CFB/L) in culture, calculated at a laboratory level. (1)	C
Laboratories shall maintain educational material bank and to enforce routine co-operation with their clinical units in order to improve preanalytical processes, including preparation of patients for delivering their urine specimens. (1)	B

Native or second language?

Visual presentation

Culturally adapted videos

+ Human councelling

Recommendations (1-2) 1 = Strong, 2 = Weak recommendation	Level of Evidence *
CONCENTRATION of single-voided urine specimens shall be measured together with the primary measurand, expressed as measurand-to-reference ratio, e.g., albumin-to-creatinine ratio, or reported separately as urine **osmolality,** relative density, or conductivity, e.g., with urine particle counts. (1)	B

Dense urine (low diuresis)  Dilute urine (high diuresis)

- Identical excretion rates of particles / proteins, but different amount of water excretion (diuresis, volume rate)

Provisional Recommendations: Specimen Collection

Recommendations (1-2)	Level of Evidence*
Measurand-to-reference ratios in single-voided urine specimens, similar to albumin-to-creatinine ratios, are recommended **to** replace timed (24-hour) urine collections for other measurands as well - in order to reduce incidence of non-conformities. Verification of an intended measurand to any new patient group is needed before clinical application. (1)	A
Preservation of urine specimens is obligatory if the sample is not analysed within 2-6 hours. Consider refrigeration if applicable. The preservation shall be verified against the given specification of the measurement procedure. (1)	B

*Levels of Evidence: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts

Suggested criteria for urine preservation

The preservation should fulfill the following criteria:

(a) For urine components with **exponential changes** in disease**,** a maximum of a two-fold change is acceptable, i.e., a maximum loss down to -50 %, or increase to +100% from the original concentration.

(b) For urine components with **linear changes** in disease, an 80% (minimum) to 90% (desirable) preservation of original concentration is recommended.

Preservatives were reviewed for urinalysis, bacterial culture, and quantitative chemical measurements - also required by the IVDR and MDR regulations.

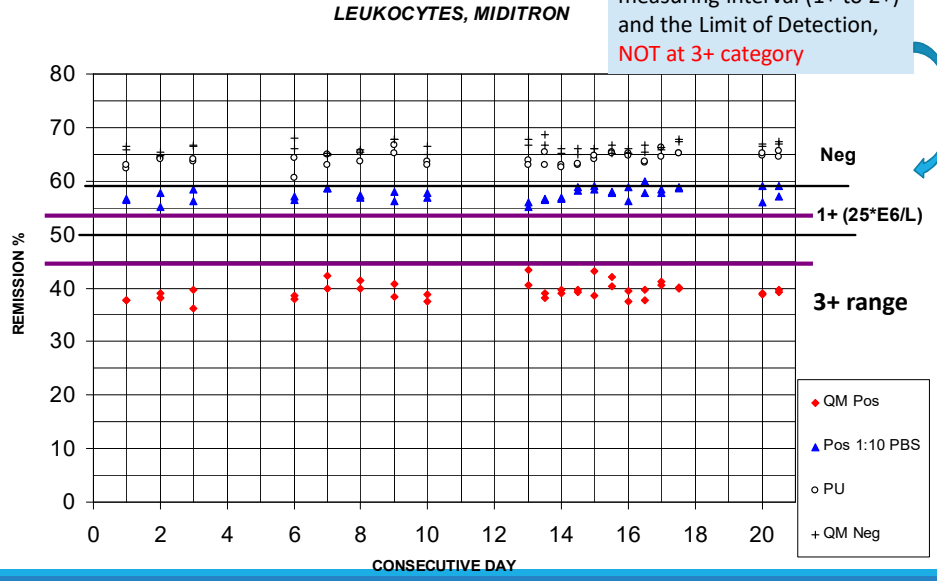
Provisional Recommendations: Chemistry

Recommendations (1-2)	Level of Evidence
Multiple (multiproperty) test strips are still recommended as screening tools for **low-risk patient** populations because of their cost-efficiency. They are NOT recommended for urine diagnostics of high-risk patients if insensitive. (1)	A
Performance of strip tests must be verified against quantitative measurements and monitored with **control solutions at the limit of positivity** (1+/2+ range). (2)	B
Sensitive detection of renal disease in high-risk groups requires measurements of both albumin, and a tubular marker in urine, such as α1-microglobulin**,** expressed as measurand-to-creatinine ratio. Measurement of urine total protein remains important in screening for free light chains in urine (*Bence Jones* proteinuria). (1)	B

Levels of Evidence: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts

Internal Quality Control of test strip reading using continuous reflectance values

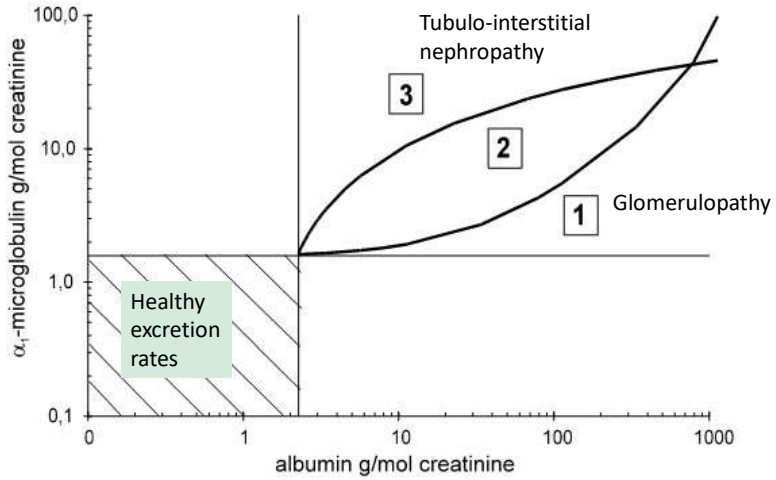
Focus on the dynamic measuring interval (1+ to 2+) and the Limit of Detection, NOT at 3+ category



Quantitative detection of kidney disease with proteinuria markers

(Hofmann W et al, 1998)

GROUPS
1. Glomerulopathy
2. Secondary glomerulo-pathy, e.g., in diabetes
3. Tubulo-interstitial nephropathy



Provisional Recommendations: Particles

Recommendations	Level of Evidence *
Laboratories shall clearly describe their basic and advanced differentiation and reporting of urinary particles. (1)	A
Automated particle analysers need to be **verified** before implemented into routine, **against published performance specifications (Level 3 procedure).** Appropriate review rules for visual microscopy need to be defined. (1)	A
Laboratories shall **decide and verify one of the (Level 2) procedures** of visual microscopy for **their routine** particle analysis. Application of phase contrast, in addition to bright field optics is strongly recommended for visual microscopy of urine particles. (1)	A
The recommended standard unit to particle counts is **particles/L (litre),** as decided at national level to avoid confusion and risks to **patient safety.** (1)	C

*Levels of Evidence: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts

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Urinalysis Guideline: Two levels of particle identification

Basic level	Advanced level
Emergency cases / Automation (24/7)	**Renal disease**
Erythrocytes, RBC	Subgroups: isomorphic and dysmorphic RBC
Leukocytes, WBC / granulocytes	WBC differentiation: lymphocytes, macrophages, granulocytes
Squamous epithelial cells Small (non-squamous) epithelial cells	**Renal tubular cells**, transitional epithelial cells (atypical cells / cytopathologists), intestinal epith cells, others
Casts: Hyaline casts Pathological = non-hyaline casts	**Differentiation of pathological casts:** RTC, RBC, WBC, Bacteria, Yeast casts, Granular, waxy, fatty, bilirubin, haemoglobin casts
Bacteria	Gram staining (MICROB laboratories)
Yeasts, trichomonas, Spermatozoa	*Schistosoma haematobium*; tropical diseases
Artefacts (hair, fibre, talc, etc)	Artefacts (hair, fibre, talc, etc)
Precipitate, crystals	**Differentiation of crystals**

eLearning, Timo Kouri

Hierarchy of Procedures (= Levels of Accuracy) in Urine Particle Analysis

Level 3: Advanced comparison level

- Kouri T, György A, Rowan RM, and the ISLH Task Force. ISLH Recommended Reference Procedure for the Enumeration of Particles in Urine. Lab Hematol 2003; 9:58-63

Level 2: Routine quantitative level

Automated quantitative counting with different technologies

Visual quantitative counting and differentiation

- Urine sediment counted in a chamber (concentration 20:1?, a precise volume)
- Chamber counting of uncentrifuged specimens (no concentration, a precise volume)
- Standardised urine sediment under a coverslip (concentration factor? -> volume?)

Level 1: Ordinal scale level

- Non-standardized urine sediment, reporting at ordinal scale (0, 1+, 2+, 3+)

Provisional Recommendations: Bacteriology

Recommendations (1-2)	Level of Evidence *
Chromogenic agar is strongly recommended as primary agar medium to identify *Escherichia coli* (most frequent uropathogen) easily, quickly, and inexpensively (no need for a panel of tests to define the species). A second agar (such as blood agar) is needed in clinically defined cases and for fastidious organisms. (1)	B
Bacterial identification using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (**MALDI-TOF MS**) is strongly recommended in medium-sized and large laboratories (> 100 specimens/day), to improve patient prognosis with (i) Accuracy and reliability of identification to the species level, and (ii) Shortened delay of reporting (from 36-48 h to 8-24 h). (1)	A
New species *Aerococcus spp., Actinotignum schaalii* and *Corynebacterium urealyticum* are proposed into the list of Type II uropathogens. (2)	B
No recommendation to the unit volume (mL or L) for reporting urine bacterial cultures. This is to be harmonised nationally to avoid confusion among professionals and patient risks. (1)	

*Levels of Evidence: A = high, B= moderate, C= low quality of evidence, D = consensus by the experts

Significance of bacteriuria and laboratory workflow

Clinical picture
- Patient population
- Symptoms and signs
- Background diseases

Way of urine collection
- Midstream specimens
- Paediatric bags and pads
- Catheterization
- Special samples

Urine specimen

Rapid screening
as locally applied

Rapid measurements (test strip, counting)
- **Leukocyturia**
- Bacteriuria

Urine bacterial culture

Colony counts (CFU/mL or CFB/L?)
- Special samples ≥ 10^2 CFU/mL (limit of positivity)
- Routine samples
  - Borderline 10^3 CFU/mL or 10^6 CFB/L?
  - Positive growth ≥ 10^4 CFU/mL

Polymicrobial growth ≥ 3 species

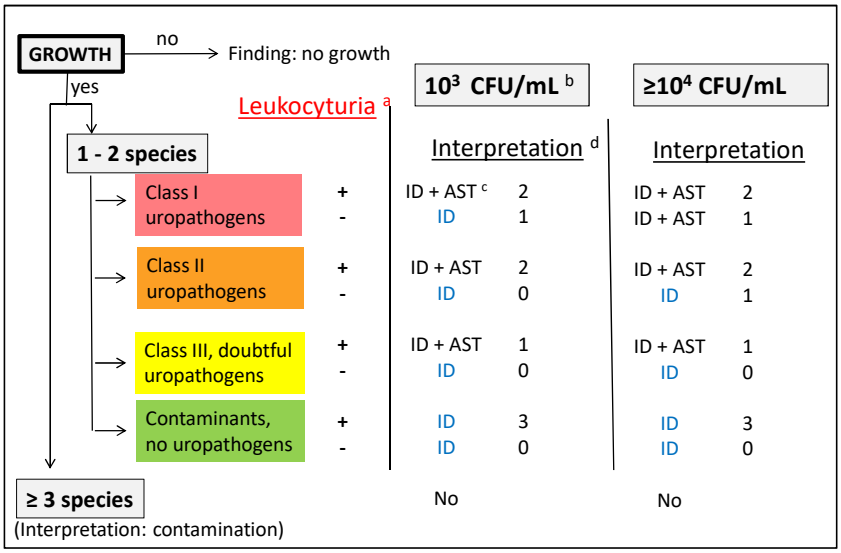
Identification of species
- Chromogenic agar 60%
- MALDI-TOF MS 40%
- Biochemistry tests occasionally

Antimicrobial Susceptibility Test

Type of grown species in culture
- Type I uropathogens (*E. coli; S. saprophyticus*)
- Type II uropathogens
- Potential uropathogens
- Contaminants

Report

Workflow of primary urine cultures from routine specimens



ID = Identification to species level

AST = antimicrobial susceptibility test

CFU = colony-forming units

Take home messages

Preanalytics: True medical indications and patient guidance create the backbone of urinalysis tests.
Hiearchy of measurements: A reference procedure of a higher level of accuracy is needed for all clinical laboratory examinations.

Chemistry: Quantitative quality control is needed also for strip tests. Kidney disease is to be screened both for albuminuria and α1-microglobulinuria (tubular proteins).

Particles: Basic level of particle identification is for 24/7 service. Verify your automated and visual procedures, using phase contrast microscopy.

Bacteriology: Consider improving your workflow, to be able to move from specimens of low-risk patients to those of high-risk patients.

Thank you for Your attention!

Appendix in the following slides

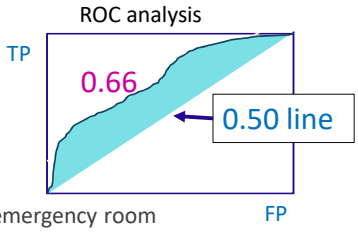
Males

Females

Available since 2000

Diagnostic performance of urine particles in renal diseases

Perazella MA, Am J Kidney Dis 2015



Acute kidney injury (AKI)

- RTC and casts in visual microscopy; AUC = 0.66
  (Useless AUC = 0.50, maximum AUC = 1.00)
- Prediction of a severe disease in AKI, AUC = 0.85
- Urine NGAL, AUC = 0.74
- Sensitivity and specificity depend on tested populations:
  Se 22 % with Sp 91% in separating AKI from non-AKI among patients at emergency room
- **Urine microscopy is more specific (but less sensitive) than NephroCheck** (combination of IGFBP7 and TIMP-2)

Chronic kidney disease

- **Particles present** more often in proliferative GN than in non-proliferative glomerulonephritis (GN)
  (Fogazzi GB et al, J Nephrol 2005)

- Urine microscopy is cheaper than specific immunochemical tests from urine
  → selection of the test reflects availability and national economy

ISLH REFERENCE MEASUREMENT PROCEDURE (Level 3)

Kouri T, Rowan RM, György A & ISLH Task force. Lab Hematol, 2003; 9:58-63

Chamber counting, no centrifugation

- 200 (RBC, WBC) or 50 counted particles (Epithelian Cells, Casts) (Poisson statistics)

Counting volume: Fuchs-Rosenthal chamber: 0.2 µm → 2x 3.2 µL;

- Bürker chamber: 0.1 µm height with 2x 0.9 µL volume, is applicable to Sternheimer staining with a background colour

Detection: phase contrast optics

Identification: phase contrast (+ Sternheimer stain if necessary)

AUTOMATED PARTICLE DIFFERENTIATION*

*Performance depends on precise cut-off limits of low counts, and patient populations

Flow cytometry (Sysmex UF-5000)

Previtali G et al, Clin Chim Acta 2017

	Sensit	Specif	Se * Sp
RBC	88,0 %	74,0 %	65,1 %
NLRBC	85,0 %	79,0 %	67,2 %
WBC	94,0 %	92,0 %	86,5 %
WBCc	95,0 %	98,0 %	93,1 %
EC	93,0 %	89,0 %	82,8 %
SEC	91,0 %	96,0 %	87,4 %
NEC	94,0 %	77,0 %	72,4 %
TEC	71,0 %	94,0 %	66,7 %
RTC	95,0 %	75,0 %	71,3 %
TOT CAST	72,0 %	83,0 %	59,8 %
HYA CAST	64,0 %	91,0 %	58,2 %
PAT CAST	91,0 %	91,0 %	82,8 %
BAC	not studied		
CRY	92,0 %	99,0 %	91,1 %
YEA	90,0 %	93,0 %	83,7 %

Image analysis in cuvette (UriSed3PRO)

Falbo R et al, Clin Chim Acta 2019

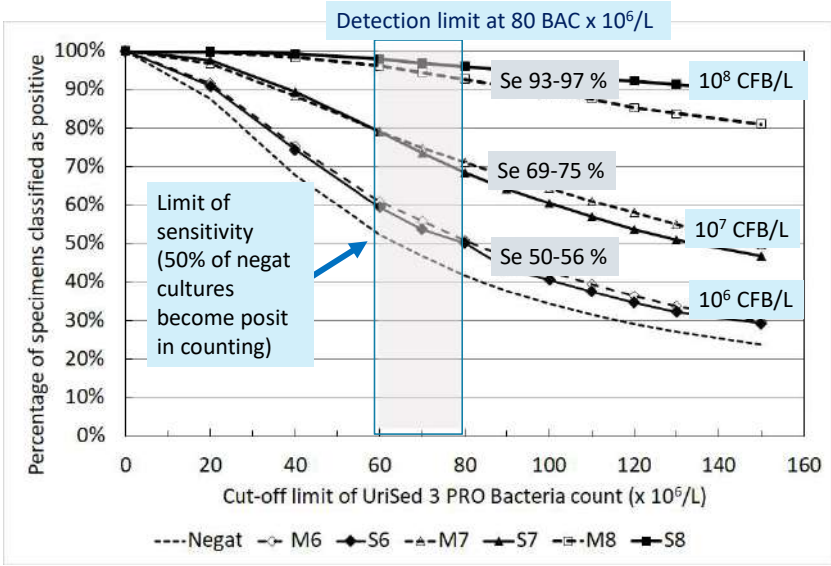
	Sensit	Specif	Se * Sp
RBC	81,5 %	96,4 %	78,6 %
WBC	89,5 %	97,0 %	86,8 %
SEC/EPI	86,5 %	94,2 %	81,5 %
NEC	84,6 %	79,9 %	67,6 %
HYA	70,6 %	97,0 %	68,5 %
PAT	61,8 %	97,2 %	60,1 %
BAC	not studied		
BACc	95,6 %	71,9 %	68,7 %
BACr	92,8 %	85,3 %	79,2 %
CRY	78,6 %	92,0 %	72,3 %
YEA	82,4 %	97,9 %	80,7 %
MUC	64,0 %	83,6 %	53,5 %

Imprecision of low particle count (x/µL) and counted volume (p): Poisson coefficients of variation for total count, CV(n), where n= p*x

Kouri T et al, Clin Chim Acta 2021; 515: 96-103

	Counted volume p (µL)						
Volume, p	*1*	*2*	*3*	*5*	*8*	*10*	*30*
Improvement factor, √p	*1*	*1,41*	*1,73*	*2,24*	*2,83*	*3,16*	*5,48*
Particle count (x/µL)							
3	57,7 %	40,8 %	33,3 %	25,8 %	20,4 %	18,3 %	10,5 %
5	44,7 %	31,6 %	25,8 %	20,0 %	15,8 %	14,1 %	8,2 %
10	31,6 %	22,4 %	18,3 %	14,1 %	11,2 %	10,0 %	5,8 %
20	22,4 %	15,8 %	12,9 %	10,0 %	7,9 %	7,1 %	4,1 %
30	18,3 %	12,9 %	10,5 %	8,2 %	6,5 %	5,8 %	3,3 %
100	10,0 %	7,1 %	5,8 %	4,5 %	3,5 %	3,2 %	1,8 %
1000	3,2 %	2,2 %	1,8 %	1,4 %	1,1 %	1,0 %	0,6 %

Sensitivity to detect bacteria by automated counting depends on clinical sample mix and definition of positive growth



Categories in culture (CFB/L)

Negat growth

M6 – M8, mixed growth at CFB 10^6 – 10^8/L

S6 – S8, identified species at CFB 10^6 – 10^8/L

CFB 10^6 – 10^8/L is equal to CFU 10^3 – 10^5/mL

Kouri T, et al. Clin Chim Acta 2021; 516: 149-156.

Automation in bacteriology

Mechanisation of preanalytics

- opening specimen containers, sample preparation, streaking, conveyors between different units

Incubators

- aerobic and CO_2 incubators / standardised temperature and atmosphere, and plate readers

  → increased sensitivity and shortened incubation time

Detection and identification

- high-resolution digital imaging of chromogenic agar plates, identification algorithms of colonies
- automated colony pickers allow inocula preparation for MALDI-TOF MS and Antimicrobial Susceptibility Testing

Turn-around time (TAT), from specimen arrival to result reporting

- reduction of TAT to 5 hours (negative), or 14 hours (positive for *E.coli*), others longer

Typical portions of species identified in a routine workflow: Chromogenic agar 60% (role of *E.coli*), MALDI-TOF Mass Spectrometry 40%, Biochemistry rarely needed after the two other procedures

Steps of Progress in 2022

The preliminary version is now on scientific review

Professionals within EFLM societies
Other medical professionals (laboratory specialists, and clinicians)
Representatives of IVD Sponsors

The official review is taken by the Chair of Committee of Science (Prof Michel Langlois) according to the EFLM procedures, and to a Public Consultation by ESCMID for endorsement.

Reviews and voting for acceptance by the EFLM national member societies, after which Official publication (Guideline is a Type 1a document) in the CCLM (probably in 2023).

Density of urine

Measurand	Hypotonic	Isotonic	Hypertonic
Osmolality, mOsm/kgH_2O	100	300	1000
Relative density (ratio to water)	1.003	1.010	1.030
Conductivity, mS/cm	2	5-7	30

Regression equation empirically:
y (urinary osmolality; mOsm/kg) = 23.83 × (urinary conductivity, µS/cm) + 181.25 (r^2 = 0.539)

Oyaert M et al, CCLM 2019;57:1169-57.

Interpretation of urine cultures

0 = Detected microorganisms **probably do not cause** a UTI (even with corresponding symptoms).

1 = Detected microorganisms **possibly** cause UTI in selected clinical presentations (immunocompromised patients, early infection…) with appropriate clinical picture.

2 = Detected microorganisms with significant colony counts. UTI is **probable** with appropriate clinical picture.

3 = No microorganisms detected with the used culture procedure. In presence of appropriate clinical picture, consider tests specific for **other microbes**, e.g., *Chlamydia*, *Mycoplasma*, *Ureaplasma*, *M. tuberculosis*, *N. gonorrhoeae*."