Sample Collection and Management Manual

for the clinics and local laboratory procedures within the

ECRAID-Base POS-cUTI

(Perpetual Observational Study on complicated Urinary Tract Infections)

Developed by: **LAB-Net (University of Antwerp, Belgium)**

In collaboration with: **Department of Clinical Microbiology, University Hospital for Infectious Diseases, Zagreb, Croatia**

Sponsor Protocol Number: **Version 3.0, Approval Date February 8th 2023**

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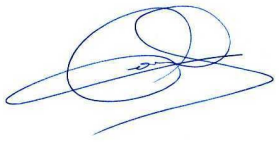
This manual has been authorised by:

Name	Signature	Date
Prof. Dr. Herman Goossens, ECRAID-Base LAB-net Coordinator (UA)		9 February, 2023
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List of abbreviations

UTI:	Urinary tract infetions
IDSA:	Infectious Diseases Society of America
ESCMID:	European Society of Clinical Microbiology and Infectious Diseases
CFU:	Colony forming units
CoNS:	Coagulase-negative staphylococci
ECRAID:	European Clinical Research Alliance on Infectious Diseases
CE:	Conformité Européenne (European Conformity)
IV:	Intravenous
BSL-2:	Biosafety level 2 laboratory
BSL-3:	Biosafety level 3 laboratory
L:	Leukocytes
HPF:	High power field
NA:	Not applicable
CLED:	Cystine lactose electrolyte deficient
ICU:	Intensive care unit
MH:	Mueller Hinton
f:	Female
m:	Male
ICF:	Informed Consent form
SoC:	Standard of care
ID:	Identification

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Document history		
Version	**Version Date**	**Summary of changes**
1.0	NA	Does not exist due to a typo in version 2.0
2.0	23/05/2022	New document
3.0	8/02/2023	Addition of Chapter 5.1.1 (Urine cultures) Addition of Chapter 5.1.2 (Blood cultures) Addition of Chapter 5.1.3 (Interpretation of the culture results) Addition of Table 7 (Interpretation of the culture results)

1.0. Introduction

This manual provides basic information on routine diagnostic procedures in processing a urine sample in a microbiology laboratory. Some procedures may be modified at the discretion of the microbiology laboratory as long as these procedures are certified.

Due to the frequent contamination of a urine sample with the periurethral microbiota, urine culture findings are not straight forward to report. After observing growth on an agar plate a decision on further workup is based on the information about the type of specimen and the colony count and morphology. As not all bacterial species have the same potential to cause urinary tract infetions (UTI), final reporting will also depend on species identification. However, final interpretation of the clinical significance of the quantitative bacteriuria report is linked to the clinical presentation of the patient. As different significance cut-off values are applicable for different UTI categories, standardized classification of urinary tract infections has a great impact on the correct interpretation of the laboratory findings.

1.1. Classification of urinary tract infections (UTI)

According to the IDSA (Infectious Diseases Society of America) and the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) guidelines for the evaluation of new anti-infective drugs for the treatment of urinary tract infection, UTIs are divided into the following categories:

- acute uncomplicated cystitis in premenopausal, non-pregnant women
- acute uncomplicated pyelonephritis
- complicated UTI (including all UTI in men)
- asymptomatic bacteriuria
- recurrent UTI (uncomplicated, no predisposing factors)

In a patient with bacteriuria the documentation of pyuria and the significant number of colony forming units (CFU) per milliliter of urine cultured constitute important evidence for UTI and inclusion in clinical trails aiming to evaluate new anti-infective drugs for the treatment of UTI. In general, findings of large numbers of bacteria in urine ($\geq 10^5$ CFU/mL) are strongly associated with true infection, whereas lower numbers usually indicate contamination. However, patient's characteristics and clinical presentation should be taken into account when interpreting the significance of the urinary colony counts (Table 1).

Table 1. **Entry criteria for studies evaluating new anti-infective drugs for the treatment of UTI**

UTI Category	Clinical symptoms	Pyuria	Significant number of bacteria in urine (CFU/ml)
Acute uncomplicated cystitis	Dysuria, urgency, frequency, suprapubic pain	**YES**	≥ 10^3 CFU/mL
Acute uncomplicated pyelonephritis	Fever, chills, flank pain; other diagnoses excluded; no history or clinical evidence of urologic abnormalities	**YES**	≥ 10^4 CFU/mL
Complicated UTI	Any combination of symptoms described above; one or more predisposing conditions for UTI	**YES**	**For women:** ≥ 10^5 CFU/mL or ≥ 10^4 CFU/mL for a urine sample obtained from an indwelling urinary catheter **For pregnant women:** ≥ 10^3 CFU/mL **For men:** ≥ 10^4 CFU/mL
Asymptomatic bacteriuria	**NO**	**YES**	**For women:** ≥ 10^5 CFU/mL, the same bacterial strain in two consecutive mid-stream urine cultures taken ≥ 24 hours apart **For men:** ≥ 10^5 CFU/mL in single mid-stream urine sample
Recurrent UTI	At least 3 episodes of acute uncomplicated infection documented by culture in last 12 mo, in women with no structural/functional abnormalities	**YES**	Uncomplicated cystitis: ≥10^3 CFU/mL Uncomplicated pyelonephritis: ≥ 10^4 CFU/mL

1.2. Urinary tract pathogens

Although almost every bacterium can cause urinary tract infection including urogenital microbiota, urinary pathogens are classified as primary, secondary and doubtful pathogens and members of microbiota that very rarely cause infections (Table 2). Originally, *Acinetobacter baumannii* was included in the group of doubtful pathogens but as this species has shown higher virulence in recent years, it is included in a group of secondary pathogens.

Table 2. Level of pathogenicity and frequency of microorganisms causing UTI

Pathogenicity in urinary system	A. Common (>10%)	B. Less 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.,* *(Leptospira,** *Mycobacteriaceae** *)*
II. Secondary pathogens		*Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *P. mirabilis, P. aeruginosa A. baumannii*	*Citrobacter* spp., *M. morganii, P. vulgaris, Serratia* spp., *S. aureus*	*C. urealyticum, C. seminalae, Haemophilus* spp.,*** Pneumococci***
III. Doubtful pathogens		*S. agalactiae,* yeasts, other CoNS****	*Acinetobacter* spp., *Pseudomonas* spp., *S. maltophilia, B. cepacia*	Microorganisms described as rare pathogens in some publications*****
IV. Urogenital microbiota		α – hemolytic streptococci, *G. vaginalis,* lactobacilli	*Bifidobacterium* spp., *Corynebacterium spp.* (non *C. diphteriae*)	

* Low concentrations are also reported, even if they are due to sample contamination

** Diagnostics is not covered with this manual

*** Most often isolated in children

**** Coagulase-negative staphylococci other than *S. saprophyticus*

***** e.g., *Aerococcus urinae*

E. coli and *S. saprophyticus* are primary pathogens that cause >80% of uncomplicated UTIs in the population with a normal function and anatomy of the urinary tract. Secondary pathogens rarely cause uncomplicated UTIs but are often the cause of complicated, especially hospital-acquired UTIs.

Some Gram-positive bacteria (*S. agalactiae, Aerococcus urinae*, coagulase-negative staphylococci other than *S. saprophyticus*) and some Gram-negative non-fermenters (*Pseudomonas* spp. except *P. aeruginosa*, *Acinetobacter* spp. except *A. baumannii*) and *Candida* spp. can be considered significant when growing in high numbers of colonies and in a repeated sample. *S. agalactiae* is considered a significant UTI pathogen in diabetic patients.

The growth of bacteria from the fourth group of pathogens, urogenital microbiota, especially in combination with the presence of epithelial cells in the Gram smear, should be interpreted as contamination during sampling. These bacteria, as well as coagulase-negative staphylococci, can be considered significant pathogens only if isolated in a sample taken by suprapubic aspiration, or if the large numbers of colonies are growing in a repeated sample and if the sample is not contaminated with other urogenital microbiota. The clinical significance of these bacteria should be discussed with the clinician before reporting.

2.0. Quality control in the laboratory

The microbiology laboratory participating in ECRAID study should conduct regular internal quality control to ensure accurate, reliable and reproducible test results. Internal quality control should be practiced according to the locally adopted good laboratory practice using standard quality control strains.

Preferably, the microbiology laboratory participating in ECRAID study should be accredited or must prove it is technically competent by taking part in the external quality assessment sheme.

3.0. Preanalytical procedures

3.1. Urine collection

Sites should collect urine specimens as per Study protocol.

Urine collection, transport, and analysis must be done in line with good laboratory practice, using materials and medical equipment for *in vitro* diagnostics with the CE [Conformité Européenne (European Conformity)] mark. Urine should be sampled in a sterile impermeable container following the aseptic technique procedure.

For urine culture, a minimum of 1 ml of urine should be sampled.

The request form should be properly filled in, including the correct type of a urine sample and correct time of urine collection.

Whenever possible, urine should be collected:

- before starting or changing antimicrobial therapy
- before rehydrating the patient either orally or by IV infusion
- early in the morning or at least 4h after the last urination

Acceptable methods of urine collection are listed in Table 3.

Table 3. Urine collection methods

Type of a urine sample	Sampling procedure	Note
Mid-stream, „clean catch" urine	Sample should be collected at least 4 hours after the last urination A nurse or other caretaker should instruct the patient how to provide a proper sample. Instructions should include the following: - Wash your hands - Unscrew the lid of the sterile container, avoiding to touch the inside - Cleanse the area around the uretral orifice ◦ In women: the external urethral orifice and the vulva should be washed properly, movement from the front to back to be repeated three times (each time using a new towelette or gauze soaked in sterile saline) ◦ in men: the glans and the external urethral orifice should be cleansed with water (the foreskin of an uncircumcised male must be retracted) - Do not dry with a towel - Collect the specimen ◦ Release the first urine flow in the toilet (about 20 ml of urine) ◦ collect the following 20-30 ml (maximum) of the urine in a sterile container without touching the edge of the container ◦ Close the container tightly and clean the outside - Wash your hands - Mark the container (write the full name of the patient) - Write the exact time of the sampling and send the sample immediately to the laboratory with a request form	◦ do not use an antiseptic to clean the urethral orifice ◦ Never take a urine sample from a bedpan
„in and out" catheterisation	- Wash your hands, use gloves - Wash the external urethral orifice with water and place a catheter ◦ Discard the first 15-30 ml of urine	◦ used in a patient unable to obtain mid-

	◦ Collect the next flow of urine in a sterile container	stream urine sample
Catheter urine (indwelling „Foley“catheter)	Check if there is enough urine in the catheter tube (10 ml) If not, squeeze the catheter tube for 10-15 minutes Sampling kit requires: gloves, 10-15 ml needle and syringe, alcohol-soaked wipe pad, sterile urine container - Wash your hands, use gloves - Disinfect the catheter collection port with 70% alcohol - Puncture the collection port with a needle attached to a syringe - Aspirate 10 mL of urine and transfer it into a sterile container, label it and prepare for transportation - Dispose the used needle and syringe in a suitable container	◦ Do not take urine from a urine collection bag! ◦ The sample from the newly placed catheter is preffered as there is a lower risk of contaminating urine sample with bacteria colonizing the catheter
Cystoscopy urine	Urine sample is obtained by a cystoscope	
Urostomy urine	- Wash your hands, use gloves - Remove the bag - Clean the area around the stoma with an antiseptic - Place a new bag - Collect the urine directly from the urostomy or with the single-used catheter (14)	
Suprapubic aspirate	- Wash your hands, use gloves - Remove hair (if present) with a clipper - Clean the skin with 70% alcohol twice with allowing alcohol to dry - Aspirate urine directly from the bladder	◦ The bladder should be full and palpable before aspiration
Urine of incontinent patient	◦ in women: - catch a sample after properlly washing the genital area or - use in and out catheterisation if the previous procedure is not feasible	

	◦ in men: - collect urine in a sterile urine collector bag - avoid catheterization	

3.2. Criteria for specimen rejection

The following specimens should be discarded:

- 24-hour urine
- catheter tip
- sample from a urine bag in the case of a catheterized patient
- sample in a damaged container
- urine sample sent for anaerobic analysis not sampled by suprapubic aspiration

Rejection of an inadequately sampled, transported or stored sample should be discussed with a clinician and if it cannot be replaced by a new sample and critically important for the patient it can be processed with a comment stating that the result is unreliable.

3.3. Specimen storage and transportation

Urine specimens should be delivered to the microbiology laboratory within 24h after collection. If not transported in a preservative tube urine should not be kept at a room temperature. If urine processing from collection to plating is not possible within 2 hours after collection, it should be stored and transported at +4°C. Do not store the urine sample for cultivation in the refrigerator for more than 24 hours. For the analysis of cellular elements (by microscopy or alternative methods) urine can be stored in the refrigerator for a maximum of 8h.

Urine should be transported in a sterile container closed in a plastic bag.

The laboratory should document date and time of specimen receipt and date and time of urine plating.

4.0. Laboratory processing of a urine specimen

Laboratory (microbiological) examination of a urine sample includes microscopy (or alternative methods for determining cellular elements) and quantitative culture or alternative non-cultivation methods. Samples are processed in BSL-2 (biosafety level 2 laboratory). Exceptionally, in case of suspicion of infection caused by *Mycobacterium* spp. (isolation of

mycobacteria is not included in these guidelines), *Salmonella* Typhi, *Salmonella* Paratyphi A, B, C, samples are processed in the BSL-3 (biosafety level 3 laboratory).

4.1. Microscopy and alternative methods for determining cellular elements

Urine microscopy or another method for the determination of cellular elements in urine is recommended for all symptomatic patients to assist in the interpretation of culture results, UTI diagnosis, assessing sample quality, and streamlining further diagnostics.

Although it is difficult to set normal range for white blood cells in urine that would account for all patient types or conditions, significant pyuria is usually defined as $>10^4$ leukocytes (L)/mL (>10 L/µL) in uncentrifuged urine, corresponding to >5 L per high power field (HPF, x400) (estimated as awerage of 10 HPFs inspected) in centrifuged urine sediment.

Hematuria may be present in patients with UTI but is not a specific indicator of infection as it may be present in a variety of non-infectious urinary tract diseases.

Squamous epithelial cells are a good indicator of sample contamination with vaginal and distal urethral microbiota.

Parasites (e.g., *Trichomonas vaginalis, Schistosoma haematobium*) and fungi (*Candida* spp.) if found by microscopic examination of the urine sample, should be reported.

The leukocyte esterase and nitrite dipstick tests are used for testing the fresh urine sample of immunocompetent patients.

The nitrite test is used for the determination of the nitrate reductase activity, which detects bacteriuria with colonies counts of ≥ 10^5 CFU/ml of urine. Test limitations include inability to detect uropathogens that do not reduce nitrates to nitrites (*S. saprophyticus*, *Enterococcus* spp., *A. bumannii*, *Candida* spp., *Pseudomonas* spp.), false negative results when urine pH <6 and false positive results in patients on a vegetarian diet.

Leukocyte esterase test is a screening test for presence of white blood cells in urine as it detects the activity of leukocyte esterase present in polymorphonuclears and macrophages. Positive test indicates the presence of ≥ 20-25 L/µL of urine. Test limitations include false negative results in patients with neutropenia and in presence of some antibiotics, and non-specific finding in patients with non-infectious kidney diseases. Testing for pyuria should be avoided in catheterized patients and in patients with neurogenic bladder as in these patients chronic pyuria is common.

4.2. Non-cultivation methods of detecting bacteriuria

Some microbiological laboratories use rapid automated non-cultivation methods (e.g., laser nephelometry) to detect bacteriuria. Cultivation of all specimens is recommended in children,

pregnant women, immunocompromised patients, and for samples obtained by an invasive method (suprapubic aspiration, cystoscopy, „in and out“catheterization) and when a new, repeated sample is required regardless of the positive and negative result of the screening.

In laboratories using an automated screening system, the microbiologist must establish a local laboratory protocol for processing samples following manufacturer's instructions and selecting samples for culture.

4.3. Urine culture

4.3.1. Quantitative plating of urine samples

Urine should always be inoculated quantitatively as the number of bacteria in mL of urine differentiates significant bacteriuria from urine contamination by the periurethral microbiota. The easiest method for quantitative inoculation is the calibrated loop technique. For routine urine processing 1 or 10 µl calibrated disposable loops are recommended.

Use a 10 µl loop to inoculate 0.01 mL of urine with the detection threshold of 10^2 CFU/mL and the maximum number of countable colonies (100-999 colonies per plate) corresponding to 10^4 CFU/mL.

Use a 1 µl loop to inoculate 0.001 mL of urine with a detection threshold of 10^3 CFU/mL and the maximum number of countable colonies (100-999 colonies per plate) corresponding to 10^5 CFU/mL.

Inoculating urine using a 10 µl or 100 µl loops is recommended for samples obtained by suprapubic aspiration or during surgery.

Interpretation of the number of colonies observed on the agar plate is presented in Table 2.

Table 4. Quantitative reporting of the urine culture results based on the number of bacterial colonies observed on the plate

Number of colonies after inoculation with the calibrated loop of a defined size			Quantification of the bacteriuria (CFU / mL)
1µL	10 µL	100 µL	
-	1-9	10-99	10^2
1-9	10-99	100-999	10^3
10-99	100-999	NA	10^4
≥100	NA	NA	$\geq 10^5$

NA = not applicable as the number of colonies >999 is not possible to count

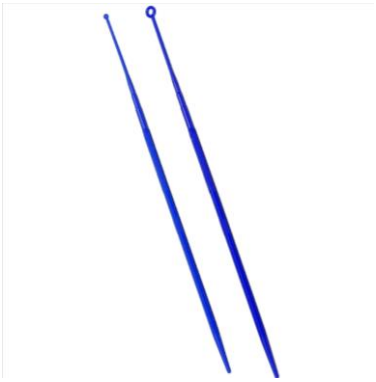

Figure 1. Recommended loops for the inoculation of the urine sampe, 1 µL and 10 µL

4.3.2. Urine inoculation procedure:

- Sterile loop is dipped vertically into the urine specimen and immersed just bellow the surface of the mixed, uncentrifuged urine. Ensure that the urine fills the loop completely.
- The loopful of urine is streaked across the surface of the agar plate first as a single line from top to bottom and then using the same loop and without dipping it again in the

urine the sample is further spread in back-and-forth horizontal streaks from top to bottom avoiding re-streaking the same areas of the plate.

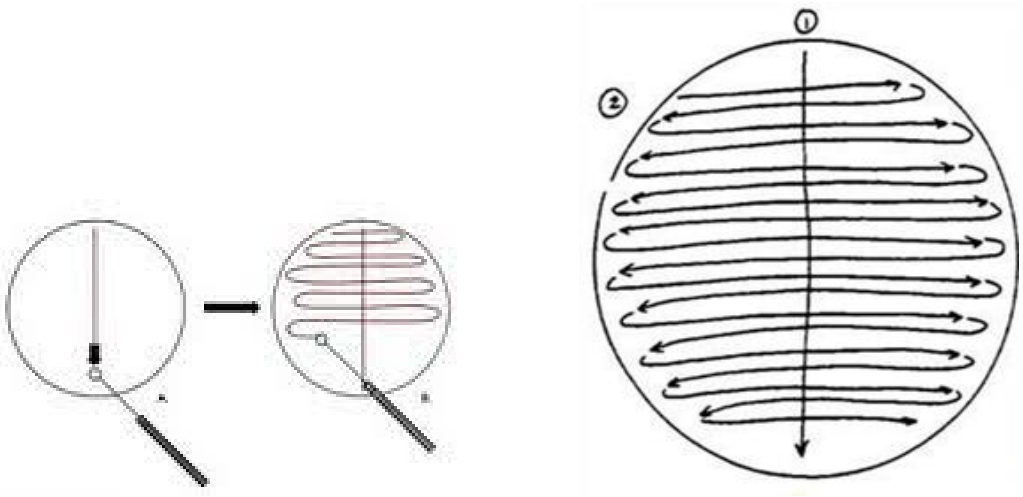

Figure 2. Plating a urine sample, the streaking patern

4.3.3. Primary agar plating media

There is no universal plating media for culturing all bacteria and fungi that may be present in urine. It is important that media selected for plating can inhibit the swarming of *Proteus* spp. and provide easy differentiation of *E. coli*, as it is possible with, for example, CLED (˝Cystine lactose electrolyte deficient˝) agar. Chromogenic media provide presumptive identification of the most common uropathogens but may be more expensive.

Proposed culture media and incubation conditions for urine cultivation are described in Table 3 but other agar plates may be used at the discretion of the microbiology laboratory to enrich isolation of other pathogens.

If a urine sample is inoculated on multiple media, a new inoculating loop is required for each new plate.

After inoculation, agar plates are placed in an incubator and urine is stored in a refrigerator at 4 °C until the analysis is done and results reported.

Table 5. Primary plating of urine samples

Clinical presentation/ condition	Standard media	Incubation			Target microorganism(s)
		Temp. (°C)	Atmosphere	Time	
UTI, Screening for asymptomatic bacteriuria in specific patient groups	chromogenic agar or CLED or blood agar	35-37	aerobic	16-24 h (48 h)	*Enterobacterales*, enterococci, *S. agalactiae, Pseudomonas, S.saprophyticus,* other CoNS, *S. aureus, Acinetobacter* spp.
In cases stated bellow, add the following:					
Urine from an ICU, paediatric ICU, burns unit, transplant unit or if yeasts are seen in microscopy	Sabouraud agar	35-37	aerobic	40-48 h	Fungi
Testing antibiotic presence in the urine	Agar for antibiotic susceptibility testing inoculated with *B.subtilis* NCTC 10400 or *E. coli* ATCC 25922	35-37	aerobic	16-24 h	Antimicrobial substance
Sterile pyuria, without antimicrobial presence in the urine	Agar for anaerobic bacteria cultivation	35-37	anaerobic	40-48 h	Anaerobic bacteria*, streptococci
	Chocolate agar	35-37	5-10%CO_2	40-48 h	Fastidious microroganisms

UTI = urinary tract infection; ICU = intensive care unit

* Applicable only for the sample obtained by suprapubic aspiration

4.3.4. Screening for the presence of antimicrobial activity in urine

Testing for the presence of an antimicrobial substance in the urine sample is important as the finding can influence the interpretation of the urine culture results.

- Mueller Hinton (MH) agar is inoculated with *B. subtilis* strain NCTC 10400 (or *E. coli* ATCC 25922). Filter agar discs, previously dipped in a urine sample, are then placed on the agar surface (multiple urine samples can be tested on one plate).
- The MH agar is incubated for 16-24 h in aerobic conditions at 35-37 °C.
- Antimicrobial substance, if present in tested urine will diffuse into the MH agar and inhibit the growth of *B. subtilis* (*E. coli*). Any zone of inhibition is considered positive on the presence of antimicrobial substances.

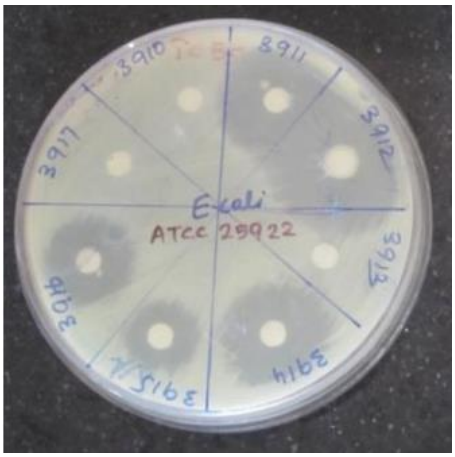

Figure 3. Screening for the presence of antimicrobial activity in urine using ATCC *E. coli* 25922

4.3.5. Urine pathogen isolation

The decision on which colonies will be chosen for further workup should be made in accordance with the following:

- Urine culture is inspected after ≥ 16 h of incubation
- The colony count for each morphotype should be determined separately
- If only urogenital or skin microbiota is observed it should be reported as “normal urogenital microbiota” without species identification or susceptibility testing
- If one type of organism is dominant but mixed with urogenital and skin microbiota representing less than 10% of the total count, microbiota can be disregarded and dominant morphotype counted as single species present
- *S. agalactiae* isolated in pregnant women and diabetic patinets is significant and should be reported regardless of the number of bacteria growing

- Positive culture plates should be kept at room temperature until the final report for possible further workup

Prolonged incubation of urine sample for up to 48 hours is required in the following situations:

- urine sample collected by invasive method (suprapubic aspiration, „in and out“ catheterization, cystoscopy)
- poor colony growth
- when the urine culture result is not correlating with the clinical presentation (sterile pyuria or UTI symptoms without positive urine culture)
- when the results of urine analysis do not correlate with the results of Gram staining (if performed)
- urine samples of immunocompromised patients or those who underwent organ transplantation
- when the mycological analysis is required or yeast is seen in microscpoy

A mixed culture in an uncomplicated patient likely indicates contamination and the crieterion of ≥ 10^5 CFU/mL indicates clinical significance in majority of specimens but the number of species to be reported depends on the type of the specimen and the colony count for each species (Table 6).

Basicly, if the specimen is a mid-stream urine, on a plate growing ≤2 primary or secondary pathogens, laboratory should identify all organisms growing ≥ 10^3 CFU/mL. Secondary pathogens are considered significant in a mixed culture if growing ≥ 10^5 CFU/mL each.

If the specimen is a catheter urine, on a plate growing ≤3 organisms, laboratory should identify all organisms growing ≥ 10^4 CFU/mL.

For details related to other types of specimens and doubtful pathogens see Table 6.

Table 6. Threshold of significant bacteriuria depending on the category of pathogen and the type of urine sample in symptomatic patients

Type of urine sample	Bacterial growth (species number / colony count)	Category of pathogen				
		Primary pathogens	Secondary pathogens		Doubtful pathogens	Urogenital microbiota
		E.coli *S.saprophyticus*	other Enterobacterales, enterococci, *P.aeruginosa, A.baumannii* *S.aureus*		*S. agalactiae, yeasts, CoNS*,* non-fermenters	Viridans streptococci, lactobacilli, *G.vaginalis*
Mid-stream urine ("Clean catch") or urine collector	Species number	1-2	1	2	1	-
	CFU/ml	each species $\geq 10^3$	$\geq 10^4$ f $\geq 10^3$ m	each species $\geq 10^5$	$\geq 10^5$	
Suprapubic aspiration	Species number	1-2 (3)[A]	1-2 (3)**		1-2 (3)**	1-2 (3)**
	CFU/ml	≥10	≥10		≥10	≥10
„in and out"catheterization or cystoscopy urine	Species number	1-2 (3)**	1-2 (3)**		1-2 (3)**	-
	CFU/ml	$\geq 10^2$	$\geq 10^2$		$\geq 10^2$	
Catheter urine (indwelling „Foley" catheter)	Species number	1-3***	1-3***		1-3***	-
	CFU/ml	$\geq 10^4$	$\geq 10^4$		$\geq 10^4$	
Ureterostomy and nephrostomy urine	Species number	1-2	1-2		1-2	-
	CFU/ml	$\geq 10^3$	$\geq 10^3$		$\geq 10^3$	

f = female, m = male

* CoNS, coagulase negative staphylococci (except *S. saprophyticus*)

** If 3 isolates are present, only 1 dominant isolate (1) is processed

*** Sampling and treatment are recommended in symptomatic patients only

Note that $\geq 10^5$cfu/mL can be detected by using 1 µL loop only and therefore this is recommended for processing the mid-stream urine. In contrast, $\geq 10^2$cfu/mL can be detected by using 10 µL loop only and therefore this is recommended for processing the "in and out" catheterisation urine sample.

4.3.6. Urine pathogen identification and antibiotic susceptibility testing

Identification of bacterial isolates should be done following the usual laboratory practice and in line with certified methods.

Antibiotic susceptibility testing should be done using international standards.

5.0. Overview of sample processing specific for study participation

- Clinical samples will only be considered study samples once the patient has signed Informed Consent form (ICF).
- Sampling materials are not provided by sponsor as collection of samples is considered as part of standard of care (SoC).
- Laboratories are encouraged to collect and store organisms that are target pathogens for clinical studies. Storage of the isolates will be done after inclusion of patient and confirmation of cUTI diagnosis.

5.1. Sample collection and processing at local microbiology laboratory

5.1.1. Urine cultures

Urine samples will be obtained:

- At Screening (Day 0)
- At Follow-up: Test of Cure (TOC) (between 14 and 21 days ±3 days after the diagnosis of cUTI)
- At Follow-up: Relaps / Reinfection (RR) only in case of new clinical symptoms (new symptoms within 30 days after the diagnosis of cUTI)

5.1.2. Blood cultures

Blood cultures are preferably taken at screening and in case of a relaps/reinfection. Blood cultures are not a requirement.

5.1.3. Interpretation of the culture results

For interpretation of culture results see Table 7.

Table 7. Interpretation of culture results

Sample	0. day	Follow up: Test of Cure (TOC) (between 14 and 21 days ±3 days after the diagnosis of cUTI)	Follow up: Relaps / Reinfection (RR) New symptoms within 30 days after the diagnosis of cUTI)
urin	pyuria	pyuria	pyuria
Mid stream urin culture *	≥10^4CFU/mL	≥10^3CFU/mL **	≥10^3CFU/mL **
Catheter urin culture *	1-3 species ≥10^4CFU/mL each	Same as above	Same as above
Blood culture	Preferably taken but not a requirement Positive or negative		Preferably taken but not a requirement Positive or negative

* For Follow up samples, the urin sample should be labeled on the requsition card as „TOC“ or „RR”
** At Follow-up vsits ≥10^3CFU/mL is considered significant if the patient is symptomatic and ≥10^5CFU/mL is considered significant in an asymptomatic patient

5.2. Storage of isolates

- Collected isolates should be labeled according to the routine laboratory procedure unless stated otherwise in the study clinical protocol. The minimum information included on the label should be the following:
  - Hospital site name
  - Subject identification number (Subject identification number is the unique ID in the eCRF, provided by the principal investigator or delegate.)
  - Sample type
  - Date of sampling
  - Isolate identification

  Ensure that the written information on the label is consistent with the information captured in the clinical record.

6.0. References:

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