



## Review

## Laboratory diagnosis of urinary tract infections: Towards a BILULU consensus guideline



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## ABSTRACT

Urinary tract infections (UTI) are very common throughout life and account for the majority of the workload in the clinical microbiology laboratory. Clear instructions for the interpretation of urine cultures by the laboratory technicians are indispensable to obtain standardized, reliable, and clinically useful results. In literature, there is often a lack of evidence-based practice in processing urinary samples in the laboratory. In this consensus document, the BILULU Study Group presents a practical approach for the implementation of existing guidelines for the culture of urine in the microbiology laboratory and offers answers for issues where no clear solution is available in the guidelines.

## 1. Introduction

The BILULU study group consists of seven microbiologists of hospital laboratories located in the region of Flanders (Belgium). A major goal of the group is to standardize diagnostic microbiology procedures based on available evidence and, in the absence of evidence, based on general microbiological principles and expert opinion. Scientific evidence of current urine culture guidelines is incomplete and at some points guidelines don't indicate clear choice.

The aim of this project was to develop a clear and unambiguous step-by-step guideline regarding the work-up of urine cultures.

Urinary tract infections (UTI) are very common throughout life, both in otherwise healthy as in immunocompromised or debilitated persons. UTI occur more frequent in women, with a lifetime occurrence rate close to 50% (Flores-Mireles et al., 2015). The diagnosis of UTI is based upon clinical signs and symptoms and is supported by laboratory evidence of pyuria and bacteriuria. Laboratory diagnosis consists of urinary WBC count, dipstick analysis and urine culture. Urinary cul-

tures represent a significant part of the workload in microbiology laboratories (Bouza et al., 2001). Clear instructions for the interpretation of urine cultures by the laboratory technicians are indispensable to obtain standardized, reliable, and clinically useful results.

Our search strategy was dual. On the one hand, we consulted reference works and searched the Internet for available guidelines on the subject. The following guidelines were withheld: 'Urine cultures' (Clinical Microbiology Procedures Handbook) (Leber, 2016); 'Laboratory diagnosis of urinary tract infections' (Cummitech) (Sharp, 2009); 'Specimen collection, transport, and processing: bacteriology' (Manual of Clinical Microbiology) (Jorgensen et al., 2015); Urinary tract infections (Mandell, Douglas and Bennett's principles and practice of infectious diseases) (Sobel and Kaye, 2009). In addition, we consulted reference guidelines of the Infectious Diseases Society of America (IDSA) and the European Association of Urology and performed specific searches on Pubmed (until May 2017) to obtain further evidence on particular items. Discrepancies and unresolved issues were finally discussed by an expert panel of 8 microbiologists.

**Abbreviations:** UTI, urinary tract infections; WBC, white blood cells; CFU, colony forming units; GBS, group B streptococci; IDSA, Infectious Diseases Society of America; CNA, colistin-nalidixic acid

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## 1.1. The pre-analytical phase: specimen collection, transportation and handling

### 1.1.1. Specimen collection

In adults, most urine specimens for laboratory examination are obtained by the clean catch-voided midstream technique. This technique is widely accepted and applied because it is simple, inexpensive and non-invasive and there is no risk of complications. Colony counts from urine specimens collected by this method correlate reasonably well with those of specimens collected by supra-pubic aspiration or straight catheterization (Stamm et al., 1982). A disadvantage of this technique is that the urine can be contaminated with commensal bacteria during its passage through the distal urethra. Simple procedures to decrease contamination rate include cleansing of skin and mucous membranes adjacent to the urethral orifice before micturition and the collection of the midstream part of the urine (Sharp, 2009). The available evidence suggests that the cleansing procedures may not decrease urine contamination rates significantly and, therefore, may be unnecessary as a routine method (Morris et al., 1979; Leisure et al., 1993; Lohr et al., 1986; Lohr et al., 1989; Lifshitz and Kramer, 2000; Prandoni et al., 1996; Jorgensen et al., 2015). Proper collection of samples by this method may be problematic in young children, elderly and disabled patients.

Supra-pubic aspiration is the best method to avoid urethral contamination, especially in young children (Tosif et al., 2012). But it is infrequently used because it is invasive, uncomfortable and time-consuming. Collection of urine by use of a single catheter (straight catheter technique) is the next-best technique to obtain urine specimens with minimal contamination risk (Wingerter and Bachur, 2011). However, the technique is not widely applied because of several disadvantages: it is labor intensive, costly and invasive. By the insertion of the catheter through the urethra, bacteria can be forced into the bladder, which involves a risk of infection (Wilson and Gaido, 2004).

Because laboratory procedures for urine cultures depend upon the type of urine specimen, it is indispensable that the collection method is specified on the laboratory request form. Other essential information includes date and time of specimen collection and any clinically relevant information (e.g. antimicrobial treatment, predisposing urological conditions such as anatomic abnormalities, stones or the presence of foreign material) (Table 1) (Leber, 2016).

### 1.1.2. Specimen transportation and storage

Several studies have demonstrated the adverse effect of delay in transportation or processing of urine specimens on laboratory results (Jefferson et al., 1975; Hindman et al., 1976; Wheldon and Slack, 1977; Delanghe and Speckaert, 2016). In each study, an increase in the number of colony forming units (CFU) per mL up to  $> 10^5$  CFU/mL was observed for a portion of the samples, thereby leading to false positive results. Current guidelines therefore recommended the inoculation of urine specimens within 2 h after collection (Table 1) (Jorgensen et al., 2015; Leber, 2016). If urine cannot be delivered to the laboratory within 2 h after collection, samples can be stored up to 24 h at 2–8 °C. An alternative is the collection of urine in tubes with preservatives, like freeze-dried boric acid-glycerol or boric acid-sodium (Eisinger et al., 2013; Lauer et al., 1979; Jorgensen et al., 2015; Leber, 2016).

### 1.1.3. Urinary specimen replication limit

Sometimes more than one urine culture is necessary for the diagnosis of UTI, for example in case of urethral contamination of a first sample or a false negative result due to excessive fluid intake (Sharp, 2009). After initiation of antimicrobial treatment, bacteria are eliminated from urine within 48 h (Oreskovic and Sembrano, 2007; Sobel and Kaye, 2009). Specimens obtained with the same collection technique within 48 h are considered as duplicate specimens and are routinely not withheld for bacterial culture (except in case of contamination). A culture to prove bacteriological eradication is not

**Table 1**

Summary of the pre-analytical phase regarding urinary specimen collection, transportation and handling.

	Description	Reference
Urinary specimen storage time	- Room temperature: max 2 h - Refrigerator (2–8 °C): max 24 h	(Leber, 2016)
Replication limit	- One sample per sample type every 24 h	(Jorgensen et al., 2015; Sharp, 2009);
Rejection or acceptance of samples for diagnosis of UTI?	- <b>Acceptable</b> sample types: ○ Clean catch-voided midstream urine ○ Supra-pubic puncture ○ Indwelling catheter ○ Single sondage ○ Urinary stoma <sup>a</sup> ○ Pedibag - <b>Suboptimal</b> sample types ○ Samples preserved > 2 h at room temperature or > 24 h at 2–8 °C ○ Samples in leaking containers - <b>Unacceptable</b> sample types ○ 24-h urine collections ○ Catheter tip/bag ○ First-voided urine fraction ○ Samples contaminated with faeces	BILULU Expert opinion; (Leber, 2016)
Definition of pyuria	- Flow cytometry techniques ○ Adults: $\geq 20$ –25 WBC/ $\mu$ L ○ Pediatrics (0–2 years of age): $\geq 10$ –25 WBC/ $\mu$ L - Microscopy techniques: ○ All patients: 10 WBC/ $\mu$ L	(Roggeman and Zaman, 2001; Manoni et al., 2013)

<sup>a</sup> High contamination rate; Abbreviations: WBC: white blood cells.

recommended, except in case of therapeutic failure (Sharp, 2009; Jorgensen et al., 2015).

### 1.1.4. Presumptive diagnosis of UTI

Urine dipstick tests (i.e. nitrate-reductase and leucocyte esterase detection), cell counts (by microscopy, flow cytometry or image recognition techniques) and microscopy of Gram-stained specimens are potential screening techniques for a presumptive diagnosis of UTI.

Dipstick tests have found to be insufficiently sensitive for detection of UTI in both retrospective and prospective studies (Baily Jr., 1995; Blum and Wright, 1992; Sultana et al., 2001). Reasons for false negative nitrite tests include insufficient bladder incubation time for conversion of nitrate to nitrite, low urinary excretion of nitrate, inability of some organisms to convert nitrate to nitrite (such as *Enterococcus faecalis*), and decreased urine pH (e.g. due to cranberry juice or other dietary supplements) (Devillé et al., 2004; St John et al., 2006; Williams et al., 2010). Conflicting results for the detection of bacteria in urinary samples by use of flow cytometry are described in literature. Depending on the sample, the concentration of bacteria for positivity ranges from 40 up to 1000/ $\mu$ L with sensitivities and specificities ranging from 74.0 to 100% and 41.9 to 98.2%, respectively (De Rosa et al., 2010; Manoni et al., 2009; Broeren et al., 2011; Brilha et al., 2010; Pieretti et al., 2010; Jolkkonen et al., 2010; Zaman et al., 2001). Gram staining of urinary samples may also be used as screening technique for UTI, but is labor intensive and requires experience. The sensitivity of the technique depends upon whether the sample is centrifuged or not (Jorgensen et al., 2015). We therefore do not recommend using dipstick tests, flowcytometric bacterial counts or Gram staining of urinary samples as selection criteria for urine culture in UTI. An overview of pre-analytical recommendations regarding the microbiological diagnosis of UTI is presented in Table 1.

**Table 2**  
Summary of urinary specimen processing.

	Description	Reference
Volume of the inoculation?	- Routine samples: at least 1 $\mu$ L - Detection of yeasts and invasive samples*: 10 $\mu$ L	(Leber, 2016; Sharp SE, 2009)
Plates that have to be inoculated?	- Routine samples: combination of a blood-containing agar plate and a MacConkey or selective/chromagar plate - Detection of yeasts: additional inoculation of a sabouraud or chromagar plate	(Jorgensen et al., 2015; Leber, 2016);
How to incubate?	- Incubation <b>temperature and atmosphere</b> <ul style="list-style-type: none"> <li>○ 35–37 °C</li> <li>○ Blood-containing agar: 5–10% CO<sub>2</sub>-atmosphere</li> <li>○ Selective plate/chromagar: ambient temperature (35–37 °C)</li> <li>○ Biplate: ambient temperature (35–37 °C), re-incubation in 5–10% CO<sub>2</sub>-atmosphere</li> </ul> - <b>Duration of incubation:</b> <ul style="list-style-type: none"> <li>○ Midstream of outpatients &lt; 65 years: at least 18 h</li> <li>○ Midstream of outpatients <math>\geq</math> 65 years: at least 36 h</li> <li>○ Inpatients: at least 36 h</li> <li>○ Indwelling catheter: at least 36 h</li> <li>○ Supra-pubic puncture: at least 42 h</li> <li>○ Culture of yeasts: at least 42 h</li> </ul>	BILULU Expert opinion; (Leber, 2016)

\*Invasive samples: supra-pubic puncture and single sondage specimens retained as invasive samples.

\*Additional inoculation of a 1  $\mu$ L plate can facilitate the count.

## 2. Specimen processing

Culture provides information regarding the number of CFU/mL and it delivers isolated colonies that can be used for identification and susceptibility testing. Culture of non-invasive specimens should allow the detection of  $10^4$  or  $10^5$  CFU/mL. This detection is usually accomplished by inoculation of 1  $\mu$ L of urine onto appropriate media (Sharp, 2009). For more invasively collected specimens (i.e. supra-pubic aspirations) or for the culture of yeasts, 10  $\mu$ L of urine should be cultured on appropriate media to achieve a detection limit of  $10^2$  CFU/mL. Inoculation of an additional routine 1  $\mu$ L sample can facilitate interpretation of heavily grown culture media (Jorgensen et al., 2015; Leber, 2016) (Table 2).

Urinary specimens can be inoculated by different inoculation methods (Jorgensen et al., 2015). Unless calibrated pipettes are used, colony counts are only approximations and can be deranged by as much as a hundred-fold (Albers and Fletcher, 1983). Especially at higher counts, one colony does not represent one CFU, nor is this accuracy necessary for urine culturing. Due to the several practical advantages, we suggest to use sterile, calibrated and disposable or automated 1 and 10  $\mu$ L loops for inoculation of urinary specimens (Table 2).

Besides MacConkey agar, a variety of selective chromogenic media are available for the identification and differentiation of urine pathogens. These chromogenic media can be used for all urine specimens or those that might be considered to be at a higher risk for contamination (e.g. indwelling catheter, pedibag) (Leber, 2016). Specific organisms will produce colored colonies, depending upon interaction between the enzymes they produce and the substrates incorporated into the medium, allowing direct identification of the most relevant urinary *Enterobacteriaceae* and *Enterococci*. In addition to MacConkey or chromogenic media, a more universal blood agar plate could be inoculated allowing the detection of Gram-positive and fastidious bacteria. Besides, a colistin-nalidixic acid (CNA) agar or phenylethyl alcohol agar could be inoculated to facilitate detection of Gram-positive organisms and suppresses the growth of swarming *Proteus* spp. and other Gram-negative bacilli that can overgrow Gram-positive cocci in the specimen (Leber, 2016). For urinary specimens obtained from outpatients, the routine inoculation of culture media selective for Gram-positive bacteria seems unnecessary, because the majority of UTI in outpatients are caused by aerobic Gram-negative bacteria (Bale and Matsen, 1981; Carroll et al., 1994). Even in patient populations in which *Staphylococcus saprophyticus* is a common cause of UTI, the use of selective media is not necessary. Urine specimens obtained from hospitalized patients are more likely to contain *Enterococci* (Wilson and Gaido,

2004). As most pathogenic yeasts grow well on blood agar plates, it is unnecessary to use consistently selective fungal media for urine cultures. In those cases where there is a high probability that a UTI is caused by a yeast or mold and, if requested, an additional selective medium (sabouraud or chromagar) can be inoculated.

With the growing evidence that fastidious bacteria like *Actinotignum schaalii* are uropathogens, we recommend the inoculation of a blood agar in combination with a selective medium such as MacConkey or chromogenic urine agar, independent of the urinary sample type and population (Table 2).

All culture media are incubated at 35–37 °C for at least 18 h for optimal growth (Sharp, 2009). MacConkey agar and chromogenic agar plates should be incubated overnight in ambient air. To enhance growth of gram-positive bacteria, blood agar containing media should be incubated under aerobic atmosphere with 5–10% CO<sub>2</sub> (Leber, 2016; Jorgensen et al., 2015). Biplates (i.e. plate composed of a selective agar on the one half and blood agar on the other half) can initially be incubated in ambient air and incubated for another 18 h in aerobic atmosphere with 5% CO<sub>2</sub> (Table 2). Culture media from midstream urine samples of outpatients aged 65 years or older, of hospitalized patients and patients with an indwelling catheter are incubated for at least 36 h (Jorgensen et al., 2015). Invasive urine specimens (i.e. supra-pubic aspiration samples), specimens from patients with suspected fungal UTI, and plates with thin or scant colonies that are barely discernible are incubated for 42 to 48 h (Leber, 2016; Joho et al., 1995; Murray et al., 1992; Aspeval et al., 2002). There is no benefit of incubating routine urine cultures for > 48 h (Table 2).

## 3. Interpretation of urinary cultures

### 3.1. Interpretation of pyuria

Pyuria is indicative for a bacterial UTI, but is not always present, especially in catheter-associated infections, infections in males and in neutropenic patients (Nicolle et al., 2005). In truly infected patients, a significant number of WBCs should generally be present (Stamm et al., 1982).

Pyuria is not a reliable predictor of infection if it is used as the only indicator. When both pyuria and bacteriuria are present, the probability of an UTI is markedly higher (National guideline Clearinghouse, 2012). We recommend to include the presence of pyuria in the microbiological diagnosis of UTI (Table 4).

The most accurate method for the quantification of white blood cells (WBCs) in urine is the manual microscopic count. A cut-off  $\geq 10$  WBC/

**Table 3**  
Classification of the different micro-organisms.

Category 1: urogenital skin flora	Viridans streptococci Commensal <i>Neisseriaceae</i> <i>Lactobacillus</i> species Coagulase-negative staphylococci other than <i>S. saprophyticus</i> <i>Corynebacterium</i> species other than <i>C. urealyticum</i> <i>Aerococcus</i> other than <i>A. urinae</i> and <i>A. sanguinicola</i>
Category 2: (common) uropathogens	Gram-negative bacilli <i>S. aureus</i> <i>S. saprophyticus</i> <i>S. pneumoniae</i> Large colony Beta-hemolytic streptococci <sup>a</sup> (excl. <i>Streptococcus anginosus</i> ) <i>Enterococci</i>
Category 3 <sup>b</sup> : rare or unusual uropathogens	Yeasts ( <i>C. albicans</i> , <i>C. glabrata</i> ) <i>Actinotignum schaalii</i> <i>Aerococcus urinae</i> <i>Aerococcus sanguinicola</i> <i>Corynebacterium urealyticum</i> <i>Gardnerella vaginalis</i>

<sup>a</sup> *Streptococcus agalactiae* in women of childbearing age (15–50 years of age) and neonates should always be mentioned as isolate.

<sup>b</sup> This group of micro-organisms is considered as uropathogen if “pure” culture or if 10 times higher concentrations of these pathogens have been grown.

μL for pyuria has a specificity for the prediction of catheter-associated UTI ( $\geq 10^5$  CFU/mL) of 90% but a sensitivity of only 37% (Tambyah and Maki, 2000). In most clinical laboratories, automated systems for urine-analysis (based on image recognition or flow-cytometry) have been introduced. For these methods, specific cut-offs ranging from 10 to 25 WBCs/μL have been suggested (Roggeman and Zaman, 2001; Manoni et al., 2013).

### 3.2. Classification of microorganisms

One of the major challenges in the interpretation of culture results of urine samples is to determine which of the bacteria in the culture is the causative organism of the infection. The identification and susceptibility testing results of the uropathogen are indispensable for a correct antibiotic treatment of serious UTI. Reporting of colonizing or contaminating micro-organisms can lead to incorrect diagnosis and/or unnecessary antibiotic treatment.

Based on the available evidence and expert opinion, we classified bacteria into three different categories according to their importance as uropathogen: common uropathogens, rare or unusual uropathogens and commensal bacteria of the skin and urogenital mucosae (Table 3).

#### 3.2.1. Common uropathogens (Category 1)

In this category, we classify a specific group of bacteria as common uropathogens. *E. coli* is the most common etiologic agent of UTI regardless of age and is responsible for up to 90% of cases of uncomplicated UTI in college-aged women, 70% of community onset cases of uncomplicated UTI, and as much as 66% of cases of UTI or acute pyelonephritis (Laupland et al., 2007; Peterson et al., 2007). *K. pneumoniae* is also considered as an important cause of UTI and may have a particular clinical importance in the renal transplant population, in which *Klebsiella* strains are often multidrug resistant (Alangaden, 2007). *P. mirabilis* and *Morganella* spp. are associated with UTI in the elderly and in patients with urolithiasis. *Pseudomonas aeruginosa* is a frequent cause of hospital acquired UTI, UTI after invasive urological procedures and UTI in the presence of foreign material (e.g. stents, catheters, etc.). *S. saprophyticus* is a major cause of uncomplicated cystitis in young, sexually active women, accounting for 10 to 15% of the infections and shares many clinical features with UTI caused by *E. coli*, but differs in pathogenesis, seasonal variation, and geographic

distribution (Latham et al., 1983; Raz et al., 2005). Some authors state that the number of micro-organisms present in a urinary sample that can cause UTI can be low, thereby making the laboratory diagnosis of UTI caused by *S. saprophyticus* challenging.

*Enterococcus* spp. has been reported in as many as 10% of all UTI (Felmingham et al., 1992), and up to 16% in the subset of nosocomial UTI (Schaberg et al., 1991). *Enterococcus* spp. and group B streptococci are commonly found in midstream urine cultures obtained from women with cystitis, but appear to rarely cause cystitis (Hooton et al., 2013). *Enterococci* can be more often associated with patients with underlying structural abnormalities or in patients who had prior urologic manipulations (Moellering, 1992). *S. aureus* can be a cause of secondary UTI. When isolated in high numbers ( $\geq 10^5$  CFU/mL) in a patient with pyuria and increased inflammatory parameters, it can be the cause of a secondary UTI.

*Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted infection in both woman and men in the US and Europe. Most Chlamydial infections are asymptomatic and remain undiagnosed and untreated (Unemo, 2013). *Neisseria gonorrhoeae* is a major cause of morbidity among sexually-active individuals worldwide. As the detection of both micro-organisms nowadays relies on antigen and molecular detection, these pathogens were not included in our consensus guideline.

Detection of yeasts (*Candida* spp.) in urine is uncommon in healthy individuals but is an increasingly important problem in hospitalized patients, especially in immunosuppressed (e.g. hematology) and intensive care unit patients. *C. albicans* is the most frequently isolated yeast from urine. The primary risk factors for candiduria include diabetes mellitus, neoplasms, urinary catheterization, periodic use of broad spectrum antibiotics or steroids, surgical procedures, female sex, increased age, and hospitalization longer than 7 days (Alvarez-Lerma et al., 2003; Kobayashi CCBA et al., 2004; Kauffman, 2005). The difficulty in assessing the clinical significance of *Candida* spp. in urine is the inability to distinguish infection from colonization (Kauffman, 2005). Studies have not been able to establish clear quantitative criteria for urine cultures in UTI due to *Candida*. For patients with indwelling urinary catheters, there seems to be no clear relation between the number of CFU of the yeast and the clinical importance:  $10^4$  CFU/mL of *Candida* spp. can represent colonization or infection (Kauffman, 2005). The presence of candiduria as an isolated observation probably doesn't have clinical significance and generally does not indicate a risk for subsequent invasive disease. Identification and reporting of the yeast is recommended only when it is present in significant quantities (see further).

#### 3.2.2. Rare or unusual uropathogens (Category 2)

In this category, we classified bacteria that are rarely encountered in urinary samples, but for which there is evidence that they are a possible cause of UTI. Bacteria that are categorized in this group are described more in detail.

*Aerococcus urinae*, *A. sanguinicola*, *Corynebacterium urealyticum* and *Actinotignum schaalii* are uncommon causes of UTI. These micro-organisms belong to the human microbioma of genitourinary tract (Hilt et al., 2014). *C. urealyticum* can be a catalyst for struvite stone formation because of its strong urease activity and has been found associated with alkaline encrusted cystitis and pyelitis in children and adults (Meria et al., 2004). This uropathogen should be looked at either when specifically requested by the ordering physician or in specific high-risk populations, such as renal transplant patients in which routine cultures are negative or in which the presence of kidney stones is mentioned on the requesting order (Aguado et al., 1993; Lopez-Medrano et al., 2008). Members of the *Aerococcus* genus have emerged as potentially significant pathogens in UTI. Colonies can bear a very close resemblance to *Enterococcus* spp., and are not easily recognized when isolated from urine cultures. Phenotypic discrimination between *A. urinae* and *A. sanguinicola* and other less pathogenic species belonging to the

**Table 4**  
Scheme for processing and workup of urine cultures based on method of collection, and number of pathogens.

Type of collection	Culture				References
	1 or 2 uropathogens		≥ 3 uropathogens		
	Colony count	Extent of workup	Colony count	Extent of workup	
Midstream Straight catheter Pedibag	≥ 10 <sup>4</sup> CFU/mL:	ID and AB	1 isolate ≥ 10 <sup>5</sup> CFU/mL and 2 isolates < 10 <sup>4</sup> CFU/mL	ID and AB of the predominant uropathogen ID of the other uropathogens No workup, ask new sample	BILULU Expert opinion (7/8)
	< 10 <sup>4</sup> CFU/mL:	ID and ask control sample (AB if pyuria)	Other cases:		
Indwelling catheter Supra-pubic sondage Nephrostomy	≥ 10 <sup>4</sup> CFU/mL:	ID and AB	1 isolate ≥ 10 <sup>5</sup> CFU/mL and 2 isolates < 10 <sup>4</sup> CFU/mL	ID and AB of the predominant uropathogen ID of the other uropathogens No workup, ask new sample	BILULU Expert opinion (7/8)
Supra-pubic aspiration	< 10 <sup>4</sup> CFU/mL:	ID and ask control sample	Other cases:		
	Always ID and AB, except for yeasts (only ID).		Contact clinician for clinical information*		BILULU Expert opinion (8/8)

\*In case of exceptional results, e.g. presence of urethral flora or ≥ 3 different types of micro-organisms.  
Abbreviations: ID: identification; AB: antimicrobial susceptibility testing; CFU: colony forming unit.

*Aerococcus* genus is difficult. However, in an era in which mass spectrometry methods are widely introduced in clinical microbiologic laboratories, identification of these species can be reliably made. In the study of Zhang et al. (Zhang et al., 2000), most patients found to be infected with *A. urinae* were elderly males with predisposing conditions and who presented with UTI. *A. schaalli* may be a more common urinary pathogen than previously described. Aerobic culture is tedious, and identification techniques have long been inadequate. In recent years, many cases of UTI have been reported, particularly among elderly patients (Prigent et al., 2016; Le Brun et al., 2015; Nielsen et al., 2010).

There are only few reports describing UTI with *Gardnerella vaginalis* (Smith et al., 1992; Andreu et al., 1994). In female patients, in which this micro-organism is part of the normal vaginal flora, isolation from urine could suggest contamination with vaginal flora. If a laboratory isolates *G. vaginalis* from a urine culture without the presence of symptoms and presence of pyuria or in mixed cultures, care should be taken before reporting this as a probable pathogen. If isolated from pure culture, consultation with the clinician is suggested before reporting it as a potential cause of UTI (Hooton et al., 2013).

Beta-hemolytic streptococci (large colony) are only rarely cultured, but are important to report, especially *Streptococcus pyogenes*. Microbiologic laboratories should provide cultures of vaginal rectal specimens to detect the presence of GBS during the third trimester of all pregnant females. In addition, urine can be used for recovery of GBS in the pregnant female. The Centers for Disease Control and Prevention (CDC) currently recommends that laboratories should report GBS in urine culture specimens when present at concentrations of ≥ 10<sup>4</sup> CFU/mL in pure culture or mixed with a second micro-organism (Centers for Disease Control and Prevention, 2010). Previous studies have shown increased risk only in those women having symptomatic UTI (Baron, 2003).

In the current Infectious Diseases Society of America (IDSA) guidelines on UTI and bacteriuria, *S. pneumoniae* is not mentioned as a possible agent for UTI (Gupta et al., 2011; Hooton et al., 2010; Nicolle et al., 2005). The same is true for the current German guideline for the diagnosis of UTI (Podbielski, 2017). Burckhardt et al. reported a case-series of 16 pediatric patients with symptoms of, where *S. pneumoniae* was the suspected uropathogen (Burckhardt and Zimmerman, 2011; Burckhardt et al., 2016). But due to the limited evidence, *S. pneumoniae* was not retained as uropathogen in our consensus guideline.

### 3.2.3. "Urogenital flora"

The ureters and bladder are usually sterile. Some urogenital flora can be found in the distal urethra. In this category, we categorize microbial flora that can be found in urine cultures.

*Lactobacillus* spp., α-hemolytic streptococci (except *S. pneumoniae*), *Neisseria* spp., *Staphylococcus* spp. (except *S. aureus* and *S. saprophyticus*), *Corynebacterium* spp. (except *C. urealyticum*) are classified as commensals of the urogenital tract (Table 3), except for *Neisseria gonorrhoeae*.

*Haemophilus influenzae* is rarely isolated from urine cultures, and its true incidence is unknown because urinary specimens are not routinely cultured on chocolate agar or other media that would support its growth. Over 24 years, 36 cases of *Haemophilus* spp. bacteriuria were found in > 5000 episodes of UTI in pediatric patients (Hansson et al., 2007). *H. influenzae* was isolated more often from girls and *H. parainfluenzae* from boys. With recent overall decreasing incidence of systemic *H. influenzae* infections, one would expect that the incidence is very rare.

### 3.3. Interpretation of the number of uropathogens

Clinical laboratories need to interpret the microbiologic relevance of growth on culture plates to determine whether further identification and antimicrobial susceptibility testing are necessary. Along with the type (cfr. supra) and number of micro-organisms, the presence of pyuria, sample type and presence of clinical symptoms need to be taken into consideration before a diagnosis of UTI can be made (Table 4).

Most culture results are interpreted readily; no growth and gross contamination are both unambiguous results. The most commonly used criterion for defining significant bacteriuria is the presence of ≥ 10<sup>5</sup> CFU/mL (Stamm et al., 1982; Kass, 1956; Kass, 1957). Despite this criterion having been established for women with acute pyelonephritis or women who were asymptomatic but had multiple urine cultures that yielded this number of bacteria, this criterion is also often applied to other patient populations. However, 30–50% of the patients with acute urethral syndrome will have colony counts of < 10<sup>5</sup> CFU/mL (Stamm et al., 1982). In symptomatic women with pyuria, lower midstream urine counts (i.e. ≥ 10<sup>2</sup>/mL) have been associated with the presence of bladder bacteriuria and thus may still be indicative for a UTI (Nitzan et al., 2015). Lower bacterial counts representing infection are also seen in men, in patients on antimicrobials, and with organisms other than *E. coli* and *Proteus* spp. On the other hand, interpretation of urine cultures that yield mixed flora in varying quantities can be difficult.

To increase the sensitivity of urinary cultures in samples that grow at most 2 uropathogens, we propose to use 10<sup>4</sup> CFU/mL as a cut-off. Midstream, straight catheter and pedibag samples that grow ≥ 10<sup>4</sup> CFU/mL are further processed (i.e. identification and antimicrobial susceptibility of the uropathogen) and the number of

**Table 5**

Overview of the different comments that can be presented on the laboratory report.

Observation culture	Report to the clinician
Only urogenital	Report: 'Urogenital flora' (+ number of CFU/mL)
If urogenital flora > uropathogens	Report: "Predominantly urogenital flora with possible presence of urinary pathogens. If clinically relevant, please send a control sample to the laboratory." (+ number CFU/mL)
Uropathogens ≥ urogenital flora	Report 'urogenital flora' (+ number of CFU/mL) and "uropathogens" (+ number of CFU/mL)
Only uropathogens (≤3 types)	Report all pathogens (+ number of CFU/mL)
Mixed flora with > 3 types of uropathogens	Report: "3 or more different types of pathogens, probably contamination. If clinically relevant, please send a control sample to the laboratory" (+ number of CFU/mL)

**Table 6**

Additional comments that can be added to a clinician report.

Additional comments in case of:	
Patients with an indwelling catheter:	Polymicrobial infections are possible in patients with an indwelling catheter. Contact the laboratory if further workup is desirable. Catheter-associated cystitis is treated with antibiotics if signs and symptoms of infection are present. Antibiotic therapy should be started after elimination of the catheter. If the catheter is needed and symptoms of infection are present, a change of catheter should be performed under appropriate antibiotic therapy.
A negative culture of a pedibag urinary sample:	A negative culture has a highly negative predictive value for absence of infection
Isolation of <i>Actinotignum schaalii</i> :	Urogenital commensal, although probable cause of UTI in predisposed patients, especially patients aged older than 60 years or with underlying pathology. Antibiotic of choice is amoxicillin. The pathogen is intrinsically resistant to ciprofloxacin and co-trimoxazol (Kristiansen R et al., 2014)
Isolation of <i>Corynebacterium urealyticum</i>	This pathogen is associated with cystitis induced by stone formation and encrusted cystitis or pyelitis (e.g. in cases of kidney transplantation (Lopez-Medrano et al., 2008)). Treatment consists of antibiotic combination therapy, acidification of urine for several weeks and surgical removal of bladder stone if present.
Isolation of <i>Corynebacterium</i> spp. (other than <i>C. urealyticum</i> )	This group of bacteria belong to the urogenital skin flora, but could be a cause of UTI. Results need to be interpreted in a clinical context. The following <i>Corynebacterium</i> spp. are associated with UTI (Jorgensen et al., 2015): <ul style="list-style-type: none"> <li>● <i>C. amycolatum</i></li> <li>● <i>C. aurimucosum</i></li> <li>● <i>C. glucuronolyticum</i></li> <li>● <i>C. minitissimum</i></li> <li>● <i>C. riegelii</i></li> <li>● <i>Arthrobacter</i> spp.</li> <li>● <i>C. pseudogenitalium</i></li> <li>● <i>C. striatum</i></li> </ul>
<i>Staphylococcus aureus</i>	<i>S. aureus</i> is not a classical uropathogen. Isolation could be due to post-operative infection? Post- uromanipulation? Hematogenous spreading? Presence of foreign body material? Further investigation is necessary (BILULU Expert opinion)
<i>Actinotignum</i> spp.	This group of bacteria belong to the urogenital skin flora, but are associated with complicated UTI (Jorgensen et al., 2015).
<i>Aerococcus urinae</i>	Mostly contaminant but possible uropathogen in predisposed patients (prostate hypertrophy, elderly patients). Nitrofurans and amoxicillin are therapeutic options in case of infection. Sensitivity to co-trimoxazole is contested. There are no interpretative criteria available for antimicrobial susceptibility testing (Jorgensen et al., 2015).
<i>Aerococcus sanguinicola</i>	Mostly contaminant, particularly associated with urosepsis. Amoxicillin is the first treatment option. Mostly, this pathogen is susceptible to cefuroxim, cefotaxim, erythromycin, tetracyclines and linezolid. There are no interpretative criteria available for antimicrobial susceptibility interpretation (Jorgensen et al., 2015).

uropathogens is reported. Samples that grow < 10<sup>4</sup> CFU/mL are only identified and reported, except for midstream, straight catheter and pedibag samples, where in case of pyuria also antibiotic susceptibility testing should be performed (Tables 4 and 5). The same is true for indwelling catheters, supra-pubic sondage and urinary stoma samples, except that pyuria is not withdrawn in the workup of these sample types. These suggestions originate from the available guidelines and are supported by expert opinion (Leber, 2016; Jorgensen et al., 2015) (Table 4).

In samples (all types of samples except supra-pubic aspirations) where 3 uropathogens are isolated, we recommend processing the predominant pathogen (≥ 10<sup>5</sup> CFU/mL) (i.e. identification and antimicrobial susceptibility of the uropathogen). For other uropathogens in these types of samples that grow concentrations < 10<sup>4</sup> CFU/mL, we propose to perform only an identification (Jorgensen et al., 2015) (Table 4). In all other cases, the clinician should be informed that 3 or more different types of pathogens are isolated (with or without mixed flora (Table 5)) that probably could indicate contamination. If clinically relevant, a control sample should be sent to the laboratory (Table 5).

Supra-pubic aspiration samples that yield growth cannot be

neglected (Stark and Maki, 1984). These types of samples always need to be processed in cases where at most 2 uropathogens are isolated. When at least 3 uropathogens are cultured, we suggest contacting the clinician to verify whether the sample was correctly drawn as supra-pubic aspiration. If this is the case, identification and antibiotic susceptibility testing of the uropathogen should be performed.

Table 4 shows a simple and adaptable urine processing and culture interpretation scheme based on the number and type of micro-organisms cultured, the number of WBCs and sample type. Specimens that are easy to collect (i.e. clean-catch midstream samples, indwelling catheter and pedibag samples), are more likely to contain colonizing organisms. More invasively collected specimens, such as straight catheter, supra-pubic aspirations, cystoscopy, and nephrostomy samples, are less likely to contain contaminating organisms.

#### 4. Reporting results

Laboratories should report culture results with interpretations and clinical comments to help the clinician to assess the clinical relevance. Beside information on patient identity, requesting physician and

clinically relevant information (e.g. antibiotic use), the laboratory report should contain information on sample type (cfr. supra).

We report cultures without growth as “negative” or “no growth of uropathogens” (Leber, 2016). For cultures with growth of uropathogens, CFU/mL are reported per pathogen (Leber, 2016). Depending on the ratio of uropathogens versus urogenital flora, different comments can be provided on the laboratory report. A summary is presented in Table 5. In particular situations (e.g. samples collected via intermittent urinary catheterization, a chronic indwelling urinary catheter, nephrostomy tube, or suprapubic catheter), additional information could inform the clinician and therefore, can be added to the laboratory report. A short summary of these possible commentaries is listed in Table 6.

## 5. Conclusion

In the last 10 years several evidence and expert based guidelines on the microbiological diagnosis of UTI have been published (Baron et al., 2013; Sharp, 2009; Nicolle et al., 2005; Hooton et al., 2010). In this document, the BILULU Study Group presents a practical approach for the implementation of existing guidelines for the culture of urine in the microbiology laboratory and we offer answers for issues where no clear solution is available in the guidelines.

This guideline can be helpful to laboratories in the development of standard operating procedures for urine culture. A schematic presentation of the presented guideline is also available through the website <http://www.bilulu.be> and is open for comment.

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