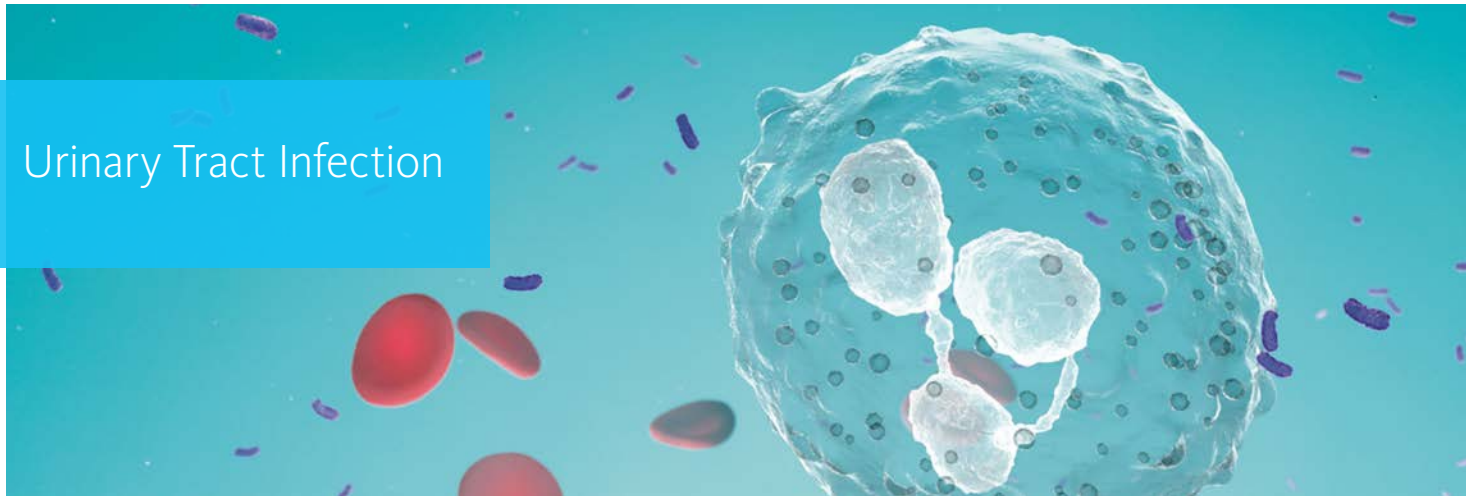
sysmex | Lighting the way with diagnostics

**URINALYSIS WHITE PAPER | May 2020**



Urinary Tract Infection

# How innovative technology leads to a faster diagnosis and more targeted treatment of UTI

## Introduction

Globally, urinary tract infections (UTI) belong to the most frequent bacterial infections, affecting around 150 million people each year [1]. This not only results in millions of medical consultations in both inpatient and outpatient settings, but also in high healthcare expenditures and social costs [2].

The pathogenic pathway can be either extraluminal by microbial contamination of the periurethral zone and subsequent colonisation towards the bladder or intraluminal by colonisation of the urinary tract via urinary catheters. Urinary tract infections are thus one of the major nosocomial infections [2].

Clinically, urinary tract infections are categorised as uncomplicated or complicated, depending on the absence or presence of underlying structural or functional abnormalities of the urinary tract, respectively [3]. Further, urinary tract infections are differentiated into lower UTI (cystitis, urethritis) or upper UTI (pyelonephritis) [4].

Besides the female gender, a recent urinary tract infection, sexual activity, diabetes, obesity and a certain genetic susceptibility are common risk factors associated with lower urinary tract infections. Complicated urinary tract infections are related to renal diseases (e.g. chronic kidney disease, renal failure, renal transplantation), obstructions of the urinary tract, urinary retention, urinary calculi, pregnancy and immunosuppression [2].

Since urinary tract infections can follow different symptomatic courses or even be asymptomatic, proper diagnosis of UTI combines patient history, urinary symptoms and laboratory diagnostics. Lower UTI manifests with alguria, pollakiuria, dysuria, acute suprapubic or abdominal pain, a general feeling of illness and occasionally haematuria, cloudy or foul-smelling urine. Upper UTI shows a more severe and systemic presentation, and in addition to the symptoms of lower UTI include costovertebral angle tenderness, fever and chills. In addition, non-specific symptoms such as tiredness, fatigue, chronic headache, persistent loss of appetite, nausea, vomiting, intermittent temperature increases and change of mental status can indicate a urinary tract infection [2]. A diagnosis solely based on the patient's history and present symptoms is still common in many countries but often inaccurate [5].

Although uropathogenic *Escherichia coli* is the most common pathogen associated with both complicated and uncomplicated urinary tract infections, various microorganisms, including Gram-negative and Gram-positive bacteria and various fungal species, can cause urinary tract infections (Fig. 1; [3]).

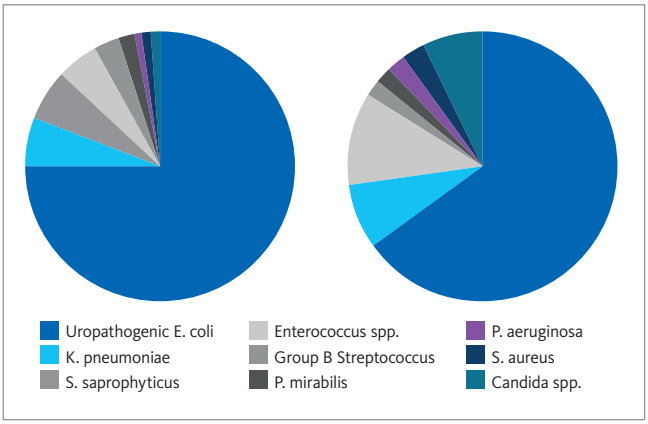



***Fig. 1*** *Epidemiology of complicated (left diagram) and uncomplicated (right diagram) urinary tract infections. Adapted from [3].*

Suspected urinary tract infections contribute to high laboratory workloads, although in the end up to 80 % of the samples are ruled out [6]. This causes the unnecessary and empirical treatment of patients with broad-band antibiotics, promoting the rise of antimicrobial resistance. Since only 17 % of all potential UTI patients who are treated with antibiotics have been tested before by proper urinalysis, re-prescription of antibiotics is often required [7].

## The classical diagnosis of UTI

The macroscopic examination of a urine specimen is often the first indicator for a suspected urinary tract infection, since abnormal colouration by macrohaematuria or pseudomonal UTI, and foul-smelling urine or turbidity due to pyuria are known urinary manifestations.

The dipstick is the most frequently used screening test for the presence of urinary tract infections. The presence of nitrite as a metabolic product derived from the reduction of urinary nitrate by certain nitrogenic species (e.g. *Escherichia*, *Proteus*, *Klebsiella*) is an indicator of bacteriuria. However, many pathogens of the urinary tract do not generate nitrate (e.g. *Enterococcus*, *Gonococcus*, *Staphylococcus*, *Pseudomonas*), which means nitrite in this context is not a reliable parameter. Leucocyte esterase, protein and blood are common parameters indicating inflammatory conditions. However, sensitivity and specificity are often relatively low, and a negative dipstick result is insufficient to rule out urinary tract infections if classical symptoms are present [5].

Microscopy of Gram-stained urine specimens is a common standard, i.e. the microscopic investigation of urine sediments that have been airdried on a microscopic slide and stained with Gram stain. The main advantage of urine microscopy is the provided information on the infectious agent to initiate antimicrobial therapy. Although the sensitivity of urine microscopy is highly reliable for samples with $\geq 10^5$ CFU/mL, reported insensitivities for lower bacterial concentrations limit its clinical utility, especially for uncomplicated UTI in outpatient settings [8].

Urine culture remains an important test in the context of UTI diagnostics, particularly for identifying the infectious microorganism. The common gold standard definition of bacteriuria is the presence of $\geq 10^5$ CFU/mL, which was established for women with acute pyelonephritis or asymptomatic UTI but was adapted for other patient groups [8]. Since many UTI patients show bacteriuria with $\leq 10^5$ CFU/mL, many laboratories already apply lower colony counts as cut-off values to increase the sensitivity of urine culture.

Positive urine cultures finally result in antibiotic susceptibility testing (antibiogram) to identify a suitable and specific antibiotic for the targeted treatment of a present microbial infection. The susceptibility testing by agar diffusion [9] is still the reliable gold standard, but indirect approaches including emerging technologies such as MALDI-TOF mass spectrometry and measuring bacterial metabolites in the presence of antibiotics are under evaluation [10].

## Detection of urinary particles by fluorescence flow cytometry

The Sysmex UF-series uses fluorescence flow cytometry to detect cellular and acellular particles, including bacteria, yeast-like cells, red blood cells, white blood cells and other parameters in urine and body fluid samples (Fig. 2).

<u>Diagnostic parameters</u>
Red Blood Cells
Non-lysed RBC
White Blood Cells
WBC Clumps
Epithelial Cells
- Squamous EC
- Non-squamous EC
- Transitional EC
- Renal Tubular EC

Casts
- Hyaline Casts
- Pathological Casts

BACT
X'TAL
Yeast-like Cells
Sperm
Mucus

<u>Research parameters</u>
Lysed RBC
Small round cells
Atypical cells
Debris
Conductivity
Osmolality

<u>Body Fluid parameters</u>
Red Blood Cells
White Blood Cells
- MN#/%
- PMN#/%

Epithelial Cells
Total Nucleated Cells
BACT

***Fig. 2*** *Overview of diagnostic, research and body fluid parameters provided by the UF-series analysers*

For detecting urinary particles, two measurement channels are available on the UF-series, the Core (CR) channel and the Surface (SF) channel. While the SF channel detects particles that do not include nucleic acid (RBC, crystals, etc.), the CR channel detects nucleic acid-containing particles. Proper particle detection requires staining of urinary particles using a diluting agent and a solution for the fluorescence labelling of subcellular structures.

In the CR channel, the membranes of WBC (Fig. 3) and the cell walls of bacteria are perforated by the diluent. These small perforations of the membranes allow the fluorescence dye to enter the cytoplasm and the nucleus and to intercalate into nucleic acid molecules.

In the SF channel, membrane components of cellular particles such as RBC are stained by the fluorescence dye without affecting the cellular integrity (Fig. 3).

The stained particles are then injected into the flow cell, where hydrodynamic focusing ensures their separation to allow accurate particle counts (Fig. 4).

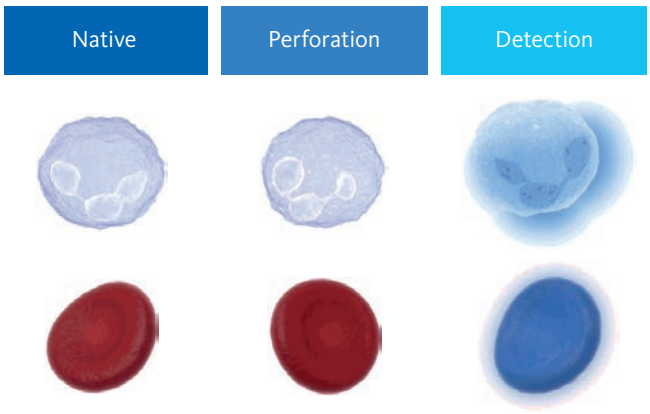



***Fig. 3*** *Particle-dependent reagent reaction for nucleic acid-containing cellular particles in the CR channel (upper row) and for nucleic acid-free cellular and acellular urine particles (lower row)*

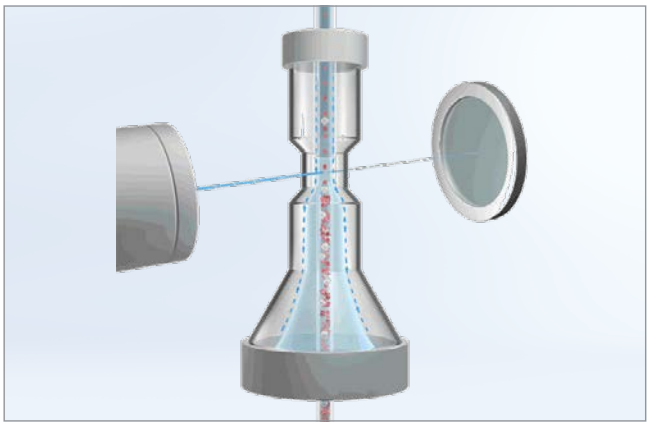


***Fig. 4*** *Hydrodynamic focusing of urine particles inside the flow cell of the UF-series instruments*

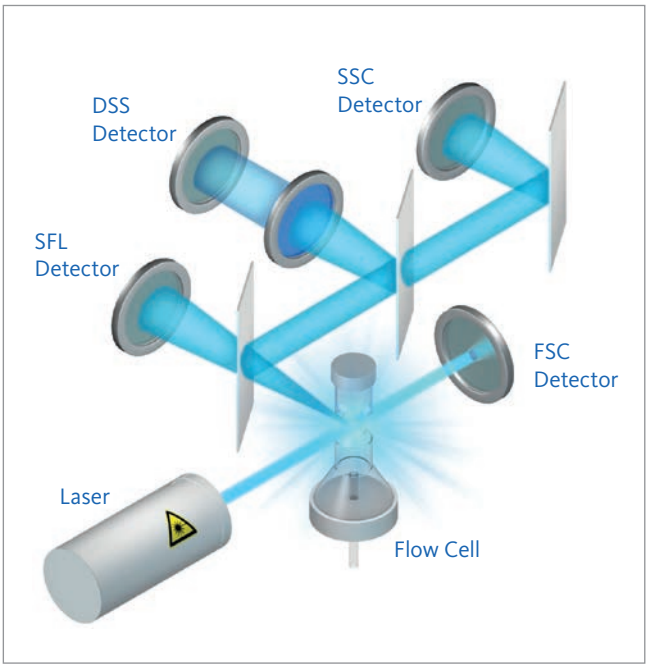



***Fig. 5*** *Fluorescence flow cytometry on the UF-series. A laser beam is directed at the flow cell, hitting all the particles passing through. Fluorescence light is emitted from excited electrons of the fluorescence dyes and, depending on the individual particle type, the oncoming laser light is characteristically diverted. Photodetectors recognise individual particles, and based on the individual signal patterns, the signals are plotted in a scattergram.*

Finally, the energy of a 488 nm laser beam excites electrons of the fluorescence dye attached to the urinary particles, elevating their energy level. Upon relaxation, photons are emitted and detected by different photodetectors (Fig. 5). Depending on the sub-structures of the different particles, the oncoming laser light can be diverted and detected by different detectors, allowing insight into the size of each cell (forward-scattered light; FSC), its intracellular complexity (side-scattered light; SSC) and its nucleic acid content (side-fluorescence light; SFL). Crystals are distinguished from RBC by using a depolarisation filter (depolarised side-scattered light; DSS).

## Improving screening for UTI

For bacteria, both quantitative and qualitative information is provided in less than a minute. This includes a reliable bacteria count and information on the Gram status.

In a representative study, the diagnostic performance of the UF-series' bacterial cell count has been identified as 0.973 (AUC). Separated between male and female patients, the diagnostic performance has been estimated as 0.988 for male and 0.959 for female patients, respectively (Fig. 6; [11]).

The investigation of different cut-off values revealed a bacteria count of ≥ 58 cells/µL as the most sensitive value for ruling out urinary tract infections with a sensitivity of 99.4 % (NPV 99.7 %) and a specificity of 78.2 % (PPV 65.4 %) [11]. However, optimal cut-off values must be established in respect to the prevailing patient population.

Samples suspicious of urinary tract infections are directly highlighted by the UTI-Info flag, based on bacteria and WBC counts to allow targeted follow-up diagnostics.

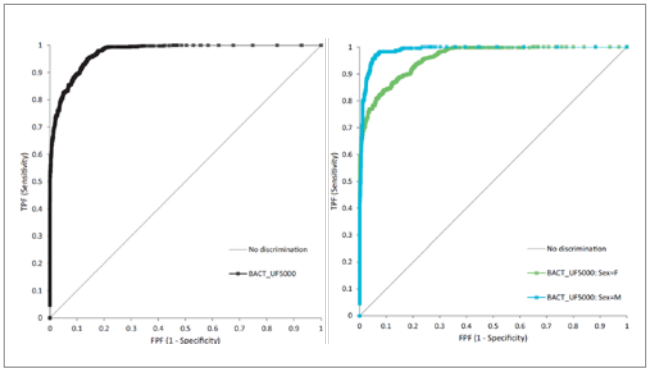



***Fig. 6*** *Diagnostic accuracy of the UF-series' bacterial count compared to quantitative urine culture from 2,714 urine samples, including 792 positive bacteriuria samples showing a bacterial growth of ≥ $10^5$ CFU/mL (adapted from [11]).*

## Insights into the Gram status

With the BACT-Info flag, the UF-series provides additional suspect information on the Gram dye affinity for samples positive for bacteriuria.

Based on the scattergram distribution, suspicious samples are highlighted with respective comments:

- **Gram Positive?**
  Based on the distribution, it can be inferred that Gram-positive bacteria are present.
- **Gram Negative?**
  Based on the distribution, it can be inferred that Gram-negative bacteria are present.
- **Gram Pos/Neg?**
  Based on the distribution, it can be inferred that Gram-positive and Gram-negative bacteria are present.
- **Unclassified**
  The class does not become clear from the distribution.

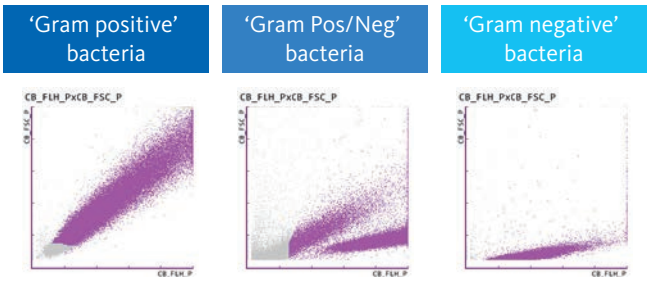



***Fig. 7*** *Detection of Gram-positive and Gram-negative bacteria by fluorescence flow cytometry on the UF-5000*

The differentiation between Gram-positive and Gram-negative bacteria is based on the composition of their cell walls. Due to the complexity of the Gram-positive cell wall, less fluorescence dye can enter the bacterial cytoplasm, resulting in a lower side fluorescence. In addition, a higher amount of laser energy is available for the forward scatter and leads – in combination with photons reflected from the thicker cell wall – to a higher FSC signal for Gram-positive bacteria.

Gram-positive bacteria are detected with a sensitivity of 78 % and a specificity of 96 %, whereas for Gram-negative bacteria, both the sensitivity and specificity reach 89 %. This high degree of sensitivity and specificity in pre-culture screening for urinary tract infections might allow an early initiation of antibiotic UTI therapy [12] and more targeted follow-up diagnostics.

## Fungal urinary tract infections

Fungal infections in adults are often related to immunocompromised individuals or other underlying conditions, such as diabetes. Therefore, funguria only represents around 7 % of complicated urinary tract infections [3]. Fungal urinary tract infections mostly manifest as lower urinary tract infections and cause classical symptoms, whereas fungal infections of the upper urinary tract are rare, except for in immunocompromised patients, caused by disseminated candidiasis [13].

Along with the exclusion of bacteriuria, a recent publication also demonstrated a high specificity of 97.7 % (NPV 98.8 %) and a good sensitivity of 89.5 % (PPV 81.0 %) for the yeast-like cell parameter [14], allowing exclusion of fungal infections and targeted diagnostics to identify the correct treatment strategy [15].

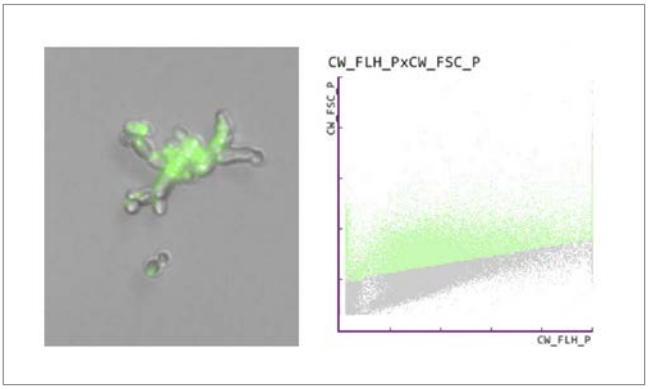



***Fig. 8*** *Yeast-like cells, detected by fluorescence microscopy (left) and on the Sysmex UF-series, displayed in the respective scattergram (right)*

## Distinguishing upper and lower UTI

The presence of renal tubular epithelial cells (RTEC) in urine is often an indicator of renal disease or tubular damage. Since RTEC line the entire renal tubule from the proximal to the distal segment, they represent a potential diagnostic marker for renal damages when other parameters are still inconspicuous [16].

As a potential clinical application, the quantification of RTEC in individuals with confirmed urinary tract infection has been shown to be a potential indicator of upper urinary tract infection (Fig. 9; [17]).

With a diagnostic accuracy of 0.923 (AUC), the RTEC count clearly outperforms known markers of upper urinary tract infection, such as $\alpha_1$-microglobulin (0.735) and γ-glutamyl transferase (0.586).

The potential diagnostic value of RTEC quantification, however, strongly depends on proper sample handling and processing, since their *in vitro* stability is impaired by storage times of two hours and more, as well as room temperature and acidic urinary pH [17].

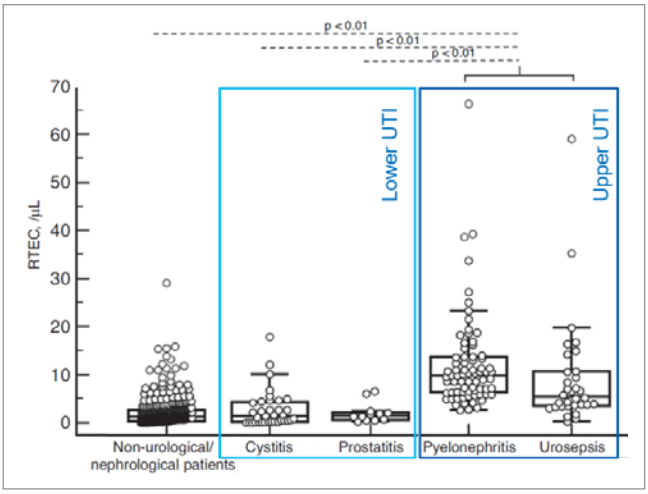



***Fig. 9*** *Renal tubular epithelial cell (RTEC) counts among non-urology/nephrology patients and patients with confirmed upper or lower urinary tract infection ([17] modified)*

## Antibiotic susceptibility testing on the UF-series

Antibiotic susceptibility testing by agar diffusion is a mandatory diagnostic procedure to identify the correct antibiotics for a persisting infection to induce targeted antimicrobial therapy and prevent antimicrobial resistances.

Briefly speaking, bacterial samples are spread on agar plates, and paper disks soaked with antibiotics are placed onto the agar. During incubation of the plate, the antibiotics will radially diffuse and inhibit the bacterial growth, depending on their antibiotic efficacy. Despite its specificity, this gold standard agar diffusion test has a high turn-around time of 18 – 48 hours [9].

A potential solution to accelerate antibiotic treatment decision has been reported for conducting antibiotic susceptibility testing on the UF-series. Aliquots of ready-to-use microbial growth broth were individually supplemented with different antibiotics and inoculated with the bacteria stemming from the patient samples. After incubation for up to four hours, the bacteria concentration within the different cultures was determined on the UF-series. A sensitivity of 83.3 % (PPV = 100 %) and a specificity of 100 % (NPV = 91.3 %) allowed, for example, the differentiation of colistin-resistant and susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolates within two hours, supported by the UF-5000 (Fig. 11; [18]).

Alternative approaches combine the diagnostics for bacteriuria on the UF-5000 with subsequent molecular testing for bacterial resistance genes [19] or mass spectrometry to identify bacteria and mediators of antibiotic resistance [20], allowing the installation of a targeted antibiotic therapy within six hours.

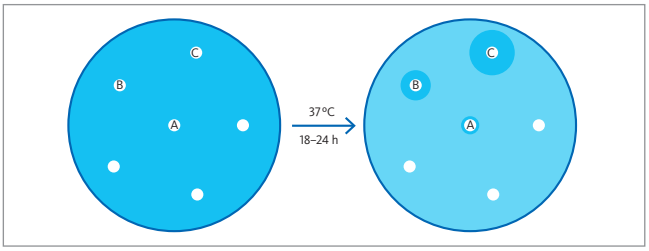



***Fig. 10*** *Schematic presentation of the antibiotic susceptibility testing by agar diffusion. The different diameters of the growth inhibition zones around the soaked paper disks correlate with (A) ineffective, (B) medium-effective and (C) highly effective antibiotics.*

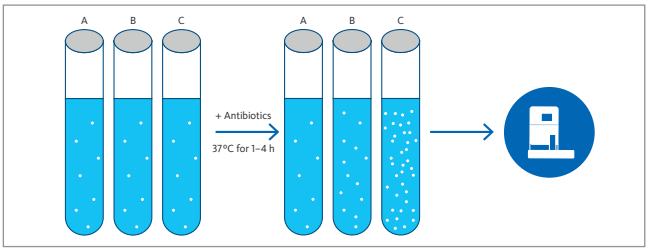



***Fig. 11*** *Alternative antibiotic susceptibility testing on the UF-5000 via growth monitoring of bacterial isolates in broth supplemented with different antibiotics. The bacteria concentration correlates to (A) highly effective, (B) medium-effective and (C) ineffective antibiotics.*

## Fighting antimicrobial resistance with targeted diagnostics

The adaptation of microorganisms to resist the actions of antimicrobial agents is widely known as antimicrobial resistance, a well-recognised problem of public health of the 21st century. Antimicrobial resistance, however, is a phenomenon that already has been reported before the discovery of penicillin. After years of extensive clinical use of the antimicrobial salvarsan for the treatment of syphilis, a waning effect for salvarsan had been observed, as well as an increase in more severe clinical pictures of syphilis [21], indicating antimicrobial resistance.

Since then, irrational use of antimicrobials (e. g. inappropriate prescriptions and self-medication), extensive use of antimicrobials in factory farming and agriculture, but also the prolonged and widespread use of antibiotics in therapy and prophylaxis augmented the numbers of resistant microorganism species [22].

With increasing antimicrobial resistance and slowed antimicrobial drug development, antimicrobial stewardship is of utmost importance. Without proper and immediate actions, the number of deaths caused by antimicrobial resistance by 2050 will surpass those of cancer [23]. Therefore, the World Health Organisation announced a global health crisis and released a global action plan [24] to fight antimicrobial resistance with the following actions:

- Create awareness and understanding
- Strengthen knowledge and scientific evidence
- Reduce infections through hygiene measures
- Optimise the use of antimicrobials in human and animal health
- Sustainable investment in new medicines, diagnostic tools and vaccines

Here, laboratory diagnostics is an important factor, as it not only aims to provide accurate information for more accurate diagnoses and clinical decision support but will also contribute to allowing a more rational use of antimicrobials.

## Summary and conclusion

Considering the total amount of suspected urinary tract infections that finally turn out to be negative, an optimised diagnostic workflow including the Sysmex UF-series can improve the efficiency of laboratory diagnostics by ruling out urinary tract infections within a short period (Fig. 12). Moreover, modern flow cytometry-based urinalysis prevents blindly prescribing unnecessary antimicrobials, and instead supports a targeted and rational use of antimicrobials, thus contributing to the much-needed antimicrobial stewardship.

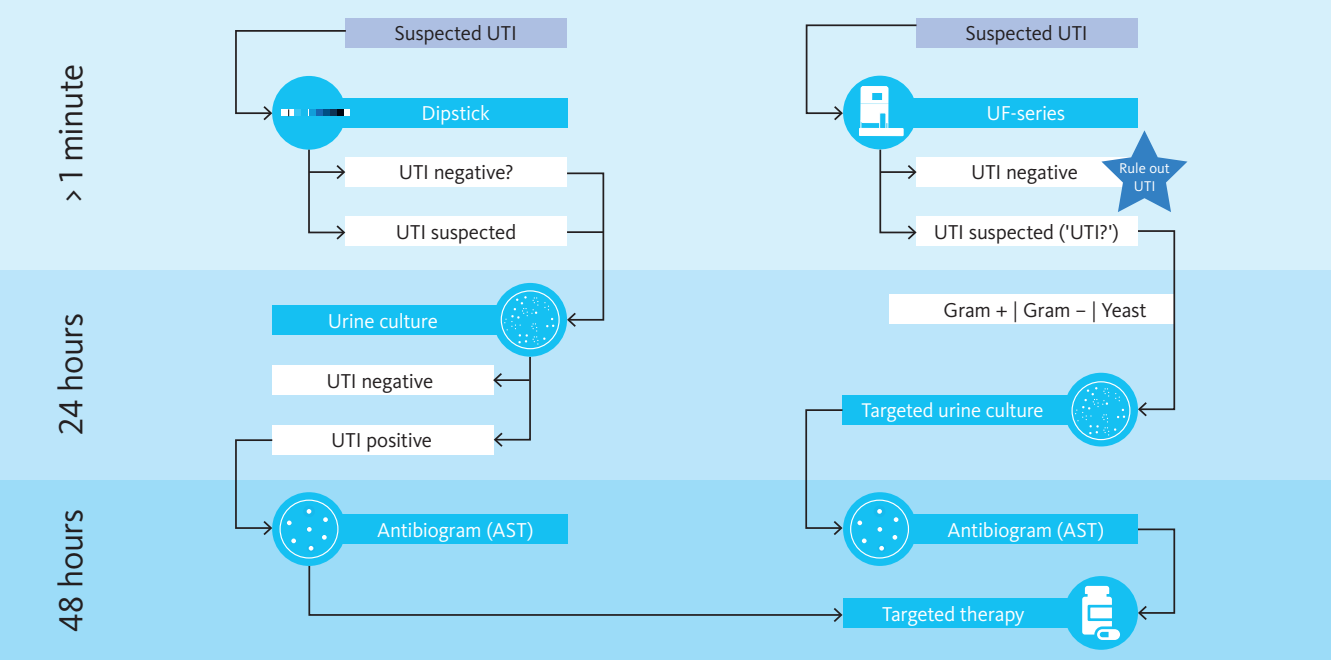



***Fig. 12*** *Overview of the diagnostic workflow for the diagnosis of urinary tract infections without (left) and with automated urine particle analysis using the UF-series (right). The UF-series allows ruling out UTI in less than a minute and reduces the unnecessary diagnostic follow-up by up to 80 % of the overall number of suspected UTI cases. For potential UTI-positive samples ('UTI?') the 'BACT Info' flag enables more targeted diagnostics to identify the presence and type of bacterial infection. Ruling out UTI at an early stage also helps to reduce the empirical prescription of antibiotics and supports antimicrobial stewardship.*

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