The ‘second generation’ Urine Fluorescence Flow Cytometers: the key to modern screening for urinary tract infections

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Indications for requesting a urine culture

There are various reasons for requesting urine cultures in the laboratory. On the one hand, they are aimed at an early detection of a possible urinary tract infection in asymptomatic patients. For immuno-suppressed patients, for patients before and after surgical interventions in the urogenital tract, for pregnant women screenings, for diabetic patients and for those with reflux, screening is often based merely on the application of the test strip before urine is sent to the lab for a culture. Cultures are then started only if test strip results were positive. If there are clinical symptoms, urine cultures are additionally requested, as a rule, for further clarification even if the test strip result was negative.

Diagnostic cornerstones

Diagnosis by the clinician is resting as a rule on four cornerstones:

- Patient case history
- Clarification of clinical symptoms
- Presence of bacteriuria
- Presence of leucocyturia

Bacteriuria

The sampling technique and type of the urine sample (mid-stream urine, bladder puncture, spontaneous urine or morning urine) will determine the interpretation of the detected bacterial count value. Information regarding the type of urine material can therefore be helpful.

Urine inside the bladder of a healthy person is normally sterile. Accordingly, the species of any detected and isolated bacteria obtained from sterile bladder puncture urine will thus be identified and reported as possibly infectious bacteria. However, puncture is not the standard sampling technique. Mostly, the samples are spontaneously voided urines, mid-stream urines or catheter urines where – due to the contact of the urine with the physiological urethral flora – a contamination is possible or, in fact, is rather the rule than the exception. If urine microscopy is performed, the number of squamous epithelial cells seen in the sediment may serve as a contamination indicator since the presence of these cells is increased in urine in case of insufficient preanalytics.

Symptoms and specimens	Species types	and number	significant colony concentration (CFU/mL)
Mid-stream urine specimen: Symptomatic urinary tract infection (UTI)	I II II II III	1–2 1 1 2 1	10^3 10^4 (women) 10^3 (men) 10^5 10^5
Mid-stream urine specimen: Asymptomatic UTI	I–III	1	10^5
Mid-stream urine specimen: Symptomatic UTI in patients with special urological diseases	I	1–3	10^2
Suprapubic aspiration specimen	I–IV	1–2	10^1
Specimen from cystoscopy or single urethral catheterisation	I–III	1–2	10^2
Specimen from indwelling catheter: Symptomatic UTI	I–III	1–3	10^4
Specimen from indwelling catheter: Asymptomatic UTI	I–III	1	10^5

Tab. 1 *Table XIII from the European Urinalysis Guideline with recommendations to significant bacterial counts, taken from the European Guidelines*

I: primary pathogenic bacteria (predominantly E. coli, S. saprophyticus)
II: secondary pathogenic bacteria (predominantly Enterobacter spp., Enterococcus spp., Klebsiella spp., P. mirabilis, P. aeruginosa)
III: facultative pathogenic bacteria (predominantly from the group of B streptococci and coagulase-negative staphylococci)
IV: Bacteria coming from the flora of the urethrogenital tract (streptococci, Gardnerella vaginalis, Lactobacillus etc.)

With mid-stream urine, many laboratory facilities have considered bacterial counts of more than 10^5 CFU/mL urine as an indication of significant bacteriuria. This established threshold value goes back to a study by Kass at the end of the 1950s and is still valid today in many lab facilities[5]. Bacterial counts of less than 10^3 CFU/mL urine are considered as not significant, and counts between 10^3 CFU/mL and 10^4 CFU/mL urine or a mixed culture with more than 2 bacteria strains are considered as an indication for contamination. However, about 25 years later, Stamm et al defined lower bacterial counts as decision criteria for the diagnostics of a urinary tract infection[12,13]. The difference between the detected values is explained by the examined sample and patient material in the various studies[1]. Kass examined the morning urine of asymptomatic patients and defined 10^5 CFU/mL urine as a significant bacteriuria whereas the later studies by Stamm exclusively examined patients with corresponding clinical symptoms. With half of the patients in the study by Stamm, a urinary tract infection was diagnosed, while only 50 % of the ill patients were found to have more than 10^5 CFU/mL urine in a urine culture[11]. Further studies demonstrated the relevance of different and partly much lower bacterial counts for diagnosing a urinary tract infection for certain patients, depending on the sampling technique, clinical symptoms and medical histories, a viewpoint which can also be found again in the European directives[17].

Leucocyturia

Increased occurrence of leucocytes is considered a further indication for an infection. If there are no leucocytes present in urine, a urinary tract infection is actually unlikely; however, it cannot be absolutely ruled out: leucocytes originally present in the urine might be lysed, for example if the urine has an alkaline pH-value, if it is hypotonic and older than 3 hours. Another possibility could be samples from immuno-suppressed patients and because of that, leucocytes are missing in these urines.

It actually happens, too, that leucocyturia is found without bacteriuria. Then, if an infection is further suspected, fungi, chlamydias, mycoplasmas, trichomonades or gonococci, and also the more rarely occurring tuberculosis bacteria, are taken into consideration as infective agents and corresponding cultures are also started.

Urinary tract infections and screening possibilities

Various possibilities for screening have become established to obtain information on a urinary tract infection as quickly and as reliably as possible, and also to reduce the number of negative cultures. They are now gaining especially importance for hospitals which are subject to a financing system from the DRG (diagnosis related groups) family – meanwhile in most of the European countries – the introduction of which is to bring about more efficient work in hospitals. From the viewpoint of the hospitals, such screening methods are desirable which cannot only minimise the unnecessary negative culture results but which can also improve the turn-around times of laboratory diagnostics.

We should not fail to mention here the savings potentials due to fewer blindly indicated antibiotics therapies. If a clinician is no longer forced to wait 1 to 2 days for the result of a culture, he may in case of an immediately available negative laboratory report for bacteria and leucocytes do without an antibiotics administration and take further steps in other directions of differential diagnostics.

***Fig. 1** Urine – The only material to be used to diagnose urinary tract infections*

A popular screening method: the test strip

Among the possible screening methods for urine samples, the test strip method is considered the most practicable. Advantages of the test strips are definitely their easy handling and fast availability of results for nitrite and leucocyte esterase.

Nitrite detection is considered a detection method with high specificity. However, sensitivity is sometimes indicated in literature to be only 30 %[4]. Of course, nitrite detection will only be positive if the infection carriers are Gram-negative and nitrite-forming agents. Thus, for example, enterococci, staphylococci and some pseudomonades as non-nitrite-forming agents cannot be detected. To be able to detect nitrite-forming agents, these bacteria must be, on the one hand, present in urine in a specific minimum concentration (10^5 CFU/mL urine) and must have had a minimum dwelling period of 4 hours in the bladder. The latter will often be difficult to achieve since patients with a urinary tract infection frequently suffer from uresiaesthesia.

In addition to nitrite, the result for leucocyte esterase is considered and included in test strip testing. This also makes it possible to detect – in addition to intact leucocytes – any already lysed leucocytes. The intracellular granulocyte esterase remains present in the urine after lysis of the cells and decomposes the indoxylester contained in the test pad to indoxyl, which then reacts with a diazonium salt to form an azo dye resulting in a violet colour change. Thus, the test strip has the advantage of being able to detect already lysed leucocytes. For a positive test strip result of leucocyte esterase, literature indicates a sensitivity of 75 to 90% [16].

If either nitrite or leucocyte esterase show a positive result, presence of a urinary tract infection can be assumed with high probability. If the nitrite and leucocyte results are negative, a urinary tract infection can, however, not be excluded, especially not for children where lower bacterial decision limits are applied [4, 7, 11, 15]. Other current studies also demonstrate that, especially for small children, negative test strip results cannot exclude a urinary tract infection.

Fig. 2 *Screening nowadays: The exclusion of urinary tract infections with Sysmex UF-series.*

A new screening method: UF-1000i and UF-500i

UF-1000i and UF-500i are fluorescence flow cytometers for the quantitative analysis of bacteria, yeast-like cells and leucocytes in human urine, in addition to the analysis of other particles classified out of urine, e.g. erythrocytes and epithelial cells. Irrespective of the bacteria's capacity for propagation, bacteria, yeast-like cells and leucocytes are counted from native urine. Two specific fluorescence dyes are used which stain the cells' nucleic acids and other distinctive cellular parts, thus making it possible within less than 2 minutes to detect bacteria, yeasts and leucocytes with high analytical sensitivity and specificity.

Every sample tube is just placed into a rack system. After pressing the start button, reading of the bar-coded sample numbers and automatic mixing and analysis of the samples on UF-1000i or UF-500i are started. Results are available after just over a minute.

This technology makes it possible to report the majority of negative samples immediately to the doctor and to thus save this part of the culture workload. But it is not only the short time in which the result of the three decisive indicators (bacteria, yeast-like cells and leucocytes) for urinary tract infections are available that make this method interesting, it is also the reliability of the results which can be obtained even down to lower concentration ranges of around 10^3 to 10^4 bacteria/mL urine [9]. The reliability of the results with their high sensitivity and specificity was confirmed by first evaluations.

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