CAT
Critically Appraised Topic

Titel: To culture or not to culture a urine sample: that's the question

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CLINICAL BOTTOM LINE

Urinary tract infections (UTI) are among the most common infections worldwide [1]. For aiding in the diagnosis of a UTI, a (bacterial) culture is still considered the gold standard. It allows identification and quantification of the causal micro-organism(s) and susceptibility testing for antimicrobials [2]. This implies that a urine culture is one of the most frequently requested tests in a microbiology laboratory. However, these tests are reasonably time-consuming, labor intensive, costly and a large proportion of these cultures shows no or insignificant growth [3]. An in hospital screening method based on dipstick analysis (nitrite and leukocyte esterase) to exclude an UTI is generally considered insufficient because of a low sensitivity and specificity [4].

For some time now, automated methods based on fluorescence flow cytometry are available for urinalysis. It is user-friendly, simple and has a very short turn-around time (TAT). Because of these advantages, it has the potential to greatly simplify the work-up for the exclusion of an UTI.

The purpose of this CAT is to determine whether the UF-4000 can be used as a fully-fledged screening alternative to rule out a UTI at an early stage. Based on an extensive literature review and our own retrospective study, we can conclude that the bacterial channel of the UF-4000 has a very good performance. The negative predictive value (NPV) for our in-house cut-off value of 45,6 bacteria/µL is 96%. As a result, the number of urine samples to be inoculated and read can theoretically be reduced by 35%. The use of the UF-4500 to screen out a UTI leads to a more efficient way of working but has no major financial impact.

CLINICAL/DIAGNOSTIC SCENARIO

1) Definitions and epidemiology

A urinary tract infection is defined as a bacteriuria with clinical signs, ranging from local (dysuria, frequency, pollakiuria, strangury, urgency) to systemic (fever, flank pain, nausea…) symptoms [5]. Different classification systems of UTI exist (CDC, IDSA, ESCMID, FDA…). The most recent guideline from the European Association of Urology uses the following classification of UTIs: uncomplicated UTI, complicated UTI, recurrent UTI, catheter-associated UTIs (CA-UTI) and urosepsis [5].

An **uncomplicated UTI** is defined as an acute, sporadic or recurrent lower (uncomplicated cystitis) and/or upper (uncomplicated pyelonephritis) UTI, limited to non-pregnant women with no known relevant anatomical and functional abnormalities within the urinary tract or comorbidities. The term **complicated UTI** is used for are all UTIs which are not defined as uncomplicated.
A **recurrent UTI** is a UTI with a frequency of at least three UTIs/year or two UTIs in the last six months. A **CA-UTI** refers to a UTI occurring in a person whose urinary tract is currently catheterized or has had a catheter in place within the past 48 hours.
Urosepsis is defined as life threatening organ dysfunction caused by a dysregulated host response to infection originating from the urinary tract and/or male genital organs [EAU].

An asymptomatic bacteriuria (ABU) refers to a situation where the bladder or urethra has been colonized by bacteria. Despite a proven bacteriuria, the patient shows no clinical symptoms. It is defined by a mid-stream urine sample showing bacterial growth ≥ 10^5 CFU/mL in two consecutive samples in women and in one single sample in men [5U]. This phenomenon is more commonly observed in the elderly population and in pregnant women and is of clinical importance in the latter population group [5].

Risk factors for the development of a cystitis include sexual intercourse (with or without use of spermicides), a history of UTI, diabetes mellitus, urine-incontinence and the presence of a urine catheter. Coitus is the most important risk factor for cystitis in women [5-6].

Dutch numbers show that the incidence of cystitis in general practice is 124 in women and 19 per 1000 patients per year in men. The incidence of cystitis shows a peak in women between 15-24 years old and is the highest in women older than 65 years. In the male gender the incidence of cystitis increases with age [6]. In Belgium, a UTI makes up 18% of all hospital infections and causes 20% of all bloodstream infections (BSI) in a hospital [7].

2) Pathogenesis and micro-organisms

A lower UTI is mainly caused by organisms from the fecal flora that colonize the bladder through the urethra. Pyelonephritis usually develops from the rise of microorganisms from the bladder. In the general practitioners' population, *E. coli* is the most common pathogen (75-85%), followed by *S. saprophyticus* (10-15%) [8]. The latter bacteria is a typical pathogen in young healthy women and disappears after menopause. Although there is a greater diversity of causative micro-organisms in UTI with systemic symptoms, *E. coli* remains the most common causative micro-organism. Data retrieved on the first of May 2021 on the ISIS-AR database shows the three most common causative micro-organisms are *E. coli*, *E. faecalis* and *K. pneumoniae* (table 1). This data represents the number of isolates of uropathogens found in first urine cultures (non-catheter) from adult, in-hospital, non-ICU patients in 2019 in the Netherlands.

Causative micro-organism	Number of isolates
Escherichia coli	24982 (52,3%)
Enterococcus faecalis	5935 (12,4%)
Klebsiella pneumoniae	4889 (10,2%)
Proteus species	3820 (8%)
Pseudomonas aeruginosa	3059 (6,4%)
Enterococcus faecium	1855 (3,9%)
Staphylococcus aureus	1738 (3,6%)
Enterobacter species	1499 (3,1%)

Table 1. Number of uropathogens in adult, in-hospital, non-ICU patients in 2019 in the Netherlands

3) Urinalysis

Diagnosing a UTI based merely on **symptoms** is still common in some countries but often inaccurate [9]. It will overestimate the presence of a UTI by approximately 20-33% [10] and leads to the unnecessary prescription of antibiotics. The increasing prevalence of antibiotic resistance is an important issue worldwide [11]. A recent meta-analysis of Costelloe et al. showed an association between antimicrobial resistance among common uropathogens and antibiotic prescriptions in primary care [12].

Urinalysis is one of the most common tests performed in a clinical laboratory because it's widely used as a screening test for diagnosing and monitoring nephrological and urological conditions [13]. In the past, **microscopic urine sediment** analysis was the most widely accepted methodology for urinalysis. The technique is however time-consuming and is highly operator-dependent. Because of this, the sediment analysis is at present time done automatically using flow cytometry or digital microscopy.

Nowadays, **dipstick** is the most frequently used screening test for the presence or absence of an UTI. It contains a variety of parameters, including nitrite and leukocytes. Nitrite is a metabolic product derived from the reduction of nitrate by certain nitrogenic species (e.g. *E. coli*, *Proteus spp.*, *Klebsiella spp.*) and is an indicator for bacteriuria. The result can be false-negative when pathogens causing the UTI do not generate nitrite (e.g. *Staphylococcus spp.*, *Enterococcus spp.*, *Pseudomonas spp.*). The presence of leukocytes indicates an infection or an inflammatory process. A meta-analysis of Devillé et al. showed that the sensitivity of the dipstick method is often relatively low and only suitable as a rule-out test when both nitrite and leukocytes are negative [4].

Urine culture remains the most important test in the context of a (complicated) UTI. The common golden standard definition of bacteriuria is the presence of $\geq 10^5$ CFU/mL (rule of Kass) in a fresh, mid-stream voided urine sample. This rule was established in a hospital environment with women with acute pyelonephritis as study population. Lower values were attributed to contamination, incorrect collection or storage of the sample before starting the urine culture. The rule of Kass is still used in some laboratories, but is nowadays more and more under discussion. Since many patients with a UTI show bacteriuria with $\leq 10^5$ CFU/mL, lower colony counts as cut-off values are applied in many laboratories to increase the sensitivity of urine culture.

4) Situation H.H. hospital Lier

a) Current workflow concerning urinalysis

At the H.H. hospital of Lier, the requesting physician has several options regarding the diagnostic examination of a urine sample. If a sediment-analysis is requested, the laboratory always performs a dipstick using the Sysmex UF-3500. Subsequently the sample is automatically transferred to the UF-4000 where the analysis of the sediment occurs. The laboratory differentiates between urgent samples (originating from the emergency department and the obstetric ward) and non-urgent samples. Urgent samples are processed immediately and non-urgent samples within one hour of reception. One can furthermore request a culture for both bacteria and yeasts. There are also some special tests possible: for example, culture of *S. agalactiae*, *Legionella* antigen detection and PCR-testing for *C. trachomatis* and/or *N. gonorrhea.*

b) Sysmex UF-4000

The Sysmex UF-4000 is a recently (June 2020) acquired, third and latest generation, fully automated urine analyzer. It can discriminate and count 17 diagnostic parameters of cells and formed elements in a urine sample. It also offers an integrated body fluid mode which contains nine parameters. The Sysmex UF-4000 has a maximum theoretical throughput of 80 samples/hour, requiring a minimum volume of 2.0 mL of uncentrifuged, urine in an automated mode (0,6 ml in STAT mode).

The technology behind this system is the globally renowned fluorescence flow cytometry (FFC). The FFC instrument aspirates and dilutes a urine sample into two different reaction volumes prior to fluorescent dye staining. In the first volume (sediment reaction) all the nucleic acid-containing cells are stained (polymethine dye) whereas in the second volume (bacterial reaction), only nucleic acids in bacteria are stained. Thereafter, the instrument utilizes fluorescence flow cytometry technology, now using a new blue semi-conductor laser at 488 nm wavelength. The FFC counts and classifies cells based on signals of forward scattered light (FSC), side scattered light (SSC), side fluorescent light (SFL) and the new, additional depolarized side scattered light (DSS).

c) Local data of H.H. hospital Lier

An overview of the annual number of urine samples can be found in figure 1 and 2 below. From 2018 we see a clear increase in the number of negative samples. A possible explanation for this increase is that up to and including 2017, the reading of the urine culture on day two was also taken into account to consider a urine culture as positive.

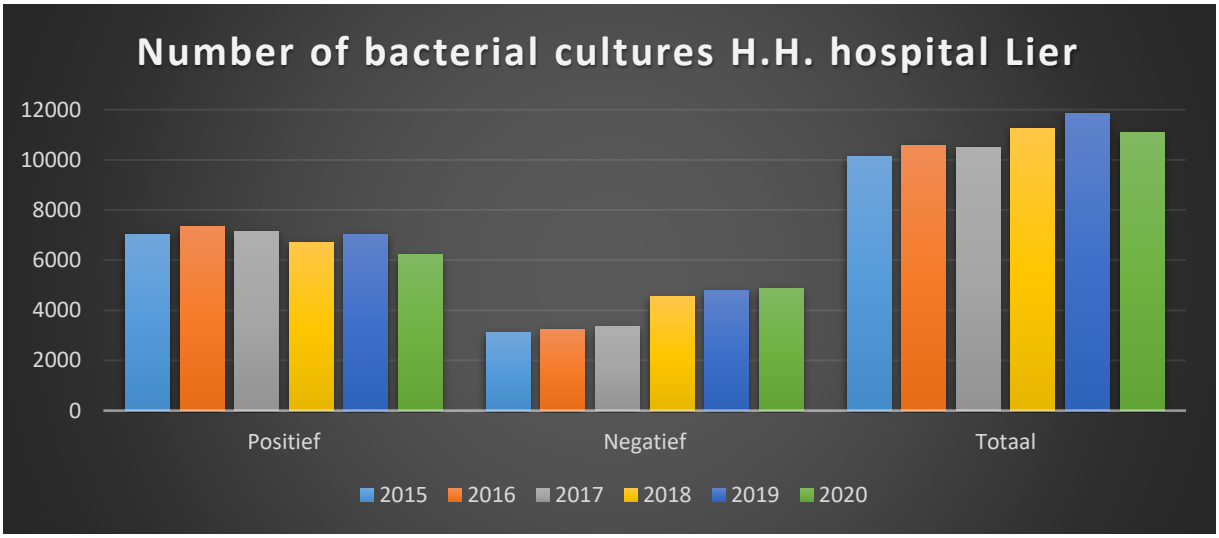

Figure 1. Number of bacterial cultures in H.H. hospital Lier

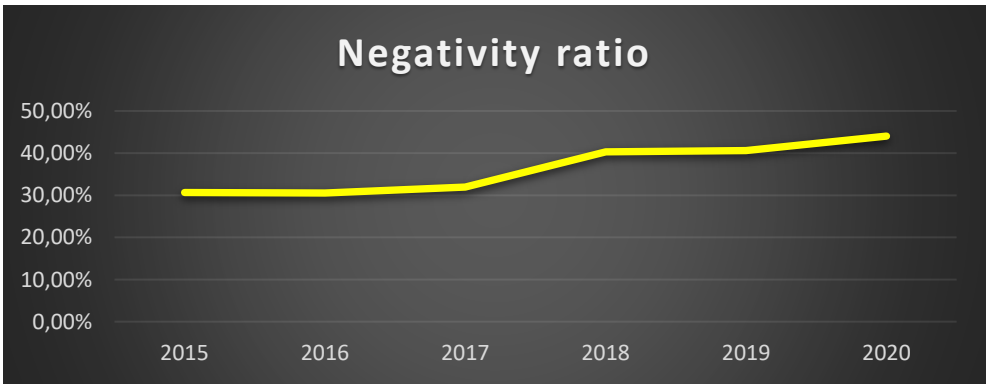

Figure 2. Percentage of negative urine cultures in H.H. hospital Lier

QUESTION(S)

Question 1: What is the diagnostic performance of the UF-4000 for ruling out a UTI?
Question 2: Is the implementation of a new UTI screening protocol feasible?

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: "("urinary tract infection"[All Fields]) AND ("flow cytometry"[All Fields])"
2) PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)
3) Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane)
4) International organizations: e.g. National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp)
UpToDate Online

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APPRAISAL

Question 1. What is the diagnostic performance of the UF-4000 for ruling out a UTI?

1) Literature study

1. Methods

One researcher (S.M.) searched for eligible studies published between January 1, 2010 and November 1, 2020 on the electronic database Medline. The following MeSH terms were used for the literature search: “urinary tract infection” and “flow cytometry”. References cited by eligible journal articles were also examined and potentially included. We only included eligible studies published in English and freely available in full-text. The inclusion criteria for the review were: 1) studies of diagnostic performance of the UF-500i, UF-1000i, UF-4000 or UF-5000, 2) both sensitivity and specificity data were available. The reviewer extracted the following data from eligible studies: the name of the first author, publication year, type of flow cytometer, total simple size, number of UTI, definition of a positive urine culture and applied cutoffs used for screening.

2. Results

In this study 20 studies were included, accounting for a total of urine 28,595 samples. Concerning the second-generation flow cytometer of Sysmex, 17 studies used the UF-500i or UF-1000i for UTI screening [1;3;14-25;27-28;30]. No studies used the UF-4000 and only three examined the performance of the UF-5000, both devices are the latest generation of flow cytometer [2;26;29]. The eligible studies are shown in table 2. All studies used urine culture as the reference standard but the cutoff values to define a urine culture as positive were sometimes different.

Nine studies evaluated UTI screening performance based on WBC counts [1-2,14,19-20,22,25-26,29], 12 studies screened using bacterial counts [1-2;14,17,19-20,22-23,25-27,29], and eight studies used strategies that combined both [3,15-18,24,28,30]. The use of a urine flow cytometer as a rule out strategy to reduce unnecessary cultures is well established according to this literature search. However, there is a wide spread of cut-off values ranging from 10 to 690 for bacteria/µL.

3. Discussion

To use a flow cytometer to rule out the diagnosis of a UTI, it is of the utmost importance that it can guarantee a high sensitivity and negative predictive value (NPV). The European Urinalysis Guidelines recommends an analytical sensitivity of > 90–95% to detect bacteriuria at 10^5 CFU/mL [3]. Kellog et al. suggests a sensitivity of ≥ 95% to use as a screening criterium and the American Society of Microbiology recommends a NPV > 98% [17;23].

The studies described above are sometimes difficult to compare with each other as the reported parameters (sensitivity, specificity etc.) depend on the definition used for a positive culture. For example, a cut-off value of 10^4 CFU/mL instead of 10^5 CFU/mL implies that a larger number of samples will have to be elaborated. However, one avoids the loss of patients for whom a bacterial count of 10^4 CFU/mL could be significant.

Regarding the usefulness of leukocyte counts as an additional parameter to discriminate between positive and negative urine cultures, there is no clear agreement. In some studies, the combination of cut-off values for leukocytes and bacteria improved the operational characteristics [3,15-16,18,24,28,30]. In other studies, the combination of leukocytes and bacteria did not yield better results (higher sensitivity and specificity) than when only the bacterial counts were used [1,2,14,17,19,20,22,26].

Because of physio-anatomical differences, UTIs are more common in women than in men [23]. Given that the length of the urethra is gender dependent, it is not unreasonable to speculate that the cutoff values for bacteria in the urine are also gender dependent [15,20,23,28]

The laboratory diagnosis of UTI is based on the detection and quantification of bacteriuria and leukocyturia. However, the presence of bacteria in the urine does not necessarily diagnose a UTI. It may also result from contamination of the sample (bad sampling) or from normal bacterial colonization of the urethra. Leukocyturia is often associated with UTI but may also derive from vaginal contamination in women or from patients with a urinary catheter.

Factors that can cause discordant results between screening using flow cytometry and culture are the following: 1) the presence of nonviable bacterial cells (due to antibiotic use prior to urine sampling) 2) viable but nonculturable bacteria (for example Mycoplasma, Ureaplasma…) [17].

To summarize, it is clear that the applicability of a flow cytometer to screen for negative urine samples and the chose cut-off value(s) strongly depend on population characteristics (taking into consideration the patient material) and the definition of a negative urine culture. The used cut-off values should also be verified on a regular basis.

Author	Year	Flow cytometer	Sample size	UTI (positive/negative samples)	Definition of positive urine culture
De Rosa et al. [3]	2010	UF-1000i	1349	346/1003	$\geq 10^4$ CFU/mL
Hu et al. [14]	2010	UF-1000i	308	109/199	$> 10^4$ CFU/ml for G^+ bacteria and $> 10^5$ CFU/ml for G^- bacteria
Jolkkonen et al. [15]	2010	UF-500i	1094	184/910	$> 10^4$ CFU/mL
Pieretti et al. [16]	2010	UF-1000i	703	217/486	$\geq 10^4$ CFU/mL
Broeren et al. [1]	2011	UF-1000i	1577	596/981	$\geq 10^5$ CFU/mL
Kadkhoda et al. [17]	2011	UF-1000i	2496	653/935 (908 contaminated)	$> 10^4$ CFU/ml of up to 2 uropathogens or $> 10^5$ CFU/ml of pure culture of nonuropathogens
Gutiérrez-Fernandez et al. [18]	2012	UF-1000i	1225	228/970	$> 10^5$ CFU/mL
Boonen et al. [19]	2013	UF-500i	281	57/224	$> 10^4$ CFU/mL
Giesen et al. [20]	2013	UF-1000i	791	102/689	$> 10^5$ CFU/mL
Gessoni et al. [21]	2015	UF-1000i	2335	598/1695 (42 contaminated)	$\geq 10^5$ CFU/mL
Martin-Gutiérrez et al. [22]	2015	UF-1000i	346	113/214 (19 contaminated)	$\geq 10^5$ CFUs/mL for women and $\geq 10^4$ CFUs/mL for men
Geerts et al. [23]	2016	UF500i	7322	1933/5389	$> 10^5$ CFU/mL or addition of antibiogram by the microbiologist
Inigo et al. [24]	2016	UF-1000i	1934	480/1377 (77 contaminated)	$> 10^2$ CFU/mL and clinical data compatible with a UTI
Middelkoop et al. [25]	2016	UF-1000i	381	143/238	$\geq 10^5$ CFU/mL
De Rosa et al. [2]	2018	UF-5000	2719	797/1922	$\geq 10^5$ CFU/mL
Kim et al. [26]	2018	UF-5000	1430	336/1094	$\geq 10^5$ CFU/mL
Conkar et al. [27]	2018	UF-1000i	546	57/489	$\geq 10^3$ CFU/mL
Millán-Lou et al. [28]	2018	UF-1000i	1220	213/1007	$\geq 10^5$ CFU/mL
Song et al. [29]	2018	UF-5000	126	41/85	$\geq 10^4$ CFU/mL
Broeren et al. [30]	2019	UF-1000i	412	63/349	$> 10^5$ CFU/mL

Table 2. Summary of eligible studies, sorted by year of publication

Author	Cut-off value(s)	SE (%)	SP (%)	PPV (%)	NPV (%)	TP (n)	TN (n)	FP (n)	FN (n)	Culture reduction (%)
De Rosa et al.	bacteria: 170/µL and WBC: 150/µL	98,8	76,5	59,2	99,5	342	767	236	4	57
Hu et al.	bacteria: 10/µL	100	36,8	45,9	100	109	73	126	0	24
	bacteria: 160/µL	81,1	83,2	73,5	89,3	89	166	33	20	55
Jolkkonen et al.	bacteria: 405/µL and WBC: 16/µL	93,4	82,3	ND	98,3	172	161	749	12	69
Pieretti et al.	bacteria: 65/µL and WBC: 100/µL	98,2	62,1	53,7	98,7	213	302	184	4	43
Broeren et al.	bacteria: 230/µL	95	80	ND	99,7	566	785	196	30	52
Kadkhoda et al.	bacteria: 20/µL	92,6	69	ND	ND	605	645	290	48	28
Gutiérrez-Fernandez et al.	bacteria: 690/µL and WBC: 38/µL	92	65	39	97	210	631	339	18	53
Boonen et al.	bacteria: 60/µL	100	62	40	100	57	139	85	0	49
Giesen et al.	bacteria: 288,9/µL	93	86	49	99	95	593	96	7	76
Gessoni et al.	bacteria: 175/µL	95	80	ND	ND	568	1356	339	30	59
Martin-Gutiérrez et al.	bacteria: 200/µL	99,1	91,6	86,15	99,5	112	196	18	1	57
Geerts et al.	bacteria: 200/µL	93	63,5	47,8	96,2	1798	3422	1967	135	49
Inigo et al.	bacteria: 460/µL and WBC: 40/µL	98,1	79,2	ND	99,2	471	1090	287	9	57
Middelkoop et al.	bacteria: 133/µL	90,2	64,3	60,3	91,6	129	153	85	14	44
De Rosa et al.	bacteria: 58/µL	99,4	78,4	65,4	99,7	787	1506	416	5	56
Kim et al.	bacteria: 15/µL	99	67,4	40,7	99,5	333	737	357	3	52
Conkar et al.	bacteria: 10/µL	100	43,5	17,1	100	57	225	264	0	41
Millán-Lou et al.	bacteria: 138,8/µL and WBC: 119,8/µL	95,3	70,4	40,5	98,6	203	709	298	10	59
Song et al.	bacteria: 50/µL and YLC: 100/µL	100	56,5	ND	100	41	48	37	0	38
Broeren et al.	bacteria: 250/µL and WBC 25/µL	97	91	90	97	61	318	31	2	78

Table 3. Design characteristics and results extracted from eligible studies (ND = no data)

2) Retrospective study H.H. hospital Lier

1. Methods

A. Patients and samples

This retrospective study was performed to evaluate the analytical performance and diagnostic accuracy of the UF-4000 for the exclusion of a UTI, compared to the standard culture. The study took place from 01/07/2020 to 30/09/2020 at the H.H. hospital in Lier. Only urine samples where both sediment and culture were requested were included in this study. All specimens were inoculated within one hour of arrival at the laboratory and analyzed immediately afterwards with the Sysmex UF-4000.

B. Microbiological analysis

A standard quantitative culture was performed on all samples with inoculation of 1 µL of urine using a sterile disposable needle on a Bi-plate consisting of a non-selective chromogenic agar (BBL CHROMagar Orientation; BD) and a more selective Columbia CNA agar. The BBL CHROMagar Orientation allows for the identification of the main urinary pathogens based on their specific enzymes. The Columbia CNA agar is a rich medium to which two antibiotics (colistin and nalidixic acid) on the one hand and 5% sheep blood on the other have been added. As a result, this medium promotes the growth of staphylococci, hemolytic streptococci and enterococci and inhibits the growth of most gram negative bacteria.

The cultures were read after 24h and 48h incubation at 37° C and interpreted according to the BILULU consensus guideline [31]. Results were expressed as the number of colony forming units per milliliter (CFU/ml). Identification of bacteria (if necessary) was done using MALDI-TOF MS (Bruker).

Then, for research purposes, initially three different categories were defined to interpret a urine culture: positive (green), negative (red) and a grey area (yellow) (attachment 1). This “grey area” was subjected to a further in depth investigation and contained eight midstream urine samples with one uropathogen < 10^4 CFU/mL and pyuria (≥ 25 WBC/µL). Only one of this eight samples was diagnosed with a UTI: it involved a middle aged woman with a S. saprophyticus who was already under levofloxacin, started by the general practitioner the day before.

The following classification was made up keeping in mind the clinical relevance of urine samples wit pyuria and after an extensive risk analysis. Positive cultures were defined as urine samples containing one to two uropathogens that exhibited at least 10^4 CFU/mL of growth or urine samples containing 3 or more uropathogens regardless of the number of CFU/mL present. A specimen was considered negative if there was no growth, only growth of urogenital flora, more urogenital flora than uropathogens or one to two uropathogens with growth less than 10^4 CFU/mL. This interpretation therefore slightly differs from the stipulated BILULU-consensus used in the laboratory: in this new model one or two uropathogens with a growth < 10.000 CFU/mL will not undergo identification nor an antibiotic susceptibility testing (in case of pyuria).

2. Data analysis

The results of the Sysmex UF-4000 BACT and WBC counts were compared with the urine cultures results using a ROC curve analysis. Sensitivity, specificity, positive and negative predictive value were calculated for the two parameters at different cut-off values with respect to the reference standard. The diagnostic accuracy of the BAC and WBC channel for UTIs was assessed by analysis of the area under the ROC curve (AUC). All data were recorded on Microsoft Excel spreadsheets. Statistical analysis was performed using Microsoft Excel's Analyse-it (version 5.80) software.

Each row of a Microsoft Excel sheet was allocated to a single urine specimen to enter detailed information about each patient and their related specimen. The information included the sample-number, name of the patient, date of birth, sex, age, clinic/ward, specimen type, BAC and WBC counts and culture interpretation (organism identification).

3. Results

During the study period 2,372 urine samples were evaluated. 57.6% and 42.4% of these samples were from female and male patients, respectively. The median age was 65 years (range: 0-98 years) and the number of hospital applications was 1436 (60,5%).

Of the 2372 samples, 1216 (51,3%) were positive according to the BILILU-consensus. Analysis of the positive urine cultures stratified by sex showed a frequency of positivity of 66,4% (808/1216) in samples from female subjects and 33,6% (408/1216) in samples from male subjects.

The causative micro-organisms identified in hospitalized patients were typical for a UTI with E. coli (43,17%), E. faecalis (20,95%) and K. pneumoniae (6,56%) as the most prevalent bacteria. A complete overview can be found in table 4.

Causative micro-organism	Number of isolates
Escherichia coli	237 (43,2%)
Enterococcus faecalis	115 (21%)
Klebsiella pneumoniae	36 (6,6%)
Proteus species	35 (6,4%)
Enterococcus faecium	18 (3,3%)
Pseudomonas aeruginosa	15 (2,7%)
Staphylococcus aureus	5 (1%)
other gram-negative rods*	56 (10,2%)
other gram-positive cocci**	31 (5,6%)

Table 4. Number of uropathogens in hospitalized patients in H.H. hospital Lier

*: Achromobacter sppp., Acinetobacter spp., Citrobacter spp., Enterobacter spp., Klebsiella spp. (except K. pneumoniae), Morganella spp., Providencia spp., Serratia spp.

**: Aerococcus spp., Staphylococcus saprophyticus, Streptococcus agalatiae, Streptococcus dysgalactiae

We sought to develop a screening strategy in which as few samples as possible needed to be cultured, while maintaining a low level of false negatives and a high negative predictive value.

We applied the following exclusion criteria to the extensive database: women aged 15-50 years, children < 1 year, patients with a nephrostomy or suprapubic catheter, samples obtained by bladder puncture or single catherization. Urine samples from patients in intensive care were also excluded. The exclusion of women between 15-50 years old is because the identification of S. agalactiae is of clinical importance in this specific population. In children under the age of one year, second and third generation flow cytometers don't have the necessary performance to screen out a UTI with a high enough degree of certainty and often a pedibag is used for collecting the urine sample. The above mentioned types of urine sampling are also excluded because the threshold to consider a urine culture can be lower than 10*4 CFU/mL and the manner of obtaining the sampling is more invasive. Lastly, samples of patients in intensive care are not withheld because they're critically ill patients. After applying these criteria, we had 1419 midstream urine samples remaining.

On this smaller database, the research categories were used to define a urine culture as positive or negative. ROC analysis was applied. The ROC curves for the Sysmex UF-4000 BACT and WBC counts are shown in figure 3. The area under the curve (AUC) for the BAC count is 0,884 (95% CI = 0,865-0,904) which is higher than that for the WBC count (0,797; 95% CI = 0,770-0,823). The AUC is a key parameter for evaluating the performance of a test as a measure of accuracy and is independent of prevalence. Furthermore, the combination of bacterial and WBC counts did not show any benefit in terms of sensitivity and specificity compared to the bacterial count alone (data not shown).

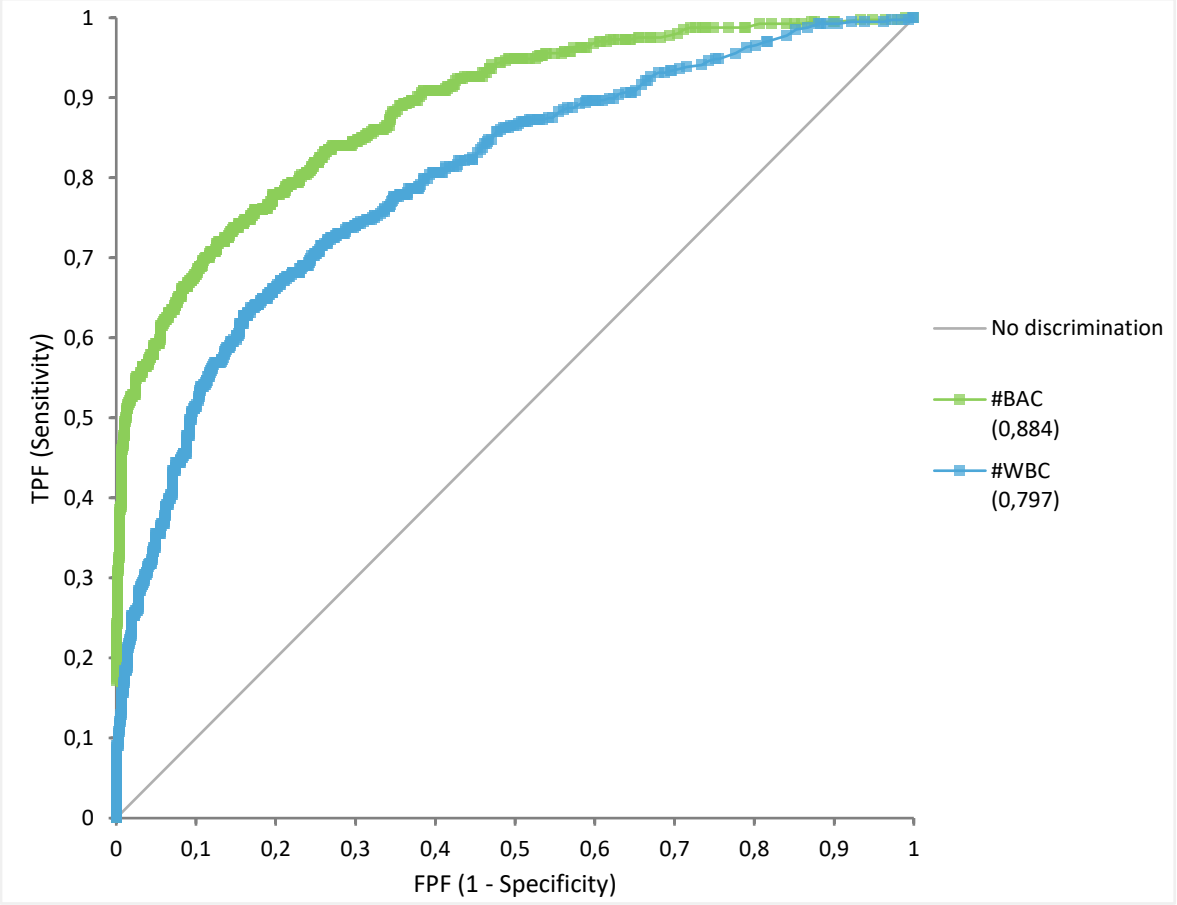

Figure 3. ROC curve for UF-4000 bacteria and leukocytes counts versus quantitative urine culture in 1419 samples

Cut-off values were determined to achieve a sensitivity of 95% and 98%. The corresponding cut-off values were 45,60 bacteria/µL and 18,40 bacteria/µL respectively. The resulting true positive, true negative, false positive and false negative values are shown in table 5.

Cut-off BAC count (cells/µL)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	TP (n)	TN (n)	FP (n)	FN (n)
45,60	95	47	96	42	388	477	534	20
18,40	98	30	98	36	400	299	712	8

Table 5. Diagnostic performance of UF-4000 in comparison of quantitative urine culture evaluated in 1419 samples

Using the cut-off of 45,6 bacteria/µL, a total of 497 urine samples (477 + 20 cases (see below); 35% from all specimens) were reported as negative with a high NPV of 96%. 534 samples (37,6% from all specimens) were cultured unnecessarily and 388 positive cases (27,3% from all specimens) were accurately diagnosed. 20 samples were found to be culture positive but UF-4000 negative (false negative rate 4,9%).

The culture results for these 20 samples were: one *S. dysgalactiae*, one *K. pneumoniae*, one sample with mixed *E. faecalis* and *P. mirabilis*, two samples with three or more uropathogens, two samples with mixed *E. faecalis and E. coli*, three *S. agalactiae*, three *E. coli* and seven E. faecalis. All had a growth between 10.000 and 100.000 CFU/mL.

We also evaluated the effect of gender on the UF-4000 performance. The ROC AUC's for BAC count were 0,882 (95% CI = 0,850-0,914) and 0,875 (95% CI = 0,847-0,903) for men and women respectively. The optimized cut-offs to ascertain a sensitivity of 95% were 23,8 bacteria/µL for men and 90,2 bacteria/µL for women respectively. The resulting true positive, true negative, false positive and false negative values listed in table 6.

Cut-off BAC count (cells/µL)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	TP (n)	TN (n)	FP (n)	FN (n)
male: 23,8	95	49	96	31	136	295	307	7
female: 90,2	95	30	92	50	252	160	249	13

Table 6. Diagnostic performance of UF-4000 in comparison of quantitative urine culture evaluated in 1419 samples for male and female separately

Based on this retrospective study, the UF-4000 could significantly contribute to the improvement of TAT in the clinical microbiology laboratory. It shortens the TAT of negative urine samples from 24h to less than 1,5h from the time of arrival to the laboratory for more than 35% of the results that can be quickly reported to the attending physician.
An extrapolation of this data to one year, means a very fast reporting of results for approximately 2000 patients.
This almost real-time reporting of negative results and reduction in unwarranted urine cultures could lead to an improvement in the quality of patient care: (1) less unnecessary empiric antibiotic treatments while waiting for culture results, (2) decreasing the risk of developing bacterial resistance and (3) possibly lower health care costs. The impact from a financial point of view is discussed in the next section.

Question 2. Is the implementation of a new UTI screening protocol feasible?

1) Proposal of a new UTI screening protocol

Initially, three theoretical test scenarios were conceived and developed. After consultation with the microbiology staff and the laboratory technicians of the microbiology lab, it was unanimously decided to continue working with one test scenario. The other two were discarded for practical reasons (disruption of lean way of working). Figure 4 shows the current way of working (left side), compared to the test scenario (right side).

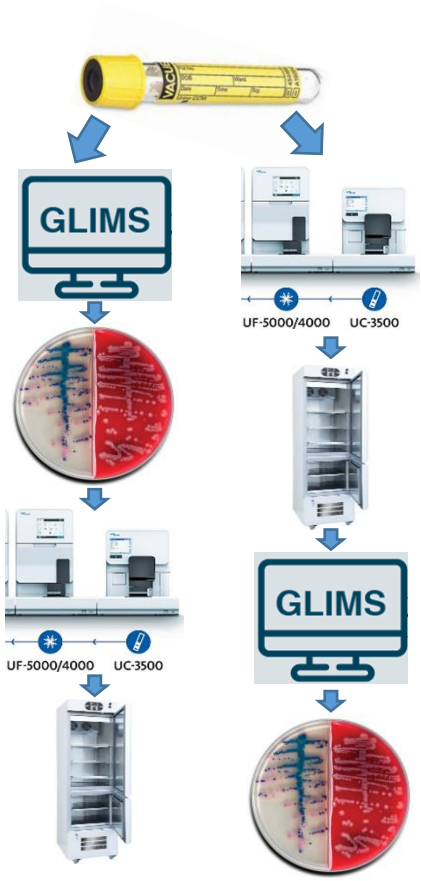

Figure 4. Overview of the diagnostic workflow (left is present workflow and right is test scenario)

Since this potentially new way of working assumes that the urine sample is first placed on the UF-4000 for analysis and then inoculated onto a nutrient medium (depending on the BAC count), we checked whether cross-contamination of cells, bacteria and/or particles would occur.

Two experiments were set up to investigate this issue. The first consisted of inoculating all urine samples (35) on a random working day before and after placement on the UF-4000. These samples were then read for two days and the results compared. Results are shown in attachment 2. Three samples of the 35 (3/35) were contaminated: two with one colony and one with two colonies.

Based on these results, a dilution series was initiated. This second experiment consisted of 10 urine samples of which five were spiked with an E. coli (2 McF, 1 McF, 0,5 McF, 0,25 McF and 0,1 McF). They were placed in a single rack alternating with a blank sample. All the samples where thereafter inoculated and read for two days. The blank sample after the sample spiked with 2 McFarland E. coli showed 2 colonies E. coli, all other blank samples showed no growth.

We also did a quick (and dirty) literary search and one article addressing this issue was found [2]. In this study four racks were prepared, each one containing a positive sample with high a bacteria count (≥ 10^6 CFU/mL) followed by three negative samples. Hereafter all tubes were cultured. They concluded that from a microbiological point of view, cross-contamination could be excluded because none of the cultured negative samples exhibited a higher growth in culture than one log10 over the bacterial count when run on the UF-5000 at the end of a series of positive samples.

Based on the results described above and after consultation with the microbiology staff, it was decided to explore the route of a deduplication (collection of two urine tubes).

2) Cost-benefit analysis

In the Belgian healthcare system, a laboratory is largely financed by means of a lump sum and a small part through fee-for-service (FFS). A cost-benefit analysis was performed to assess the financial impact of the possibly implemented new workflow (test scenario). A search on the RIZIV/INAMI database showed three possible sources of income, related to urinalysis (table 7).

	description	code number		B-value	fee 100%	fee 25%
		AMB	HOS			
B = 0,032012	microscopic examination of urine sediment	126512	126523	70	€ 2,24	€ 0,56
B = 0,032012	aerobic culture of urine	549312	549323	200	€ 6,40	€ 1,6
B = 0,032012	determination of the sensitivity to antibiotics	550734	550745	400	€ 12,80	€ 3,20

Table 7. Sources of FFS- income related to urinalysis

Possible savings as a result of omitting cultures for urine samples that were classified as negative by the UF-4000 were calculated by extrapolating data on technician hands-on time and costs of the used materials for a single urine sample.
The hands-on time of the technician included retrieving the barcode label and BI-plate, inoculation of the media, transporting the media to the appropriate incubator, putting the urine sample on the UF-4000, recollecting the media (for the next two days), analyzing the media for growth (for the next two days) and entering the data in the laboratory information system. Because not every urine sample is processed individually, we retrieved data from a random workweek (Monday to Friday) and calculated the average time a technician spends on a single urine sample during office-hours (8h-19h). This analysis showed that a technician spends on average 63 seconds processing a urine sample, as described in the first part of this paragraph. The used material calculation consisted of a urine tube, a urine transfer device, a Bi-plate, an inoculating loop and reagents of the UC-3500/UF-4000. In case of the test scenarios an extra urine tube was invoiced because of the need to collect two urine tubes.

Based on the potential costs and revenues described above, a cost-benefit analysis was performed on the 1419 urine samples that would qualify for this potentially new way of working (table nr 8 and 9).

	Material cost	Personnel cost	Total cost
Reference scenario	€ 4.459,56	€ 1.191,96	€ 5.651,52
Test scenario (sensitivity 95%)	€ 4.111,57	€ 846,05	€ 4.957,61
Test scenario (sensitivity 98%)	€ 4.292,88	€ 978,29	€ 5.271,17

Table 8. Costs related to urinalysis

	Total revenue (25% fee)	Total revenue (100% fee)	Total expenses
Reference scenario	€ 3.065,04	€ 12.260,16	€ 5.651,52
Test scenario (sensitivity 95%)	€ 2.269,84	€ 9.079,36	€ 4.957,61
Test scenario (sensitivity 98%)	€ 2.573,84	€ 10.295,36	€ 5.271,17

Table 9. Overview of revenues and expenses related to urinalysis

Based on these data, we can conclude that are no major changes in revenues nor in expense. The ratio of both remains approximately status quo. However, we must keep in mind that 497 and 307 fewer urine samples had to be cultured, respectively. This still makes for a more efficient way of working and has a major clinical impact.

TO DO/ACTIONS

1) Practical implementation of the new workflow
2) Review plan to periodically evaluate the used cut-off value

ATTACHMENTS

Attachment 1

Groei (aflezen)	GLIMS				Werkwijze (voor de pathogenen)
	Isolaat	Beoordeling		Commentaar ("Insert" en kies)	
		Code	Aantal kiemen CFU/mL [1]		
Geen groei	--	--	--	--	--
Enkel urogenitale flora	&UF	K	< 10.000	--	--
		T	10.000 – 100.000		
		G	> 100.000		
Urogenitale flora > pathogenen	&UF	K	< 10.000	UF_overw *Overwegend urogenitale flora met mogelijk aanwezigheid van pathogenen. Graag controlestaal indien klinisch relevant.*	--
		T	10.000 – 100.000		
		G	> 100.000		
Pathogenen ≥ urogenitale flora (1 of 2 soorten)	&UF en naam pathogenen intikken	K	PYURIE [2]: < 10.000	--	ID/AB
		K	GEEN PYURIE: < 10.000	AB_aanvr *Antibiogram wordt enkel uitgewerkt op specifieke vraag.*	ID
		T	10.000 – 100.000	--	ID/AB
		G	> 100.000		
Pathogenen ≥ urogenitale flora (≥ 3 soorten)	&UP	K	< 10.000	UP_meng *3 of meerdere kiemsoorten; vermoedelijk contaminatie. Graag controlestaal indien klinisch relevant.*	--
		T	10.000 – 100.000		
		G	> 100.000		

[1] Aantal kolonies op de URI-plaat * 1000
[2] I.g.v. verblijfskatheter: geen rekening houden met pyurie, steeds AB op aanvraag!

Attachment 2

Original result	Test result	Concordance analysis
no growth	no growth	concordant
no growth	no growth	concordant
UF t	UF t	concordant
no growth	no growth	concordant
no growth	no growth	concordant
E. coli & UF t	E. coli & UF t	concordant
UF k	UF k	concordant
UF > enc	UF > enc	concordant
UF t	UF t	concordant
UF > E. coli	UF > E. coli	concordant
E. faecalis k	E. faecalis k	concordant
K. pneumoniae t & E. faecalis t	K. pneumoniae t & E. faecalis t	concordant
M. morganii t & UF t	M. morganii t & UF t	concordant
UF k	UF k	concordant
UF k	UF k	concordant
no growth	no growth	concordant
E. faecalis k	E. faecalis k	concordant
UF t	UF t	concordant
no growth	no growth	concordant
S. aureus t	S. aureus t	concordant
UF k	UF k + 1 kol S. aureus	discordant
UF t	UF t	concordant
UF t	UF t	concordant
UF k	UF k	concordant
no growth	no growth	concordant
E. coli g & P. mirabilis t	E. coli g & P. mirabilis t	concordant
no growth	2 kol E. coli	discordant
no growth	no growth	concordant
E. coli g & S. agalactiae k	E. coli g & S. agalactiae k	concordant
UF t	UF t + 1 kol E. coli	discordant
UP meng	UP meng	concordant
E. coli g & UF t	E. coli g & UF t	concordant
E. coli g	E. coli g	concordant
no growth	no growth	concordant