

A new concept and a comprehensive evaluation of SYSMEX UF-1000i flow cytometer to identify culture-negative urine specimens in patients with UTI

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Abstract Urinary tract infections (UTIs) are among the most common bacterial infections in men and urine culture is gold standard for diagnosis. Considering the high prevalence of culture-negative specimens, any method that identifies such specimens is of interest. The aim was to evaluate a new screening concept for flow cytometry analysis (FCA). The outcomes were evaluated against urine culture, uropathogen species and three conventional screening methods. A prospective, consecutive study examined 1,312 urine specimens, collected during January and February 2012. The specimens were analyzed using the Sysmex UF1000i FCA. Based on the FCA data culture negative specimens were identified in a new model by use of linear discriminant analysis (FCA-LDA). In total 1,312 patients were included. In- and outpatients represented 19.6% and 79.4%, respectively; 68.3% of the specimens originated from women. Of the 610 culture-positive specimens, *Escherichia coli* represented 64%, enterococci 8% and *Klebsiella spp.* 7%. Screening with FCA-LDA at 95% sensitivity identified 42% (552/1312) as culture negative specimens when UTI was defined according to European guidelines. The proposed screening method was either superior or similar in comparison to the three conventional

screening methods. In conclusion, the proposed/suggested and new FCA-LDA screening method was superior or similar to three conventional screening methods. We recommend the proposed screening method to be used in clinic to exclude culture negative specimens, to reduce workload, costs and the turnaround time. In addition, the FCA data may add information that enhance handling and support diagnosis of patients with suspected UTI pending urine culture.

Keywords UTI · Screening · Urine culture · Flow-cytometry · Sysmex UF-1000i

Introduction

Urinary tract infections (UTIs) are among the most common infections in community and hospitalized patients with more than 175 million UTI incidences worldwide [1]. UTI is caused by pathogenic microorganisms which induce infection and an inflammatory response with presence of leukocyturia and erythrocyturia. However, UTIs are often harmless and self-eradicated [2]. Nevertheless, UTIs are associated with high morbidity and costs and especially among patients with diabetes [3]. In the United States UTIs account annually for more than 7 million physician visits, more than 1 million emergency department visits and more than 100,000 hospitalizations. The estimated annual cost for treatment is calculated to more than US \$1 billion and the indirect cost estimated to approximately \$1.6 billion [4, 5]. Furthermore, 15% of all antibiotics prescribed to outpatients and data from some European countries also suggest a similar rate [6, 7].

In a large German study on patients with diabetes mellitus type 2 the cost was estimated to €316 per UTI event with a total increased cost of €3916 per patient in the UTI group compared with the non UTI group [3].

The original version of this article was revised: The original version of this article unfortunately contains an error in the abstract.

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For diagnosis of UTI symptoms such as urgency, dysuria, frequent urination, back pain, leukocyturia and a positive nitrite test are considered reliable indicators [2, 8]. However, urine culture is the gold standard for diagnosis of UTI but is laborious and moderately costly, with a turnaround time of 24 to 48 h [9].

In clinical laboratories, urine specimens are among the most commonly encountered and approximately a quarter to more than half are considered culture negative [9, 10]. For this reason, any screening method that identifies and excludes urine specimens with non-significant bacteriuria would be of great interest [11–13].

Flow cytometry analysis (FCA) is a promising method to identify and enumerate bacteria, leukocytes, erythrocytes and other particles in urine. The second generation automated FCA instrument, the Sysmex UF-1000i (Medical Electronics, Kobe, Japan) has a separate detection channel for bacteria with improved sensitivity (SE) and specificity (SP) [11–15].

Using FCA, we have recently evaluated the inflammatory response of leukocytes and erythrocytes in urine for different pathogens in patients with suspected UTI [9]. Based on the dataset we now aimed to set up and assess a new screening model to identify and rule out culture negative urine specimens in patients with suspected UTI prior to culture. The prerequisites for the screening model are high SE to prevent specimens with significant bacteriuria (SBU) from being erroneously classified as negative (i.e. false negatives) and a relatively high SP to prevent unnecessary culturing. The present screening model was evaluated against three conventional screening methods [11, 16, 17].

Material and methods

Collection of urine specimens

A prospective consecutive multicenter study was conducted during January and February 2012 which analyzed urine specimens from in- and outpatients. The specimens were collected in non-preservative tubes, stored and transported at ≤ 6 °C to the Department of Clinical Microbiology at the University Hospital of Umeå for analysis. All specimens were from the county of Västerbotten, Sweden.

Urinalysis

All specimens underwent FCA and urine culture within 3 h of arrival to the laboratory. Specimens that arrived after 4 PM were analyzed with flow cell analysis (FCA) and stored at +6 °C until cultured the following morning. Excluded were specimens from pregnant women, urinary catheter and those that lacked complete FCA data.

Flow cytometry analysis was performed using the UF-1000i instrument (Sysmex, TOA Medical Electronics, Kobe, Japan), supported by the Sysmex software version of 00–15 [9]. The screening model was built on the observed bacterial and leukocyte counts.

Urine culture

Gram-negative and Gram-positive uropathogens were identified by Brilliance™ UTI agar (Oxoid Ltd., Basingstoke, UK) biochemical tests, and urine culture was performed as previously described [9].

Species identification

Isolates were identified in specimens with presence of $\geq 10^6$ colony forming units/L (CFU/L) and those with mixed flora (with both gram negative and gram positive bacteria) with a dominating pathogen (i.e. bacterial count at least 10 times higher than any other species) [9].

Significant bacteriuria

SBU was defined in accordance with European guidelines (at $\geq 10^6$ CFU/L of an uropathogen with acute uncomplicated cystitis) [7] and was the gold standard in the present article [18, 19]. In patients with $< 10^8$ ($\geq 10^6$ and 10^7 CFU/L) and where the presence or absence of UTI symptoms could not be determined, the specimens were classified indeterminate.

Evaluations were also done when SBU was defined as $\geq 10^7$ or $\geq 10^8$ CFU/L of a uropathogen, respectively, irrespective of presence of UTI symptoms.

Ethical approval

All procedures were in accordance with institutional and national ethical standards and the Helsinki declaration.

Statistical analysis

Urine specimens analyzed with FCA and urine culture were used to derive a decision rule that based on FCA data determines which specimens should be cultured. Screening methods based on bacterial counts (BC) and white blood cell counts (WBC) have been suggested by Jolkkonen, Manoni and De Rosa [11, 16, 17]. Here, specimens are cultured if either $BC > a$ or $WBC > b$, where a and b are the method specific cut-off values: Jolkkonen: $a = 405$, $b = 16$; Manoni: $a = 125$, $b = 40$; De Rosa: $a = 170$, $b = 150$.

We suggest the FCA-LDA approach that uses linear discriminant analysis (LDA) on the FCA variables BC and WBC [20]. The FCA-LDA rule was derived using data from specimens that were concluded to be significant bacteriuria or

non-significant bacteriuria and with $BC < 5000$. A LDA-model was fitted to the log-transformed FCA-values, assuming homoscedasticity and proportional priors, which resulted in a decision rule: $[\ln(BC) > \alpha + \beta \ln(WBC)]$. The intercept α was tuned so that the rule had the desired sensitivity (SE). The decision rule is a line, where specimens above the line are cultured (Fig. 1).

The methods were evaluated in terms of their SE and specificity (SP), negative and positive predictive values (PPV and NPV), the number of cultured specimens and the relative cost (RC) compared to the standard procedure. RC was calculated under the assumption that the running cost of culture is five times as high as running FCA. The evaluations were performed for different definitions of UTI.

In a similar style as described above a LDA-model, based on BC and the variable B-FSC (the forward scatter / “particle size”), was derived to determine if the specimens’ bacteria were gram positive or gram negative. This resulted in a classification rule: $[\ln(BC) > \alpha + \beta \ln(B-FSC)]$ where specimens were predicted gram positive if the inequality was valid and gram-negative otherwise.

Results

In total, 1587 urine specimens were eligible for FCA and urine culture. Two hundred seventy-five specimens (17.3%) were

excluded: 125 urinary catheters, 128 specimens from pregnant women, 18 that lacked clinical information and four specimens with candiduria. In total, 1312 specimens were enrolled in the present study (Fig. 1).

Among genders, 68.3% ($p < 0.001$) of the specimens originated from women with a mean age of 58.9 years (standard deviation 25.5) versus 31.7% and 61.7 years (20.8) for men. Outpatients represented 79.4% and inpatients 20.6%. The demographic information of the dataset is presented in Table 1.

When UTI was defined according to European guidelines, 36.0% (473/1312 specimens) had significant bacteriuria, 56.5% (741/1312 specimens) had non significant bacteriuria and 7.5% (98/1312 specimens) were classified as indeterminant, due to lack of clinical information.

E. coli was the most predominant uropathogen, representing 71.2% (337/473) of the uropathogens followed by *Enterococcus faecalis* 6.8%, *Klebsiella pneumoniae* 6.1% and coagulase-negative staphylococci 5.1% (Tables 1 and 2).

FCA-LDA decision rules, using BC and WBC as the predictors and controlling the SE at 90%, 95% and 98%, respectively, were derived, i.e.

$$\text{FCA-LDA}_{98}: \ln(BC) > 10.46 - 0.65 \ln(WBC)$$

$$\text{FCA-LDA}_{95}: \ln(BC) > 8.22 - 0.65 \ln(WBC)$$

$$\text{FCA-LDA}_{90}: \ln(BC) > 7.07 - 0.65 \ln(WBC)$$

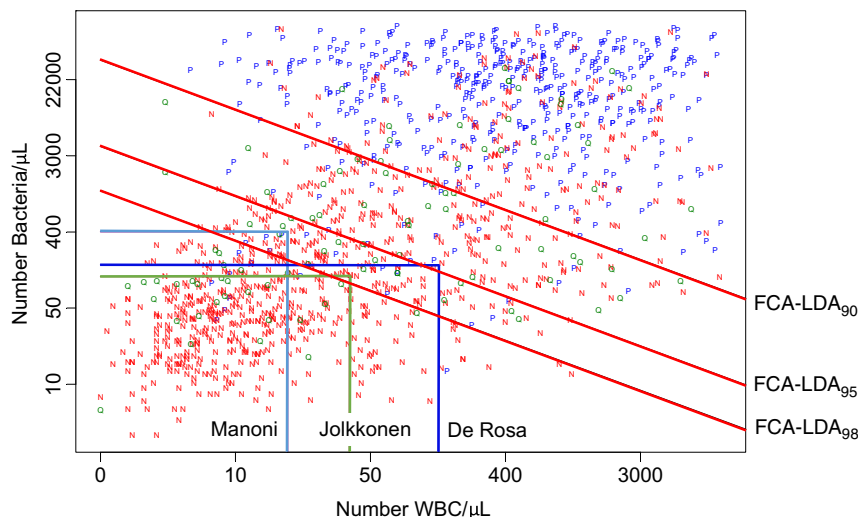


Fig. 1 Plot of 1,312 urine specimens with significant, non-significant and indeterminant bacteriuria and the classification criteria or the different screening methods examined. Specimens above the classification line were predicted as positives and send to culture. P (blue color) = “positive specimens”, i.e. specimens with significant bacteriuria when urinary tract infection was defined according to European guidelines. N (red colour) = “negative specimens”, i.e. specimens with non-significant bacteriuria when urinary tract infection was defined according to European guidelines. Q (green colour) = “indeterminant/questionable” specimens, i.e. specimens with indeterminant significant bacteriuria when urinary tract infection was defined according to European guidelines (mainly

specimens that lacked information regarding UTI symptoms), number of bacteria (Bact = Y-axis) and white blood cells (WBC = X-axis) estimated by flow cell instrument per uL. Red lines represent the cut off for linear discriminant analysis at: 98% sensitivity (FCA-LDA₉₈), 95% sensitivity (FCA-LDA₉₅) and 90% sensitivity (FCA-LDA₉₀). Dark blue color represents the cut-off values according to De Rosa et al. (cut off: bacteria 170, white blood cells 150). Green color represents the cut-off values according to Manoni et al. (cut off: bacteria 125, white blood cells 40). Light blue color represents the cut-off values according to Jolkkonen et al. (cut off: bacteria 405, white blood cells 16)

Table 1 Demographic data of 1,312 patients and urine specimens enrolled in the study

| Characteristic | Total ^a | Women ^a | Men ^a |
|---|--------------------|--------------------|------------------|
| Age (mean) | 58.9 | 57.6 | 61.7 |
| Outpatients (%) | 79.4 | 82.4 | 72.3 |
| Specimens included, <i>n</i> (%) | 1312 | 896 (68) | 416 (32) |
| Growth distribution at culture | | | |
| > 10 ⁸ colony forming units per liter | 447 (34) | 359 (80) | 88 (20) |
| 10 ⁷ –10 ⁸ colony forming units per liter | 370 (28) | 271 (73) | 99 (27) |
| 10 ⁶ –10 ⁷ colony forming units per liter | 288 (22) | 193 (67) | 95 (33) |
| No growth | 207 (16) | 73 (35) | 134 (65) |
| SBU ^b , <i>n</i> (%) | 473 | 380 (80) | 93 (20) |
| Gram-negative | 405 (86) | 337 (83) | 68 (17) |
| <i>Escherichia coli</i> | 337 (71) | 288 (85) | 49 (15) |
| <i>Klebsiella</i> spp. | 29 (6) | 24 (83) | 5 (17) |
| Other | 39 (8) | 25 (64) | 14 (36) |
| Gram-positive | 68 (14) | 43 (63) | 26 (37) |
| <i>Staphylococcus</i> spp. | 24 (5) | 17 (71) | 7 (29) |
| <i>Enterococcus</i> spp. | 32 (7) | 18 (56) | 14 (44) |
| Other | 12 (2) | 8 (67) | 4 (33) |
| Indeterminant SBU ^b , <i>n</i> (%) | 98 | 68 (69) | 30 (31) |
| Gram-negative | 74 (75) | 50 (69) | 24 (31) |
| <i>Escherichia coli</i> | 54 (55) | 37 (69) | 17 (31) |
| <i>Klebsiella</i> spp. | 13 (13) | 9 (69) | 4 (31) |
| Other | 7 (7) | 4 (57) | 3 (43) |
| Gram-positive | 24 (25) | 18 (76) | 6 (24) |
| <i>Staphylococcus</i> spp. | 2 (2) | 1 (50) | 1 (50) |
| <i>Enterococcus</i> spp. | 15 (15) | 10 (67) | 5 (33) |
| Other | 7 (7) | 7 (100) | 0 (0) |
| Non significant SBU ^b , <i>n</i> (%) | 741 | 448 (60) | 293 (40) |
| Gram-negative | 10 (1) | 7 (70) | 3 (30) |
| <i>Escherichia coli</i> | 2 (0) | 1 (50) | 1 (50) |
| <i>Klebsiella</i> spp. | 3 (0) | 2 (66) | 1 (33) |
| Other | 5 (1) | 4 (80) | 1 (20) |
| Gram-positive | 29 (4) | 24 (83) | 5 (17) |
| <i>Staphylococcus</i> spp. | 5 (1) | 2 (40) | 3 (60) |
| <i>Enterococcus</i> spp. | 4 (1) | 4 (100) | 0 (0) |
| Other | 20 (3) | 18 (90) | 2 (10) |
| Mixed flora Gram-negative | 202 (27) | 148 (73) | 54 (27) |
| Mixed flora Gram-positive | 293 (39) | 196 (67) | 97 (33) |
| No growth | 207 (28) | 73 (35) | 134 (65) |

^a The percentage (%) represents the figures within each sub-group. The columns *Women* and *Men* represent the relative distribution between the genders

^b Significant bacteriuria according to European guidelines (SBU), indeterminate according to European guidelines and non-significant bacteriuria according to European guidelines

Recall that a specimen is cultured if the inequality is fulfilled. The FCA-LDA-rules were compared to the bivariate decision rules suggested by Jolkkonen, Manoni and De Rosa

[11, 16, 17]. The six decision rules and their outcome are shown in Fig. 1.

Evaluation when SBU was defined according to European guidelines

Overall, when the FCA-LDA₉₈ decision rule was used and UTI was defined according to European guidelines (at $\geq 10^6$ CFU/L of an uropathogen with acute uncomplicated cystitis) [7] then the screen resulted in 98% SE (464 true positive specimens), 52% SP (385 true negative specimens) and 32% (421 specimens) were identified as culture negative (Fig. 2, total data). The corresponding numbers for the more restrictive inclusion rules FCA-LDA₉₅ and FCA-LDA₉₀ were (95%, 65%, and 42%) and (90%, 83%, and 56%), respectively (Fig. 2, Table 2). In comparison, the screening methods by Jolkkonen, Manoni and De Rosa had similar SE as for FCA-LDA₉₈; however, the Jolkkonen's and Manoni's methods had lower SP (41%) compared to FCA-LDA₉₈ (52%) and De Rosa (51%). The outcome of the six decision rules are presented in Table 2. In general, the screening methods had relative high SE for *E. coli* (0–3% above average SE for the examined methods) and *Klebsiella/Citrobacter* groups (KC-group 0–5% above average SE). Relative low SE was found for the enterococci group (EN group –1 to –18% below average SE) and the streptococci group (SR group –18 to –30% below average SE for the examined methods; Table 2).

Screening at SBU defined according to European guidelines yielded higher SE and SP than UTI defined at $\geq 10^7$ and $\geq 10^8$ /CFU/L (Table 3).

At FCA-LDA₉₈, FCA-LDA₉₅ and FCA-LDA₉₀ screening 9, 23 (9 + 14) and 47 (23 + 24) specimens proved false negative, respectively, representing 0.7, 1.7 and 3.6% of the specimens. Information related to the false negative specimens is presented in Table 4. For the FCA-LDA₉₅ screen, seven of the false negative specimens had 10⁸ CFU/L, eight 10⁶ and 10⁸ CFU/L each. Patients 2, 3, 7, 9, 10, 16 had all low WBC- and elevated bacterial counts indicating asymptomatic bacteriuria in these patients. Patient 8 had “high” WBC and red blood cell (RBC) counts but low bacterial counts despite presence of *Proteus mirabilis* at 10⁸ CFU/L at culture. Of the 47 false negative specimens, 72% (34/47) originated from women.

Evaluation when SBU was defined at $\geq 10^7$ and $\geq 10^8$ CFU/L

Overall, defining UTI at $\geq 10^7$ CFU/L of an uropathogen, irrespective of presence of UTI symptoms, resulted in lower sensitivities (3–9%) and similar specificities in comparison when SBU was defined according to European guidelines (Tables 3 and 5). Defining UTI as $\geq 10^8$ CFU/L (irrespective of presence of UTI symptoms) resulted in higher sensitivities

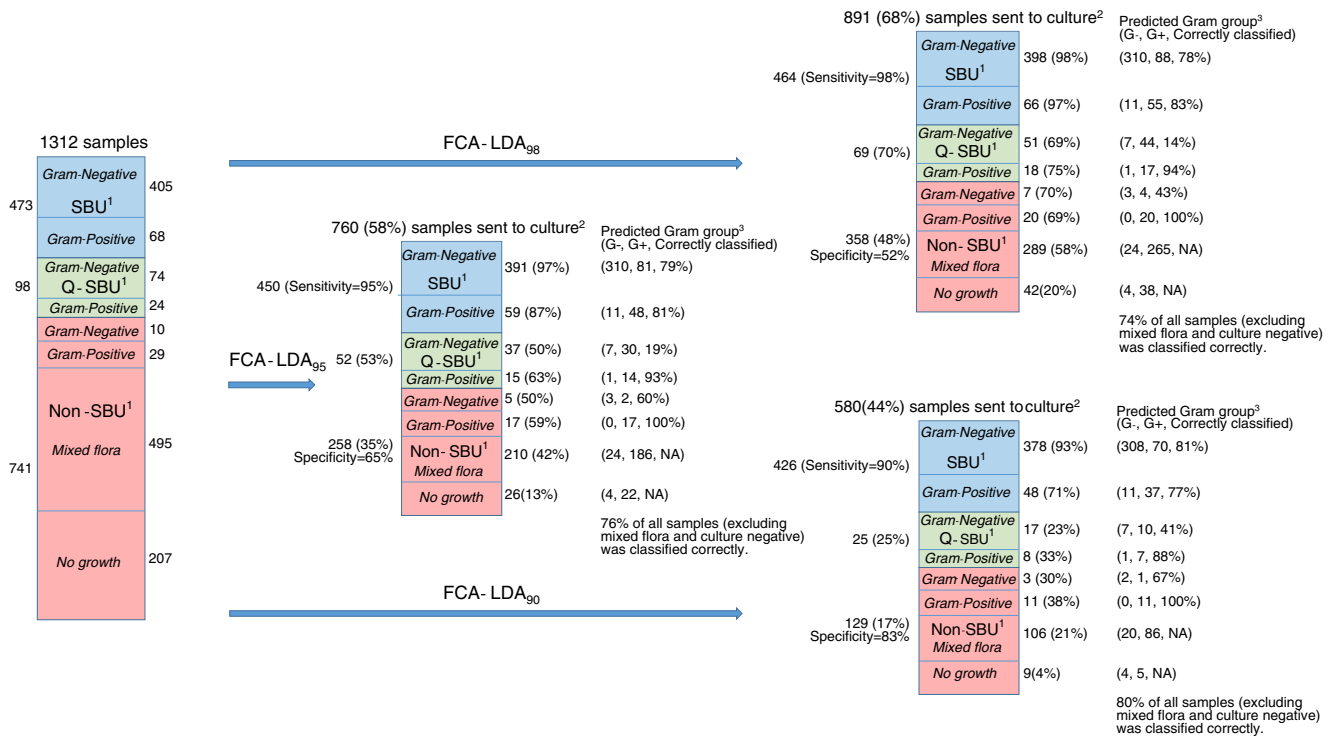


Fig. 2 Outcome of the 1312 urine specimens examined by flow cytometry analysis and samples sent to culture after screening using linear discriminant analysis at 90, 95 and 98% sensitivity when significant bacteriuria was defined according to European guidelines. Blue arrows: represent the outcome of linear discriminant analysis at 98, 95 and 90% sensitivity, respectively (FCA-LDA₉₈, FCA-LDA₉₅, FCA-LDA₉₀) when urinary tract infection was defined according to

European guidelines. G+ = gram positive species. G- = gram negative species. ¹SBU = significant bacteriuria, blue-colored boxes; Q-SBU = indeterminant (questionable) SBU, green-colored boxes; Non SBU, red-colored boxes. ²Number of specimens sent to culture when FCA-LDA selection was applied. ³Specimens sent to culture was classified as Gram negative or Gram positive bacteria

(1–5%) and lower specificities (2–4%) in comparison to European guidelines (Tables 3 and 6).

Also at these screening methods SE had great impact on the proportion of specimens identified as culture negative (26–56%; Tables 2, 3, 5, and 6). For all methods a higher SE was observed for *E. coli* (0–5% above average SE), and lower SE for the enterococci (0 to –18% below average SE) and streptococci groups (0 to –25%) (Tables 2, 5, and 6).

Prediction of gram groups

Bacteria were predicted to their gram group according to their FCA results such that $\ln(BC) > -8.49 + 3.82 \ln(B-FSC)$ were classified to be gram-positive otherwise to be gram-negative. In specimens sent to culture, 74–80% of the bacteria were correctly classified to their gram group (Fig. 2).

Calculation of the relative cost in a model including FCA screening found a 5–36% cost reduction compared with non-selective culture depending on the screening method used (Table 3). Implementation of the FCA-LDA₉₅ estimated a 22% cost reduction when UTI was defined according to European guidelines (Table 3).

Discussion

UTIs are a common infection and one of the most commonly analyzed specimens in clinical microbiological laboratories. The aim of the present study was to present and evaluate a new model for identification of culture negative urine specimens identified by FCA.

The basis of the new screening model was to establish the cut off for bacterial and leukocyte counts based on LDA which differs from conventional screening models based on fixed cut off’s for bacteria- and WBC counts (Fig. 1) [11–13, 15–17, 20–22]. The new model was evaluated with respect to: SE, definitions of significant bacteriuria, different uropathogens, group of uropathogens, mixed flora and culture negative specimens and three conventional screening methods [12, 16, 17]. At evaluation the new LDA method proved superior to the Jolkkonen and Manoni methods but similar to that described by De Rosa et al. [17]. The prerequisite for the presented screening method was high SE since diagnosis was later confirmed by urine culture. Since the outcome for SBU defined according to European guidelines was superior compared with 10⁷ CFU/L, irrespective of UTI symptoms. For this reason is the European guidelines recommended.

Table 2 Outcome of the four screening methods of 1,312 urine specimens examined with flow cytometry analysis when UTI was defined according to European guidelines

| Group ^a | Bacteria | SBU | | Methods evaluated ^b | | | | | | | | | | | | | | | | | | |
|--------------------|-------------------------------------|-----|-----|--------------------------------|------------|------------|------------|-----|-----|---------|------------|------------|-----------------------|-----|-----|-----------------------|------------|------------|-----------------------|-----|-----|-----|
| | | | | Jolkkonen | | | Manoni | | | De Rosa | | | FCA-LDA ₉₈ | | | FCA-LDA ₉₅ | | | FCA-LDA ₉₀ | | | |
| | | | | Pos ^c | Neg | Q | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | |
| EC | <i>Escherichia coli</i> | 337 | 2 | 54 | 99 | 0 | 16 | 99 | 0 | 15 | 98 | 0 | 23 | 99 | 0 | 20 | 97 | 0 | 38 | 93 | 100 | 70 |
| KC | <i>Acinetobacter spp.</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 100 | 1 | | 0 | 0 | | 100 | 1 | | 100 | 1 | |
| KC | <i>Citrobacter spp.</i> | 9 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | | 100 | 0 | 0 | 100 | 0 | | 100 | 0 | 0 | 89 | | 1 |
| KC | <i>Citrobacter freundii</i> | 1 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | | 100 | 0 | 0 | 100 | 0 | | 100 | 0 | 0 | 100 | | 0 |
| KC | <i>Enterobacter spp.</i> | 2 | 1 | 2 | 100 | 0 | 1 | 100 | 0 | 1 | 100 | 0 | 1 | 100 | 0 | 1 | 100 | 0 | 1 | 100 | 0 | 1 |
| KC | <i>Other Klebsiella spp.</i> | 1 | 0 | 1 | 100 | 1 | 1 | 100 | | 1 | 100 | 1 | 1 | 100 | | 1 | 100 | 1 | 1 | 100 | | 1 |
| KC | <i>Klebsiella oxytoca</i> | 8 | 1 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 1 |
| KC | <i>Klebsiella pneumoniae</i> | 20 | 2 | 11 | 95 | 100 | 8 | 100 | 50 | 6 | 100 | 50 | 7 | 95 | 100 | 8 | 95 | 100 | 9 | 95 | 100 | 9 |
| KC | <i>Pantoea spp.</i> | 1 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| KC | Aggregated | 42 | 5 | 15 | 98 | 40 | 10 | 100 | 20 | 8 | 100 | 40 | 10 | 98 | 40 | 10 | 98 | 60 | 12 | 95 | 60 | 14 |
| PR | <i>Morganella morganii</i> | 2 | 1 | 2 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 2 | 100 | 100 | 3 |
| PR | <i>Proteus mirabilis</i> | 9 | 1 | 2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 1 | 89 | 0 | 1 | 89 | 100 | 2 | 89 | 100 | 3 |
| PR | <i>Proteus vulgaris</i> | 2 | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| PR | <i>Providencia rettgeri</i> | 1 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| PR | Aggregated | 14 | 3 | 4 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 67 | 2 | 93 | 33 | 2 | 93 | 67 | 4 | 93 | 67 | 6 |
| PS | <i>Pseudomonas aeruginosa</i> | 11 | 0 | 0 | 91 | 1 | 1 | 100 | | 0 | 100 | 0 | 0 | 91 | | 1 | 91 | 1 | 1 | 91 | | 1 |
| PS | Other <i>Pseudomonas spp.</i> | 0 | 0 | 1 | | 0 | 0 | | | 0 | | 0 | 0 | | | 0 | | 0 | 0 | | | 0 |
| PS | Aggregated | 11 | 0 | 1 | 91 | 1 | 1 | 100 | | 0 | 100 | 0 | 0 | 91 | | 1 | 91 | 1 | 1 | 91 | | 1 |
| ST | CoNS | 13 | 4 | 0 | 100 | 0 | 0 | 100 | 25 | 1 | 100 | 25 | 1 | 100 | 25 | 1 | 100 | 25 | 1 | 85 | 25 | 3 |
| ST | <i>Staphylococcus aureus</i> | 8 | 1 | 1 | 88 | 0 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 75 | 0 | 2 | 63 | 100 | 5 |
| ST | <i>Staphylococcus saprophyticus</i> | 3 | 0 | 1 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 67 | | 1 |
| ST | Aggregated | 24 | 5 | 2 | 96 | 0 | 1 | 100 | 20 | 1 | 100 | 20 | 1 | 100 | 20 | 1 | 92 | 20 | 3 | 75 | 40 | 9 |
| EN | <i>Enterococcus faecalis</i> | 28 | 4 | 10 | 93 | 0 | 6 | 96 | 0 | 4 | 93 | 0 | 6 | 96 | 0 | 5 | 82 | 25 | 11 | 71 | 75 | 17 |
| EN | <i>Enterococcus faecium</i> | 4 | 0 | 5 | 100 | 2 | 2 | 100 | | 1 | 100 | 2 | 2 | 100 | | 2 | 100 | 3 | 3 | 75 | | 6 |
| EN | Aggregated | 32 | 4 | 15 | 94 | 0 | 8 | 97 | 0 | 5 | 94 | 0 | 8 | 97 | 0 | 7 | 84 | 25 | 14 | 72 | 75 | 23 |
| SR | Alpha-hemolytic streptococci | 3 | 6 | 0 | 67 | 17 | 2 | 67 | 17 | 2 | 67 | 17 | 2 | 67 | 17 | 2 | 67 | 17 | 2 | 67 | 33 | 3 |
| SR | <i>Gemella haemolysans</i> | 1 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| SR | <i>Streptococcus</i> Group A | 0 | 0 | 1 | | 0 | 0 | | | 0 | | 0 | 0 | | | 0 | | 0 | 0 | | | 1 |
| SR | <i>Streptococcus</i> Group G | 0 | 0 | 3 | | 0 | 0 | | | 0 | | 0 | 0 | | | 0 | | 1 | 1 | | | 2 |
| SR | <i>Streptococcus</i> Group C | 1 | 0 | 3 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 0 | | 2 |
| SR | Aggregated | 5 | 6 | 7 | 80 | 17 | 2 | 80 | 17 | 2 | 80 | 17 | 2 | 80 | 17 | 2 | 80 | 17 | 3 | 60 | 33 | 8 |
| GB | <i>Streptococcus agalactiae</i> | 6 | 14 | 0 | 83 | 36 | 6 | 100 | 29 | 4 | 100 | 36 | 5 | 100 | 50 | 7 | 67 | 64 | 11 | 50 | 79 | 14 |
| OT | Diphtheroid rod | 1 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| OT | <i>Haemophilus influenzae</i> | 1 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| OT | Aggregated | 2 | 0 | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 | 100 | 0 | 0 | 100 | | 0 |
| NE | Culture negative | 0 | 207 | 0 | | 64 | 132 | | 64 | 132 | | 77 | 160 | | 80 | 165 | | 87 | 181 | | 96 | 198 |

Table 2 (continued)

| Group ^a | Bacteria | SBU | | Methods evaluated ^b | | | | | | | | | | | | | | | | | | |
|--------------------|----------------|-----|-----|--------------------------------|-----------|------------|------------|-----|-----------|------------|-----------|-----------|-----------------------|------------|----|-----------------------|-----------|-----------|-----------------------|----|----|-----|
| | | | | Jolkkonen | | | Manoni | | | De Rosa | | | FCA-LDA ₉₈ | | | FCA-LDA ₉₅ | | | FCA-LDA ₉₀ | | | |
| | | | | Pos ^c | Neg | Q | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | |
| MP | Mixed flora G+ | 0 | 293 | 0 | 34 | 100 | 37 | 107 | 43 | 127 | 45 | 133 | 57 | 166 | 78 | 229 | | | | | | |
| MN | Mixed flora G- | 0 | 202 | 0 | 31 | 62 | 27 | 55 | 41 | 82 | 36 | 73 | 59 | 119 | 79 | 160 | | | | | | |
| All | Total | 473 | 741 | 98 | 98 | 41 | 339 | 99 | 41 | 330 | 98 | 51 | 420 | 98 | 52 | 421 | 95 | 65 | 552 | 90 | 83 | 732 |

SBU significant bacteriuria, SE sensitivity, SP specificity, NC not cultured, i.e. specimens screened as culture negative, spp species, CoNS coagulase negative staphylococci

^a Group = bacteria or bacterial groups. EC = E. coli; KC = *Klebsiella/Citrobacter* group; PR = *Proteus* group; PS = *Pseudomonas* group; ST = Staphylococcal group; SR = Streptococci group; GB = *Streptococcus agalactiae* (GBS); OT = other bacteria; NE = culture negative; MP = mixed Gram-positive flora; MN = mixed Gram-negative flora; All = total

^b Methods evaluated: Jolkkonen et al., Manoni et al., De Rosa et al., linear discriminant analysis at 98, 95 and 90% sensitivity, respectively (FCA-LDA₉₈, FCA-LDA₉₅, FCA-LDA₉₀)

^c Pos = SBU when urinary tract infection was defined according to European guidelines; Neg = non SBU according to European guidelines; Q = indeterminate (questionable) significant bacteriuria according to European guidelines

Currently, the instrument is mainly used for screening to identify and rule out culture negative specimens. However, screening can be done at different levels of SE, e.g. at lower SE for non hospitalized patients as UTIs are often harmless

and self eradicating [2] or at higher SE (or omitted) in high risk patients in intensive care, immunosuppressed patients, pregnancy and those with pyelonephritis. In these patients, urine culture is recommended irrespective of the outcome of

Table 3 Outcome of the four screening methods to rule out culture negative urine specimens among patients with suspected UTI

| SBU ^a | Measures (%) | Methods evaluated ^b | | | | | |
|------------------------|-----------------|--------------------------------|--------|---------|-----------------------|-----------------------|-----------------------|
| | | Jolkkonen | Manoni | De Rosa | FCA-LDA ₉₈ | FCA-LDA ₉₅ | FCA-LDA ₉₀ |
| European Guidelines | SE | 98 | 99 | 98 | 98 | 95 | 90 |
| | SP | 41 | 41 | 51 | 52 | 65 | 83 |
| | PPV | 51 | 52 | 56 | 56 | 63 | 77 |
| | NPV | 96 | 98 | 98 | 98 | 95 | 93 |
| | NC ^c | 26 | 25 | 32 | 32 | 42 | 56 |
| ≥10 ⁷ CFU/L | RC ^d | 94 | 95 | 88 | 88 | 78 | 64 |
| | SE | 94 | 96 | 94 | 94 | 90 | 81 |
| | SP | 42 | 42 | 53 | 52 | 67 | 84 |
| | PPV | 56 | 56 | 61 | 61 | 68 | 80 |
| | NPV | 91 | 93 | 92 | 92 | 89 | 85 |
| ≥10 ⁸ CFU/L | SE | 99 | 100 | 100 | 99 | 98 | 95 |
| | SP | 38 | 38 | 48 | 48 | 62 | 81 |
| | PPV | 44 | 44 | 49 | 49 | 56 | 71 |
| | NPV | 99 | 100 | 100 | 99 | 99 | 97 |

SE sensitivity, SP specificity, PPV positive predictive value, NPV negative predictive value

^a SBU = Definition of significant bacteriuria according to European guidelines, ≥10⁷ and ≥10⁸ colony forming units per liter in urine at culture

^b Comparing screening methods according to Jolkkonen et al., Manoni et al., De Rosa et al. Flow cell analysis-linear discriminant analysis at 98% sensitivity (FCA-LDA₉₈), FCA-LDA₉₅ = at 95% sensitivity, and FCA-LDA₉₀ = at 90% sensitivity

^c NC = not cultured (%): specimens identified as culture negative by the screening method

^d RC = relative cost of the screening method (%) compared to the gold standard procedure (100%) when all specimens are cultured. A calculated cost of, e.g. 78%, represents a 22% cost reduction when FCA screening was included in its cost

Table 4 Data associated with the 47 false negative urine specimens present in linear discriminatory analysis at 98%, 95% and 90% sensitivity

| Sensitivity (%) | Patient | Gender | Patient age (years) | Unit | Specimen | 10 ⁿ CFU/L ^a | Bacteria ^b | Nitrit-test ^c | UTI ^d Symptom | Comments / clinical information | RBC ^e | WBC ^f | Bacterial counts ^g |
|-----------------|---------|--------|---------------------|-------|----------|------------------------------------|--------------------------------------|--------------------------|--------------------------|---|------------------|------------------|-------------------------------|
| 0.98 | 1 | M | 32 | HCC | IC | 7 | α -hemolytic str ^m | | | IC | 2 | 5 | 49 |
| 0.98 | 2 | F | 6 | HCC | MSU | 7 | <i>Escherichia coli</i> | | P | BSU | 5 | 6 | 127 |
| 0.98 | 3 | M | 75 | Hosp. | IC | 6 | <i>Escherichia coli</i> | | | | 10 | 4 | 101 |
| 0.98 | 4 | M | 73 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | Increased CRP, BSU | 4 | 6 | 69 |
| 0.98 | 5 | F | 80 | HCC | MSU | 6 | <i>Escherichia coli</i> | | | 500 WBC ^h | 22 | 18 | 122 |
| 0.98 | 6 | M | 91 | HCC | MSU | 6 | <i>Ent. faecalis</i> | | P | Urgency | 4 | 5 | 38 |
| 0.98 | 7 | F | 78 | HCC | MSU | 8 | <i>Klebsiella pneum.</i> | | | | 1 | 7 | 224 |
| 0.98 | 8 | F | 23 | Hosp. | MSU | 8 | <i>Proteus mirabilis</i> | | | Smelly urine, increased WBC ^h | 65 | 170 | 9 |
| 0.98 | 9 | F | 29 | HCC | MSU | 8 ^{M7} | <i>Ps. aeruginosa</i> | | | Ante-natal clinic | 36 | 8 | 172 |
| 0.95 | 10 | F | 54 | HCC | MSU | 7 | <i>Escherichia coli</i> | | | | 37 | 11 | 343 |
| 0.95 | 11 | M | 60 | HCC | MSU | 7 | <i>Escherichia coli</i> | | P | Urgency | 74 | 69 | 76 |
| 0.95 | 12 | F | 5 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | Cystitis symptom | 18 | 423 | 62 |
| 0.95 | 13 | F | 36 | HCC | MSU | 6 | <i>Escherichia coli</i> | | + | | 4 | 81 | 131 |
| 0.95 | 14 | F | 68 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | Urgency, 1 h BID | 20 | 244 | 33 |
| 0.95 | 15 | F | 75 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | Urgency | 7 | 334 | 40 |
| 0.95 | 16 | M | 69 | Hosp. | MSU | 8 | <i>Ent. faecalis</i> | | | | 8 | 8 | 290 |
| 0.95 | 17 | M | 79 | HCC | MSU | 8 ^{M6} | <i>Ent. faecalis</i> | | | | 110 | 98 | 179 |
| 0.95 | 18 | F | 89 | Hosp. | MSU | 7 ^{M6} | <i>Ent. faecalis</i> | | P | Urgency | 12 | 65 | 152 |
| 0.95 | 19 | F | 6 | HCC | MSU | 7 ^{M6} | <i>Ent. faecalis</i> | | P | Frequency | 5 | 20 | 357 |
| 0.95 | 20 | F | 79 | Hosp. | MSU | 8 | <i>Str. agalactiae</i> | | P | BSU, urgency | 18 | 14 | 364 |
| 0.95 | 21 | F | 50 | HCC | MSU | 8 | <i>Str. agalactiae</i> | | | | 10 | 32 | 220 |
| 0.95 | 22 | F | 89 | Hosp. | MSU | 7 | <i>S. aureus</i> | | | 100 WBC ^h | 6 | 11 | 236 |
| 0.95 | 23 | F | 37 | HCC | MSU | 7 | <i>S. aureus</i> | | P | Urgency | 7 | 29 | 414 |
| 0.90 | 24 | F | 29 | HCC | MSU | 6 | <i>Citrobacter spp.</i> | | | | 4 | 144 | 348 |
| 0.90 | 25 | M | 70 | Hosp. | IC | 7 | CoNS | | | Fever | 108 | 79 | 1085 |
| 0.90 | 26 | M | 79 | HCC | IC | 7 | CoNS | | | UTI symptom. Neurogenic urine bladder disease | 70 | 2495 | 82 |
| 0.90 | 27 | M | 20 | HCC | MSU | 8 ^{M7} | <i>Escherichia coli</i> | | P | BSU | 4 | 210 | 467 |
| 0.90 | 28 | F | 26 | HCC | MSU | 8 | <i>Escherichia coli</i> | | | 500 WBC ^h | 4 | 190 | 234 |
| 0.90 | 29 | M | 30 | HCC | MSU | 8 | <i>Escherichia coli</i> | | | | 1 | 89 | 471 |
| 0.90 | 30 | F | 36 | HCC | MSU | 8 | <i>Escherichia coli</i> | | - | Urgency, | 26 | 55 | 1334 |
| 0.90 | 31 | F | 47 | HCC | MSU | 8 | <i>Escherichia coli</i> | | P | Urgency, BSU | 2 | 10 | 7478 |
| 0.90 | 32 | F | 47 | HCC | MSU | 8 | <i>Escherichia coli</i> | | | | 87 | 28 | 2122 |
| 0.90 | 33 | F | 74 | HCC | MSU | 8 ^{M6} | <i>Escherichia coli</i> | | + | | 5 | 20 | 3684 |
| 0.90 | 34 | F | 92 | HCC | MSU | 8 | <i>Escherichia coli</i> | | | UTI? | 26 | 122 | 1382 |
| 0.90 | 35 | F | 35 | HCC | MSU | 7 ^{M6} | <i>Escherichia coli</i> | | | 500WBC ^h , 25RBC ^h | 43 | 893 | 101 |
| 0.90 | 36 | F | 48 | HCC | MSU | 7 | <i>Escherichia coli</i> | | | 500 WBC ^h , 50 RBC ^h | 4 | 288 | 785 |
| 0.90 | 37 | F | 70 | HCC | MSU | 7 | <i>Escherichia coli</i> | | + | | 8 | 139 | 186 |
| 0.90 | 38 | F | 33 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | 500 WBC ^h , 200 RBC ^h | 14 | 1113 | 151 |
| 0.90 | 39 | F | 71 | HCC | MSU | 6 | <i>Escherichia coli</i> | | P | BID 4 h, BSU, Urgency | 6 | 52 | 284 |
| 0.90 | 40 | M | 62 | HCC | MSU | 8 | <i>Ent. faecalis</i> | | | Urgency | 2 | 6 | 1913 |

Table 4 (continued)

| Sensitivity (%) | Patient | Gender | Patient age (years) | Unit | Specimen | 10 ⁿ CFU/L ^a | Bacteria ^b | Nitrit-test ^c | UTI ^d Symptom | Comments / clinical information | RBC ^e | WBC ^f | Bacterial counts ^g |
|-----------------|---------|--------|---------------------|-------|----------|------------------------------------|-------------------------|--------------------------|--------------------------|---------------------------------|------------------|------------------|-------------------------------|
| 0.90 | 41 | M | 72 | HCC | MSU | 8 | <i>Ent. faecalis</i> | | | Fever | 17 | 25 | 981 |
| 0.90 | 42 | F | 73 | HCC | MSU | 8 ^{M7} | <i>Ent. faecalis</i> | | P | Urgency | 12 | 35 | 2449 |
| 0.90 | 43 | F | 56 | Hosp. | MSU | 8 ^{M6} | <i>Ent. faecium</i> | | | Neutropenia | 78 | 12 | 1116 |
| 0.90 | 44 | F | 82 | HCC | MSU | 8 ^{M6} | <i>Str. agalactiae</i> | | P | Urgency | 42 | 49 | 2339 |
| 0.90 | 45 | F | 70 | Hosp. | IC | 8 | <i>S. aureus</i> | | | Infection? | 40 | 6 | 2440 |
| 0.90 | 46 | F | 26 | HCC | MSU | 8 ^{M6} | <i>S. saprophyticus</i> | | P | | 9 | 155 | 1119 |
| 0.90 | 47 | F | 60 | HCC | MSU | 8 | Streptococci | | | | 14 | 273 | 410 |

M male, F female, HCC health care centre, Hosp hospital, MSU mid stream urine, IC intermittent catheterized, α -hemolytic *str* alpha haemolytic streptococci, BSU burning sensation during urination, CoNS coagulase negative staphylococci

^a M6, M7 represents mixed flora at 10⁶, 10⁷ / colony forming units/L, respectively 10ⁿ the numbers 6, 7, 8 represents 10⁶, 10⁷ and 10⁸ colony forming units/L, respective of bacteria quantified at urine culture. M = mixed flora, i.e. 8^{M7} = specimens with 10⁸ colony forming units/L of the dominating uropathogen and mixed flora 10⁶ colony forming units/L.

^b Bacteria/uropathogen identified at culture

^c + = positive test, - = negative test

^d UTI (urinary tract symptoms): + P = present

^e RBC = red blood cells counted in flow cytometry analysis $\times 10^6$ /L

^f WBC = white blood cells counted in flow cytometry analysis $\times 10^6$ /L

^g Bacterial counts estimated in the flow cytometry analysis $\times 10^6$ /L^h WBC = white blood cells, RBC = red blood cells estimated at clinic/health care centre

the FCA screening to avoid missed diagnosis in those deemed high risk. Since *Streptococcus agalactiae* is a potential threat to the unborn child and mother, we always recommend urine culture at pregnancy to identify colonization at low colony counts ($<10^6$ CFU/L).

Overall, we found that FCA-LDA₉₅ provided a good balance between high SE and specimens excluded as culture negative (> 40%). At our laboratory, nearly 12,600 (of 30,000) specimens could be excluded from culture providing a significant reduction in workload, costs and turnaround time. The predicted cost reduction in the present model (5–36%) is in accordance with others [23]. In addition, FCA analysis adds important information to clinicians in identifying or rejecting patients for antimicrobial treatment. For those treated, the microorganisms Gram group adds further information for treatment success.

The microorganism's Gram stain identity was predicted by use of B-FSC [24, 25] with an accuracy of 76% (74–80%) in the present study. Rod-shaped bacteria are predicted at higher accuracy than for cocci, >90% and 29%, respectively [26]. Use of NaOH-sodium dodecyl sulfate (SDS) has been proven to facilitate discrimination between Gram positive and Gram negative bacteria [27].

Excluded specimens (urinary catheters and those from pregnant women) were re-examined with a similar outcome as those included (data not shown). LDA screening seems therefore also to be useful for these specimens but further studies are warranted.

Of Among the false negative specimens patient 8 had “high” WBC counts and low bacterial counts despite presence of *Proteus mirabilis* at 10⁸ CFU/L at culture. We speculate that the bacterial FCA-staining may have failed in this specimen.

The strength of the present study is the new screening approach, the large cohort and the comprehensive evaluations and against three conventional screening methods [11, 16, 17]. Also the outcomes are presented for different uropathogens, groups of bacteria, negative cultures and pinpoint the strength and weakness in the different methods as the impact of the definition of SBU.

Similar evaluations are, to our knowledge, not reported for SBU defined according to the European definition (at $\geq 10^6$ CFU/L of an uropathogen with acute uncomplicated cystitis) [7]. In addition we also evaluated the outcome at $\geq 10^7$ and $\geq 10^8$ CFU/L irrespective of presence of UTI symptoms.

The distribution of uropathogens was in accordance with other studies [20, 28, 29] and sampling was carried out to minimize the destruction of RBC and WBC [30]. Also, the sampling approach possessed a very low risk for contamination with improved specimen quality.

A weakness is the cohort's population structure with respect to gender, age, in-/outpatients, type of specimens, etc., which differs from other studies and explains the differences in outcome [11–13, 15–17, 19–22, 31]. However, the pathophysiology of UTI is expected to be quite similar despite some differences between species [9] and cohorts. However, the

Table 5 Outcome of the four screening methods of 1,312 urine specimens examined with flow cytometry analysis when UTI was defined as growth of $\geq 10^7$ colony forming units per liter at culture

| Group ^a | Bacteria | SBU | | Methods evaluated ^b | | | | | | | | | | | | | | | | | |
|--------------------|---------------------------------|-----|-----|--------------------------------|-----|-----|--------|-----|-----|---------|-----|-----|-----------------------|-----|-----|-----------------------|-----|-----|-----------------------|-----|-----|
| | | | | Jolkkonen | | | Manoni | | | De Rosa | | | FCA-LDA ₉₈ | | | FCA-LDA ₉₅ | | | FCA-LDA ₉₀ | | |
| | | | | Pos ^c | Neg | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE |
| EC | <i>Escherichia coli</i> | 374 | 19 | 97 | 26 | 16 | 98 | 32 | 15 | 96 | 47 | 23 | 97 | 37 | 20 | 94 | 84 | 39 | 86 | 100 | 70 |
| KC | <i>Acinetobacter spp.</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 1 | 0 | 0 | 0 | 0 | 100 | 1 | 0 | 100 | 100 | 1 |
| KC | <i>Citrobacter spp.</i> | 8 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 1 |
| KC | <i>Citrobacter freundii</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 0 |
| KC | <i>Enterobacter spp.</i> | 4 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 |
| KC | Other <i>Klebsiella</i> | 2 | 0 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 | 50 | 1 |
| KC | <i>Klebsiella oxytoca</i> | 10 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 90 | 1 |
| KC | <i>Klebsiella pneumoniae</i> | 29 | 4 | 86 | 100 | 8 | 90 | 75 | 6 | 86 | 75 | 7 | 86 | 100 | 8 | 83 | 100 | 9 | 83 | 100 | 9 |
| KC | <i>Pantoea spp.</i> | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| KC | Aggregated | 55 | 7 | 91 | 71 | 10 | 93 | 57 | 8 | 91 | 71 | 10 | 91 | 71 | 10 | 89 | 86 | 12 | 87 | 100 | 14 |
| PR | <i>Morganella morganii</i> | 4 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 75 | 100 | 2 | 50 | 100 | 3 |
| PR | <i>Proteus mirabilis</i> | 11 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 1 | 91 | 0 | 1 | 91 | 100 | 2 | 82 | 100 | 3 |
| PR | <i>Proteus vulgaris</i> | 3 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| PR | <i>Providencia rettgeri</i> | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| PR | Aggregated | 19 | 2 | 100 | 50 | 1 | 100 | 50 | 1 | 100 | 100 | 2 | 95 | 50 | 2 | 89 | 100 | 4 | 79 | 100 | 6 |
| PS | <i>Pseudomonas aeruginosa</i> | 11 | 0 | 91 | 1 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 91 | 1 | 91 | 1 | 91 | 1 | 91 | 1 | 1 |
| PS | Other <i>Pseudomonas</i> | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| PS | Aggregated | 12 | 0 | 92 | 1 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 92 | 1 | 92 | 1 | 92 | 1 | 92 | 1 | 1 |
| ST | CoNS | 17 | 0 | 100 | 0 | 94 | 1 | 94 | 1 | 94 | 1 | 94 | 1 | 94 | 1 | 94 | 1 | 82 | 3 | 3 | 3 |
| ST | <i>Staphylococcus aureus</i> | 9 | 1 | 89 | 0 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 89 | 0 | 1 | 56 | 100 | 5 |
| ST | <i>Staph. saprophyticus</i> | 4 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 75 | 1 | 1 | 1 |
| ST | Aggregated | 30 | 1 | 97 | 0 | 1 | 97 | 0 | 1 | 97 | 0 | 1 | 97 | 0 | 1 | 93 | 0 | 2 | 73 | 100 | 9 |
| EN | <i>Enterococcus faecalis</i> | 38 | 4 | 87 | 25 | 6 | 92 | 25 | 4 | 87 | 25 | 6 | 89 | 25 | 5 | 76 | 50 | 11 | 63 | 75 | 17 |
| EN | <i>Enterococcus faecium</i> | 9 | 0 | 78 | 2 | 89 | 1 | 78 | 2 | 78 | 2 | 78 | 2 | 78 | 2 | 67 | 3 | 33 | 6 | 6 | 6 |
| EN | Aggregated | 47 | 4 | 85 | 25 | 8 | 91 | 25 | 5 | 85 | 25 | 8 | 87 | 25 | 7 | 74 | 50 | 14 | 57 | 75 | 23 |
| SR | Alpha-hemolytic streptococci | 9 | 0 | 78 | 2 | 78 | 2 | 78 | 2 | 78 | 2 | 78 | 2 | 78 | 2 | 78 | 2 | 67 | 3 | 3 | 3 |
| SR | <i>Gemella haemolysans</i> | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| SR | <i>Streptococcus</i> Group A | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 1 |
| SR | <i>Streptococcus</i> Group G | 3 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 67 | 1 | 33 | 2 | 2 | 2 |
| SR | <i>Streptococcus</i> Group C | 4 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 50 | 2 | 2 | 2 |
| SR | Aggregated | 18 | 0 | 89 | 2 | 89 | 2 | 89 | 2 | 89 | 2 | 89 | 2 | 89 | 2 | 83 | 3 | 56 | 8 | 8 | 8 |
| GB | <i>Streptococcus agalactiae</i> | 18 | 2 | 72 | 50 | 6 | 83 | 50 | 4 | 78 | 50 | 5 | 67 | 50 | 7 | 44 | 50 | 11 | 28 | 50 | 14 |
| OT | Diphtheroid rod | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| OT | <i>Haemophilus influenzae</i> | 1 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| OT | Aggregated | 2 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| NE | Culture negative/no | 0 | 207 | 64 | 132 | 64 | 132 | 77 | 160 | 80 | 165 | 88 | 181 | 96 | 198 | | | | | | |
| MP | Mixed flora G+ | 0 | 293 | 34 | 100 | 36 | 107 | 43 | 127 | 45 | 133 | 57 | 166 | 78 | 229 | | | | | | |
| MN | Mixed flora G- | 2 | 200 | 100 | 31 | 62 | 100 | 28 | 55 | 100 | 41 | 82 | 100 | 37 | 73 | 100 | 59 | 119 | 100 | 80 | 160 |
| All | Total | 577 | 735 | 94 | 42 | 339 | 96 | 42 | 330 | 94 | 53 | 420 | 94 | 53 | 421 | 90 | 67 | 552 | 81 | 84 | 732 |

SBU significant bacteriuria, SE sensitivity, SP specificity, NC not cultured (i.e. specimens screened as culture negative), spp. species, CoNS coagulase negative staphylococci

^a Group = bacteria or bacterial groups. EC = *E. coli*; KC = *Klebsiella/Citrobacter* group; PR = *Proteus* group; PS = *Pseudomonas* group; ST = Staphylococcal group; SR = Streptococci group; GB = *Streptococcus agalactiae* (GBS); OT = other bacteria; NE = culture negative; MP = Mixed grampositive flora; MN = mixed gramnegative flora; All = total

^b Methods evaluated: Jolkkonen et al., Manoni et al., De Rosa et al., Flow cell analysis-linear discriminant analysis at 98% sensitivity (FCA-LDA98), FCA-LDA95 = at 95% sensitivity, FCA-LDA90 = at 90% sensitivity

^c Pos = SBU when urinary tract infection was defined as growth of $\geq 10^7$ colony forming units per liter at culture

relatively large differences in outcome between studies is probably due to different cut off's rather than differences between cohorts, which is supported in the present study where similar outcome was found for the present and De Rosa's screening model. Also at an optimal cut off we expect a similar outcome, unless one method is superior. If so, a universal cut off can be implicated which significantly enhances

implementation of the FCA screening method in clinic, but further studies are warranted.

Also, the delay from initiation until publication is a weakness but the distribution of uropathogens causing UTI is quite conserved over decades [2, 20, 28, 29, 32].

The present cohort may not reflect urine specimens from an average population but rather a selected cohort of specimens

Table 6 Outcome of the four screening methods of 1,312 urine specimens examined with flow cytometry analysis when UTI was defined as growth of $\geq 10^8$ colony forming units per liter in urine at culture

| Group ^a | Bacteria | SBU | | Methods evaluated ^b | | | | | | | | | | | | | | | | | | | |
|--------------------|-------------------------------------|-----|-----|--------------------------------|-----|-----|-----|-----|--------|-----|-----|---------|-----|-----|-----------------------|-----|-----|-----------------------|-----|-----|-----------------------|----|----|
| | | | | Jolkkonen | | | | | Manoni | | | De Rosa | | | FCA-LDA ₉₈ | | | FCA-LDA ₉₅ | | | FCA-LDA ₉₀ | | |
| | | | | Pos ^c | Neg | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC | SE | SP | NC |
| EC | <i>Escherichia coli</i> | 311 | 82 | 100 | 20 | 16 | 100 | 18 | 15 | 100 | 28 | 23 | 100 | 24 | 20 | 100 | 48 | 39 | 98 | 77 | 70 | | |
| KC | <i>Acinetobacter spp.</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 1 | 0 | 0 | 0 | 0 | 100 | 1 | 0 | 100 | 1 | 100 | 1 | |
| KC | <i>Citrobacter spp.</i> | 7 | 2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 50 | 1 | | |
| KC | <i>Citrobacter freundii</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| KC | <i>Enterobacter spp.</i> | 2 | 3 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | | |
| KC | <i>Other Klebsiella</i> | 1 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | 100 | 100 | 1 | | |
| KC | <i>Klebsiella oxytoca</i> | 9 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 1 | | |
| KC | <i>Klebsiella pneumoniae</i> | 20 | 13 | 95 | 54 | 8 | 100 | 46 | 6 | 100 | 51 | 7 | 95 | 54 | 8 | 95 | 62 | 9 | 95 | 62 | 9 | | |
| KC | <i>Pantoea spp.</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| KC | Aggregated | 41 | 21 | 98 | 43 | 10 | 100 | 38 | 8 | 100 | 48 | 10 | 98 | 43 | 10 | 98 | 52 | 12 | 98 | 62 | 14 | | |
| PR | <i>Morganella morganii</i> | 2 | 3 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 33 | 1 | 100 | 67 | 2 | 100 | 100 | 3 | | |
| PR | <i>Proteus mirabilis</i> | 7 | 5 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 20 | 1 | 86 | 0 | 1 | 86 | 20 | 2 | 86 | 40 | 3 | | |
| PR | <i>Proteus vulgaris</i> | 3 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| PR | <i>Providencia rettgeri</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| PR | Aggregated | 13 | 8 | 100 | 13 | 1 | 100 | 13 | 1 | 100 | 25 | 2 | 92 | 13 | 2 | 92 | 38 | 4 | 92 | 63 | 6 | | |
| PS | <i>Pseudomonas aeruginosa</i> | 11 | 0 | 91 | 0 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 91 | 0 | 1 | 91 | 0 | 1 | 91 | 0 | 1 | | |
| PS | <i>Other Pseudomonas spp.</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| PS | Aggregated | 11 | 1 | 91 | 0 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 91 | 0 | 1 | 91 | 0 | 1 | 91 | 0 | 1 | | |
| ST | CoNS | 10 | 7 | 100 | 0 | 0 | 100 | 14 | 1 | 100 | 14 | 1 | 100 | 14 | 1 | 100 | 14 | 1 | 100 | 43 | 3 | | |
| ST | <i>Staphylococcus aureus</i> | 6 | 4 | 100 | 25 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 25 | 1 | 83 | 100 | 5 | | |
| ST | <i>Staphylococcus saprophyticus</i> | 3 | 1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 67 | 0 | 1 | | |
| ST | Aggregated | 19 | 12 | 100 | 8 | 1 | 100 | 8 | 1 | 100 | 8 | 1 | 100 | 8 | 1 | 100 | 17 | 2 | 89 | 58 | 9 | | |
| EN | <i>Enterococcus faecalis</i> | 23 | 19 | 96 | 26 | 6 | 100 | 21 | 4 | 100 | 32 | 6 | 100 | 26 | 5 | 91 | 47 | 11 | 78 | 63 | 17 | | |
| EN | <i>Enterococcus faecium</i> | 4 | 5 | 100 | 40 | 2 | 100 | 20 | 1 | 100 | 40 | 2 | 100 | 40 | 2 | 100 | 60 | 3 | 75 | 100 | 6 | | |
| EN | Aggregated | 27 | 24 | 96 | 29 | 8 | 100 | 21 | 5 | 100 | 33 | 8 | 100 | 29 | 7 | 93 | 50 | 14 | 78 | 71 | 23 | | |
| SR | Alpha-hemolytic streptococci | 2 | 7 | 100 | 29 | 2 | 100 | 29 | 2 | 100 | 29 | 2 | 100 | 29 | 2 | 100 | 29 | 2 | 100 | 43 | 3 | | |
| SR | <i>Gemella haemolysans</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| SR | <i>Streptococcus</i> Group A | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 1 | | |
| SR | <i>Streptococcus</i> Group G | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 1 | 0 | 67 | 2 | | | |
| SR | <i>Streptococcus</i> Group C | 1 | 3 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 33 | 2 | | |
| SR | Aggregated | 4 | 14 | 100 | 14 | 2 | 100 | 14 | 2 | 100 | 14 | 2 | 100 | 14 | 2 | 100 | 21 | 3 | 75 | 50 | 8 | | |
| GB | <i>Streptococcus agalactiae</i> | 6 | 14 | 83 | 36 | 6 | 100 | 29 | 4 | 100 | 36 | 5 | 100 | 50 | 7 | 67 | 64 | 11 | 50 | 79 | 14 | | |
| OT | Diphtheroid rod | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| OT | <i>Haemophilus influenzae</i> | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| OT | Aggregated | 2 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | | |
| NE | Culture negative/no | 0 | 207 | 64 | 132 | 64 | 132 | 77 | 160 | 80 | 165 | 87 | 181 | 96 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| MP | Mixed flora Gram+ | 0 | 293 | 34 | 100 | 36 | 107 | 43 | 127 | 45 | 133 | 57 | 166 | 78 | 229 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| MN | Mixed flora Gram- | 2 | 200 | 100 | 31 | 62 | 100 | 28 | 55 | 100 | 41 | 82 | 100 | 37 | 73 | 100 | 59 | 119 | 100 | 80 | 160 | | |
| All | Total | 436 | 876 | 99 | 38 | 339 | 100 | 38 | 330 | 100 | 48 | 420 | 99 | 48 | 421 | 98 | 62 | 552 | 95 | 81 | 732 | | |

SBU significant bacteriuria, SE sensitivity, SP specificity, NC not cultured (i.e. specimens screened as culture negative), spp. species, CoNS coagulase negative staphylococci

^a Group = bacteria or bacterial groups. EC = *E. coli*; KC = *Klebsiella/Citrobacter* group; PR = *Proteus* group; PS = *Pseudomonas* group; ST = Staphylococcal group; SR = Streptococci group; GB = *Streptococcus agalactiae* (GBS); OT = other bacteria; NE = culture negative; MP = Mixed gram positive flora; MN = mixed gramnegative flora; All = total

^b Methods evaluated: Jolkkonen et al., Manoni et al., De Rosa et al., Flow cell analysis-linear discriminant analysis at 98% sensitivity (FCA-LDA98), FCA-LDA95 = at 95% sensitivity, FCA-LDA90 = at 90% sensitivity

^c Pos = SBU when urinary tract infection was defined as growth of $\geq 10^7$ colony forming units per liter at culture

from patients with suspected UTI, pyelonephritis and failure or control after treatment. However, the specimens are representative of the urine samples analyzed at our laboratory.

Flow cytometry is a promising method to identify and rule out culture negative urine specimens prior to culture [11–13, 15–17, 19–22, 31]. The present screening model can be recommended in clinic for pre-screening of UTI

specimens prior to culture to improve laboratory management of UTI specimens and service to clinicians. By an early identification of culture negative specimens, patients are excluded for unnecessary antimicrobial treatment and its associated side effects.

In summary, a new screening concept is presented based on LDA of FCA data that excludes 42% as culture negative

urine specimens prior to culture when UTI was defined according to European guidelines. The present LDA screening method was superior or similar to three conventional methods and is recommended for clinical use to reduce workloads, costs, turnaround time and time to diagnosis.

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Compliance with ethical standards

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Conflict of interest The authors declare no conflict of interest.

Ethics approval The article was conducted without participation of human or animals.

Informed consent This study did not involve human subjects or samples; therefore, no informed consent is required.

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