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UriSed 3 PRO automated microscope in screening bacteriuria at region-wide laboratory organization

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ABSTRACT

Background and aims: We assessed the possibility to rule out negative urine cultures by counting with UriSed 3 PRO (77 Elektronika, Hungary) at Helsinki and Uusimaa Hospital District.
Materials and methods: Bacteria counting of the UriSed 3 PRO automated microscope was verified with reference phase contrast microscopy against growth in culture. After acceptance into routine, results of bacteria and leukocyte counting from 56 426 specimens with eight UriSed 3 PRO instruments were compared against results from parallel samples cultured on chromogenic agar. Laboratory data including preanalytical details were accessed through the regional database of the Helsinki and Uusimaa Hospital District.
Results: A combined sensitivity of 87–92% and a negative predictive value of 90–96% with a specificity of 54–50% was reached, depending on criteria. Preanalytical data (incubation time in bladder) combined with the way of urine collection would improve these figures if reliable.
Conclusions: Complex patient populations, regional logistics and data interfases, and economics related to increased costs of additional particle counts against costs of screening cultures of all samples, did not support adaptation of a screening process of urine cultures. This conclusion was made locally, and may not be valid elsewhere.

1. Introduction

Rapid screening of bacteriuria has interested both clinical units and laboratories, since urinary tract infections (UTI) are prevalent in emergency patients, and laboratories are willingly improving their turn-around times and efficiency by avoiding unnecessary cultures of negative samples [1]. Application of flow cytometry to rule out negative bacterial cultures has been developed during the last 20 years [2–9]. Sufficient sensitivity for such a use is also claimed for some automated image analysers, including previous models of UriSed/sediMAX analysers [10–13]. Definition of a significant growth varies between 10^5–10^8 CFB/L (colony-forming bacteria/liter, equivalent to 10^2–10^5 CFU/mL)

in the criteria of the ECLM European urinalysis guidelines [14]. Also the American guideline for clinical trials on new anti-infective drugs for UTI recommends a combined approach in the interpretation of positive urine cultures from mid-stream samples, including clinical presentation (cystitis vs pyelonephritis), symptoms related to UTI such as dysuria, gender and age, as well as the types of isolated species or mixed growth [15]. Furthermore, economic factors of local health care system affect the outcome of any assessments of bacteriuria screening [16].

We verified the performance of a new model of UriSed automated microscope called UriSed 3 PRO (also sediMAX conTRUST PRO; 77 Elektronika, Budapest, Hungary) with phase-contrast images on urine particles [17] for routine particle analysis in HUSLAB regional

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laboratories. In addition to particle counting in laboratories of the Department of Clinical Chemistry, a total of eight UriSed 3 PRO instruments at Helsinki and Uusimaa regional laboratories were tested in their capability to rule out negative bacterial cultures at the Department of Clinical Microbiology of HUSLAB, Helsinki, for a possibility to screening about 310 000 urine cultures annually.

2. Materials and methods

2.1. Urine samples

Parallel urine specimens, one for particle analysis and the other for bacterial culture, were collected into 10 ml boric acid, sodium formate and borate preservative tubes (C&S, BD Preanalytical Solutions, Eysins, Switzerland, cat no 364955), and transported regionally from patients' homes, primary care sites, or regional hospital units to the nearest regional laboratory, or to the Meilahti central laboratory at room temperature (with temperature loggers inside the boxes that were located within the warmed spaces of the vans). Particle analysis was carried out within 8 h from sample collection [18], and bacterial cultures were initiated within 24 h from collection.

2.2. Automated microscopy with UriSed 3 PRO

The automated UriSed 3 PRO has been previously described [17]. The model 3 PRO with improved phase contrast optics was first launched in April 2018, and HUSLAB received its instruments among the first customers (eight for Helsinki and Uusimaa Hospital District and two for Kymenlaakso Hospital District, not included in this study). Bacteria (BAC) are not counted individually by UriSed 3 PRO, but by software assessing details in the 15 grids of 960×1280 pixels. In that sense, BAC count is an arbitrary value related to concentration of bacteria-like particles. That is why we wanted to verify the performance of the first UriSed 3 PRO instrument ("Gold") against growth in culture, and compare those figures to reference microscopy of limited number of urine samples against culture, before taking into routine use.

After verification, local flagging limits (=autoverification rules) were adopted for routine particle counting to solve uncertainties or review flags provided by the instrument in about 30% of HUSLAB routine samples (see Supplementary material A for detail of the HUSLAB flagging limits). The eight UriSed 3 PRO were verified and taken into routine use with a software version 4.1.29 after modifications in data transfer. Results of region-wide samples of this study were taken from patient database, i.e., after reviews by human operators and releases to laboratory information system in HUSLAB laboratories. In internal quality control, commercial control solutions (KOVA®Liqua-Trol™ with Microscopics; Kova International, Amsterdam, The Netherlands) were used. Day-to-day repeatability of RBC counts was 8.4% at 350 RBC $\times 10^6$/L and 15.7% at 55 RBC $\times 10^6$/L using the control material. The corresponding figures for WBC counts were 8.4% at 211 $\times 10^6$/L and 15.2 at 48 WBC $\times 10^6$/L. Imprecision targets included consideration of theoretical Poisson distributions of low counts. Linearity was verified for natural RBC up to 2000 $\times 10^6$/L and for WBC up to 3600 $\times 10^6$/L with $R^2 = 1.00$. No carry-over was demonstrated to RBC, WBC or BAC counts.

Laboratory report included white blood cells (WBC), red blood cells (RBC), bacteria (BAC), squamous epithelial cells (SEC), non-squamous epithelial cells (NEC), and casts (CAST, combining hyaline, HYA, and pathological, PAT casts), while other particle classes provided by the instrument were used for flagging limits only. Ordinal scale agreements with weighted kappa coefficients were the following: WBC 0.94, RBC 0.89, SEC 0.88, CAST 0.59 and NEC 0.49. Agreement of BAC counts with reference procedures is reported in this paper.

2.3. Visual microscopy

In primary verification for laboratories in Helsinki and Uusimaa

Hospital District, automated results of the first UriSed 3 PRO instrument (called "Gold") were verified against visual microscopy. The other seven instruments (called "Silver" instruments) were verified sequentially against the first one using parallel samples. The reference microscopy with phase contrast optics was performed by four authors, each sample counted only once and by one author, but the interobserver variability of the experienced microscopists was controlled before the study, especially with regard to bacteria quantitation. We used a modified ISLH reference procedure with disposable 1 μL Bürker chambers (Cell Vision Technologies, SB Heerhugowaard, The Netherlands; product code CV-1000-B) and a modification from the ISLH reference procedure [19]: a maximum of 1 μL of uncentrifuged, unstained urine sample was counted after resuspension, independently of particle counts. As a novelty, quantitative estimates on bacteria counts were obtained by using either B or D squares of the Bürker chamber with 1/160 μL or 1/4000 μL volume, respectively (see Supplementary material B for detail). Two bacteria in B square corresponded to 320 bacteria/μL (limit of a "positive" result), and two bacteria in D square corresponded to 8000 bacteria/μL (limit of an "abundant" result).

2.4. Urine bacterial cultures

Parallel specimens were available for bacterial culture at the Department of Clinical Microbiology. The urine samples were cultured by using automated inoculation with 1 μL loop (WASP® automated microbiology instrument, COPAN Wasp, Brescia, Italy) on chromogenic agar plates (CHROMagar™ Orientation medium, RT413-25, CHROMagar, Paris, France), following incubation at 35 °C for 18 h. Limit of significant growth was defined as (1) generally at 10^7 CFB/L (SI unit, corresponding to 10^4 CFU/mL) or more, and 1–2 identified species of common urinary pathogens or mixed growth, independently of type of collection, based on the current practice, or (2) experimentally, using other definitions of significant growth, and provisionally a short incubation time (less than 4 hours) in bladder as a sign of dysuria for midstream urine (MSU) specimens, as provided by the electronic requests. In those MSU specimens, a significant growth was additionally defined as 10^6 CFB/L (SI unit, corresponding to 10^3 CFU/mL) or more with 1–2 identified species reflecting the ECLM European urinalysis guidelines [14]. Mixed growth was treated either as negative or positive result as indicated in the text or tables.

After the primary verification of bacteria counting of the Gold instrument with 396 of samples (see Supplementary material B) in September and October 2018, the other seven "Silver" instruments were verified against it with parallel samples before clinical use by March 2019. The district-wide evaluation of performance of bacteria screening was carried out within the Helsinki and Uusimaa Hospital District by comparing 56 426 reported routine results (after reviews of the operators based on created flagging limits) from eight UriSed 3 PRO instruments with parallel bacterial culture results from April to September 2019. Samples with no parallel results or no quantitative counts (e.g., crowded sample, or too much amorphous precipitate) from either procedure were excluded.

2.5. Data analysis

Data were collected from different analyzers and combined on Microsoft Excel spreadsheets. Sample IDs were used to combine the results from parallel samples and the preanalytical data of the encrypted patients at the IT department of the Helsinki and Uusimaa Hospital District. Statistics from Analyze-It® software (Analyse-it Software Ltd., England) was used as a module of Microsoft Excel spreadsheets as needed.

Non-parametric Spearman's correlation coefficients were calculated for quantitative counts (WBC and RBC). Ordinal scale agreement was assessed by weighted Cohen's kappa coefficients from cross-tabulated classified data.

The large regional database with encrypted personal IDs was requested and constructed by utilizing the comprehensive database of the Helsinki and Uusimaa Hospital district (including laboratory data), out of approximately 310 000 annually cultured samples. This allowed comparisons of patients' demographic data, ways of sample collection, bladder incubation times, and particle counting results with those from screening cultures and identified microbial species. Automated formulas on Excel spreadsheets were created to calculate sensitivities and specificities against culture growth, with different thresholds of UriSed 3 PRO counts for WBC and bacteria.

Results were received from 4935 children (less than 15 years of age, 61.4% girls) and 51 591 adults (15–100 years of age, 51.7% women). The specimens were sent from hospital emergency rooms in 30%, hospital departments in 45%, and from various primary care sites in 25% of cases. The way of collection was explicitly reported in the database for 13 630 samples (24.2% of all cases). Of those samples, midstream urine (MSU) collection constituted 76.5%, single catheterization 10.7% and indwelling catheter 8.8% of cases. From the reported 1007 pediatric samples, 80.3% were MSU collections, 9.6% bag urine and 5.8% spontaneously voided infant specimens. Bladder puncture specimens were excluded from the study.

3. Results

3.1. Verification of UriSed 3 PRO bacteria counting

Detailed performance of UriSed 3 PRO bacteria counting was not extensively studied when we received that instrument model in 2018. As a necessary step before clinical use, the performance of the first UriSed 3 PRO instrument ("Gold") bacteria counting was assessed against urine bacterial cultures. In addition, a parallel study was performed with a developed reference visual microscopy in a Bürker chamber with different patient samples (see Supplementary material B). At a ruling-out limit of 80×10^6/L of BAC counts, UriSed 3 PRO reached a sensitivity of 90% with a specificity of 39% against culture, and at a ruling-in limit of 800×10^6/L, a specificity of 96% with a sensitivity of 24% against culture. The performance shown by a receiver-operating characteristic (ROC) curve was remarkably similar to that obtained by visual reference microscopy with phase contrast optics (see the Supplementary material B). The other seven UriSed 3 PRO instruments ("Silver" instruments) in HUSLAB laboratories of Helsinki and Uusimaa Hospital District were assessed then against the primary instrument (Gold) before clinical use.

3.2. Comparison of multiple UriSed 3 PRO instruments against bacterial culture

The capability of UriSed 3 PRO to screen bacteriuria from clinically reported samples was assessed using 56 426 specimens with parallel requests and tubes for urine particle analysis and bacterial culture, and quantitative results from both assays. Urine cultures resulted in 29 279 (51.9%) samples with no growth, 16 055 (28.5%) samples with mixed growth, and 11 092 (19.7%) samples with identified species (Table 1) and antimicrobial susceptibility testing. The patient populations from emergency rooms, specialized and primary health care had a 55% occurrence of *E. coli* isolates.

True positive (TP) or false positive (FP) fractions against bacterial culture were calculated separately for each category of culture growth at each limiting BAC count of UriSed 3 PRO (Fig. 1). In this way, it was possible to see the impact of increasing colony counts 10^6 – 10^8 CFB/L (or 10^3 – 10^5 CFU/mL) and types of growth (1–2 identified species, or mixed growth) on detection of bacteria. When counting unselectively bacteria-like particles, the specificity (rate of FP cases) against selective urine cultures becomes important. The shaded zone of 60–80 BAC $\times$ 10^6/L delineated the range where FP rate from negative cultures reached the limit of a useful test of about 50% (FP rate increased from

Table 1
Identified species from urine cultures. In cases with two identified species, the more important uropathogen was taken into the list.

|	N	%
Escherichia coli	6092	54.9%
Enterococcus faecalis	815	7.3%
Klebsiella pneumoniae	743	6.7%
Staphylococcus. not sapr. not aureus	523	4.7%
Pseudomonas aeruginosa	310	2.8%
Gram positive cocci. not specified	269	2.4%
Streptococcus agalactiae	240	2.2%
Klebsiella oxytoca	199	1.8%
Proteus mirabilis	192	1.7%
Lactobacillus spp.	180	1.6%
Citrobacter spp.	164	1.5%
Enterobacter cloacae	138	1.2%
Staphylococcus aureus	122	1.1%
Staphylococcus saprophyticus	112	1.0%
Enterococcus faecium	107	1.0%
Streptococcus viridans spp.	96	0.9%
Citrobacter freundii	95	0.9%
Aerococcus urinae	86	0.8%
Enterobacter aerogenes	66	0.6%
Morganella morganii	55	0.5%
Candida albicans	51	0.5%
Gram negative coliform rods	35	0.3%
Serratia marcescens	34	0.3%
Enterobacter spp.	30	0.3%
Proteus vulgaris	29	0.3%
Staphylococcus lugdunensis	28	0.3%
Raoultella ornithinolytica	25	0.2%
Gram negative rods. not specified	22	0.2%
Klebsiella spp.	19	0.2%
Acinetobacter spp.	18	0.2%
Aerococcus sanguinicola	17	0.2%
Others	180	1.6%
TOTAL amount of specimens	11,092	100%
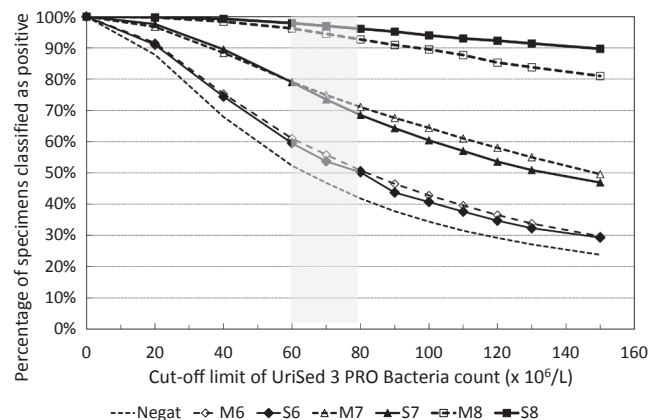
Fig. 1. Diagnostic performance of UriSed 3 PRO Bacteria (BAC) counting against urine bacterial culture (n = 56 426 specimens). Cumulative percentage of true positives (sensitivity) at each limiting BAC count against culture is shown for each category of positive growth, and that of false positives (1-specificity) against negative culture. Culture results were converted to a SI unit colony-forming bacteria/liter (CFB/L) by adding three exponentials, e.g., 10^7 CFB/L = 10^4 CFU/mL. Labels (and the corresponding numbers of specimens) of each category of growth were as follows: Negative culture, Negat (29 279 specimens), M6 = 10^6 CFB/L Mixed growth (9 139), S6 = 10^6 CFB/L with 1–2 Species identified (622), M7 = 10^7 CFB/L Mixed growth (5 701), S7 = 10^7 CFB/L with Species identified (2 202), M8 = 10^8 CFB/L Mixed growth (1 215), and S8 = 10^8 CFB/L with Species identified (8 268 specimens). The limiting zone for specificity ≥50% with UriSed 3 PRO BAC at 60–80 × 10^6/L is marked with a grey shading.
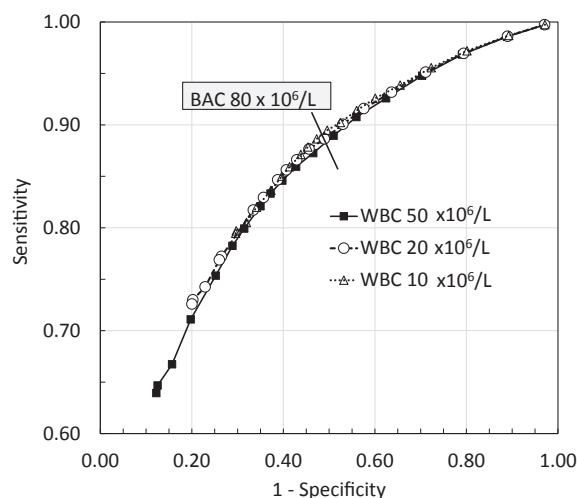
Fig. 2. Performance of UriSed 3 PRO combined BAC and WBC counts to detect significant growth in culture, defined as 10^7 CFB/L or more colonies of identified species or mixed growth, according to the local practice. Out of the 56 426 urine samples, 16.8 % grew at 10^8 CFB/L and 14.0% at 10^7 CFB/L or more in culture, as combined from the caption of Fig. 1. The receiver-operating characteristic (ROC) curves were focused around the sensitivity of 90%. The ROC curves show diagnostic performance of various BAC counts at the limiting WBC concentrations of 10, 20 or 50 $\times 10^6$/L. The cut-off point with a specificity of 50% at BAC 80 $\times 10^6$/L is shown.

42% to 52%) (Fig. 1). The colony count used to define a positive result in culture had a major role in defining the sensitivity (TP fraction) by UriSed 3 PRO, while almost no difference was observed in sensitivities between cultures with identified species and those with mixed growth (Fig. 1). With bacterial growth at 10^8 CFB/L (both identified species and mixed growth), a combined sensitivity of 93–97% was reached by using a BAC cut-off of 70–80 $\times 10^6$/L. A stepwise reduction of sensitivity was seen down to 69–75% at 10^7 CFB/L, and to 50–56% at 10^6 CFB/L with a similar specificity when each category of growth was assessed separately (Fig. 1).

Detection of bacteriuria might be improved by using both WBC and BAC counts in screening. Detection of bacterial growth at 10^7 CFB/L (10^4 CFU/mL) or more, including mixed growth, succeeded with UriSed

3 PRO with a sensitivity of 64% and specificity of 85% at a cut-off of 30 WBC $\times 10^6$/L alone, by UriSed 3 PRO. A WBC count of 15 $\times 10^6$/L was the limit of quantitation of patient samples with UriSed 3 PRO (unpublished results). At the highest cut-off of 10^8 CFB/L (10^5 CFU/mL) in culture, a sensitivity of 82% with a specificity of 80% was obtained at 30 WBC $\times 10^6$/L by UriSed 3 PRO. The sensitivity of WBC to detect bacteriuria at 10^6 CFB/L (10^3 CFU/ml) was 75%, i.e., better than that at 10^7 CFB/L, when cultures with mixed growth were ignored. The detail at different cut-offs of WBC counts at 20–50 $\times 10^6$/L alone, and definitions of positive growth is shown in the Supplementary material C.

3.3. Screening of bacteriuria with combined UriSed 3 PRO results

An optimum combination to rule out negative bacterial cultures was sought by repeated calculations with automated Excel formulas at variable cut-off limits of BAC and WBC counts, using results from 56 426 regional samples. The performance of UriSed 3 PRO against all growth at 10^7 CFB/L (the current practice) or more is shown in a set ROC curves, modified by WBC counts (Fig. 2). It is seen that WBC counts had almost no impact in the outcome of performance. To guarantee a specificity >50%, the following UriSed 3 PRO limits were selected for performance assessment for regional practice: WBC 50 $\times 10^6$/L and BAC 80 $\times 10^6$/L.

The definition of significant bacteriuria was varied from the current local practice, yielding into different sensitivities (Table 2). By using the chosen UriSed 3 PRO limits, an estimate of 41% of routine samples (23 087/ 56 426) could be ruled out from urine cultures, and 59% would be cultured after counting. At HUSLAB, this would mean 127 000 samples sorted out of 310 000 annual routine cultures, leaving 183 000 samples for culture. A sensitivity of 87% with the Panel A (defining all growth significant at 10^7 CFB/L or more) could be increased to 92% by using the Panel B (all growth at 10^8 CFB/L significant, but samples with lower CFB at 10^6/L or 10^7/L defined significant if specific species were identified only). This would reduce the number of false negative (FN) samples from 2214 to 980 cases and lead into a NPV of 96%. Further improvement of sensitivity of bacteriuria screening up to 98%, and NPV up to 99% would be seen by classifying only the cultures at 10^8 CFB/L as significant (Panels C-D), with no major difference in classification of samples with mixed growth (Table 2). Thus, the sensitivity increased from 87% to 98%, while the specificity fell from 54% to 48% with different criteria. Negative predictive value (NPV) increased concomitantly from 90% to 99%. The highest thresholds were calculated for technical comparison

Table 2
Diagnostic performance of UriSed 3 PRO combined BAC and WBC counts in detecting bacteriuria by using different definitions of significant growth (A-D). Cut-off limits of positive UriSed 3 PRO counts were BAC 80 $\times 10^6$/L and WBC 50 $\times 10^6$/L. NPV and PPV = negative and positive predictive values.

A. Significant growth 10^7 CFB/L or more, including mixed growth				B. Significant growth 10^6 CFB/L or more with identified species plus mixed growth at 10^8 CFB/L			
UriSed3PRO	POSIT	Culture NEGAT	Total	POSIT	Culture NEGAT	Total	
Posit	**15,172**	**18,167**	33,339	**11,327**	**22,012**	33,339	
Negat	**2214**	**20,873**	23,087	**980**	**22,107**	23,087	
Grand Total	17,386	39,040	56,426	12,307	44,119	56,426	
Prevalence	30.8%			21.8%			
Sensitivity	87.3%	NPV	90.4%	92.0%	NPV	95.8%	
Specificity	53.5%	PPV	45.5%	50.1%	PPV	34.0%	

C. Significant growth 10^8 CFB/L or more, including mixed growth				D. Significant growth 10^8 CFB/L or more with identified species only			
UriSed3PRO	POSIT	Culture NEGAT	Total	POSIT	Culture NEGAT	Total	
Posit	**9248**	**24,091**	33,339	**8102**	**25,237**	33,339	
Negat	**235**	**22,852**	23,087	**166**	**22,921**	23,087	
Grand Total	9483	46,943	56,426	8268	48,158	56,426	
Prevalence	16.8%			14.7%			
Sensitivity	97.5%	NPV	99.0%	98.0%	NPV	99.3%	
Specificity	48.7%	PPV	27.7%	47.6%	PPV	24.3%	

only as they do not comply with the European and American guidelines for significant growth.

Additionally, several guidelines point out the importance of clinical symptoms of UTI and preclinical detail of urine specimens in assessing significance of bacteriuria. We modelled symptoms of patients by using the available bladder incubation times in electronic laboratory requests to classify urgency. For MSU samples, a lower level at 10^6 CFB/L should be considered significant growth if patients suffered from dysuria (short incubation time in bladder), while a higher level of 10^8 CFB/L is applicable for patients with no urgency symptoms (reflected by an incubation time at least for 4 h) [14]. From the 980 FN cases (Panel B in Table 2), all MSU specimens with information on a bladder incubation time for 4 h or more (absence of urgency) were reclassified as true negatives if the growth in culture was less than 10^8 CFB/L. By this criterion, the amount of FN cases was reduced to 532 patients, and NPV was increased from 96% to 98%.

3.4. Prediction of mixed growth by squamous epithelial cell (SEC) counts

High concentrations of SEC in urine have been suggested to predict non-significant result in culture. Distributions of SEC counts in the available 39 035 MSU specimens (when no coded information was assumed to represent a MSU collection) with different categories of growth is shown in Fig. 3. Among MSU samples with negative culture result, the 95% upper reference limit was 9 SEC $\times 10^6$/L. With increasing CFB/L in culture, the 95th percentile of SEC counts increased up to 55 SEC $\times 10^6$/L in samples with mixed growth at 10^8 CFB/L. The 90th percentile in the last group was 40 SEC $\times 10^6$/L (marked in Fig. 3). SEC at 40 SEC $\times 10^6$/L or more were seen in 1.2% of the MSU samples. These were divided as follows: 160 in 2531 specimens with mixed growth (6.3% sensitivity to predict mixed growth), 279 /7619 (3.7%) in specimens with identified species, and 208/28,885 (0.7%) in samples that remained negative in culture. The positive predictive value (PPV) of the SEC cut-off at 40 $\times 10^6$/L in indicating mixed growth was only 24.7%. Using a cut-off of 60 SEC $\times 10^6$/L, a sensitivity of 2.7% to detect mixed cultures was obtained, with a PPV still at 25.5% only (detail not shown).
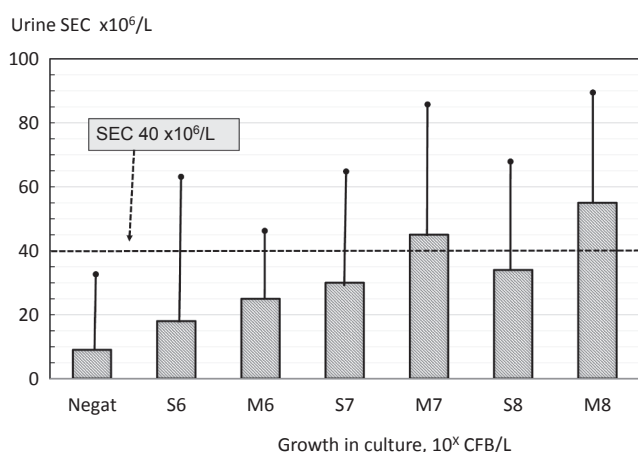

Fig. 3. Value of squamous epithelial cells (SEC) in predicting mixed growth in culture assessed from available mid-stream urine (MSU) specimens (n = 39 035). The bars show 95th percentiles and the whiskers 99th percentiles of urine SEC counts in each category of growth. The number of MSU specimens in each group was as follows (given in brackets): Negat = Negative culture (28 885), S6 = 10^6 CFB/L with Species identified (504), M6 = 10^6 CFB/L Mixed growth (465), S7 = 10^7 CFB/L with Species identified (1 635), M7 = 10^7 CFB/L Mixed growth (1 367), S8 = 10^8 CFB/L with Species identified (5480), and M8 = 10^8 CFB/L Mixed growth (699 specimens).

4. Discussion

4.1. Analytical assessment

In the verification, the installed UriSed 3 PRO instruments fulfilled the quality specifications of trueness and precision for counting of urine RBC, WBC, and SEC [27]. Improved detection of bacteria was developed in visual microscopy, by using the D squares in Bürker chambers (1/4000 μL), and peer practicing to reach reproducible counts among observers (Supplementary material B). A sensitivity of 89% as compared to 10^7 CFB/L in culture exceeds microscopy in most routine laboratories. A similar sensitivity and specificity against urine cultures was reached with phase contrast optics of UriSed 3 PRO, which was important in confirming the accuracy of pixel-based BAC counts of the instrument. The flagging limits (Supplementary material A) adapted for routine counting with UriSed 3 PRO included visual confirmation of all abundant BAC counts (800 or more $\times 10^6$/L), and improved bacteria counting of samples with amorphous precipitates by dilution of specimens, by using on-screen reviews of automated images, and occasional visual microscopy as needed.

The performance of BAC parameter of the eight UriSed 3 PRO instruments in screening for bacteriuria was tested with laboratory data of 56 426 samples from the region-wide patient database of Helsinki and Uusimaa Hospital District. The identified bacterial species was *E. coli* in 55% of 11,092 samples (Table 1). In combined definitions of significant growth, the fraction of samples with low colony counts, and classification of mixed growth into significant or non-significant have a major impact in the observed sensitivity of screening against urine culture. When testing the growth categories separately, we reached a sensitivity of 93–97% at 10^8 CFB/L, a sensitivity of 69–75% at 10^7 CFB/L, or a sensitivity of 50–56% at 10^6 CFB/L with a similar specificity of about 50–55% by UriSed 3 PRO BAC counts (Fig. 1). In addition to sensitivity, details of the measurement principle in a particle analyzer set the frame for specificity of bacteria detection achieved against cultures that are carried out on selective media.

Antimicrobial treatment might have affected specificity of BAC counting, but we were not able to evaluate the magnitude of that effect. In Finland, there is a general guidance to obtain a sample for urine culture before an empiric treatment, but information on current antimicrobial treatment is rarely recorded in the electronic requests. That is why samples with antimicrobial treatment could not be studied separately, to see the role of dead bacteria on the performance.

At the highest threshold of significant growth at 10^8 CFB/L (both identified species and mixed growth from Fig. 1), a sensitivity of 96% with a specificity of 52% was reached with UriSed 3 PRO by using the BAC channel alone. As a comparison, the latest flow cytometer technology of Sysmex UF-5000 has provided a sensitivity of 99% with a specificity of 80% at the same cut-off limit of growth, using samples from hospital patients [8]. A stepwise reduction of sensitivity was seen with lower CFB/L limits, as shown also for UF-5000 [20]. Our primary definition for a significant bacteriuria was the current practice with routine screening of all growth at 10^7 CFB/L or more. By the BAC channel alone, bacteriuria at that limit was shown with a sensitivity of 87% and a specificity of 51%.

Leukocyte detection has traditionally been used to screen for bacteriuria with test strips or microscopy. A sensitivity of 58–69% with a specificity of 89–81% was seen by using WBC counts at a range of 20–50 $\times 10^6$/L against any growth at 10^7 CFB/L or more in culture (Supplementary material C). UriSed BAC counts have been shown to be more sensitive than WBC counts in detecting bacteriuria also in another study of patients at a university hospital [12]. BAC and WBC counts analyzed with the Sysmex UF-500i flow cytometer at a general hospital have shown similar results [21]. Large centralized laboratories seem to receive specimens from a wide range of clinical situations, including differential diagnostics of various symptoms. The insensitivity of WBC counts in detecting bacteriuria is not associated with capability to count

WBC that are most easily counted by all particle analyzers. Also UriSed 3 PRO reached a sensitivity of 95% with a specificity of 90% at 30 WBC $\times$ 10^6/L, or a sensitivity of 92% with a specificity of 77% at 10 WBC $\times$ 10^6/L against our reference microscopy [27]. The apparent insensitivity of WBC is associated with lack of pyuria among samples sent to urine bacterial culture. Foudraine *et al.* have shown that in an academic center almost half of the samples may be requested from patients not suspected for UTI [22].

A combined performance of BAC and WBC counts is usually of value when maximizing the sensitivity of automated particle counting in bacteriuria screening. In our collection of regional results, the ROC curves with various WBC concentrations did not, however, show any clear difference in the sensitivity or specificity of bacteriuria detection with our definition of significant growth (Fig. 2). The ROC curves also showed how the achieved sensitivity is obtained with loss of specificity against culture. With the ROC curve, we could compare performance of UriSed 3 PRO with that of an older version of the UriSed instrument: The current version provided a sensitivity of about 72% with a specificity of 80%, as compared with a sensitivity of 56% and a specificity of 81% in 2013, although those authors do not reveal the size of the fraction of low-colony counts included in their study [23]. Optimized screening with BAC counts at 80 $\times$ 10^6/L and WBC counts at 50 $\times$ 10^6/L by UriSed 3 PRO would result in a sensitivity of 87% and NPV 90% (Panel A in Table 2) that is similar to the values without WBC counts. A sensitivity of 92% and NPV of 96% at a prevalence of 22% of positive cases was reached at 10^6 CFB/L or more by including samples with identified species only, and also mixed growth at the highest level of 10^8 CFB/L (Panel B of Table 2). Compared to the current practice (Panel A), the verified sensitivity was considered insufficient, because more than 10% of cultures with significant growth remained negative. Additionally, about 50% of isolates at the lowest 10^6 CFB/L category (including 622 samples with identified species) would have remained undetected if screened with the UriSed 3 PRO (Fig. 1), creating a need of special requisition and laboratory processes for clinically important suspicions of low-colony count bacteriuria if applied. The highest cut-off of 10^8 CFB/L with a sensitivity of 98% and NPV of 99% (Panels C and D in Table 2) were calculated for technical comparisons against other instruments. Mixed growth in culture is suggested to represent borderline significance for high-risk patients who may not be able to provide non-contaminated samples in all clinical situations, despite being considered non-significant in the assessment of new drugs for UTI [15].

Elimination of samples with high SEC counts from the assessment of bacteriuria has been suggested to improve the diagnostic performance of automated counting [24], since those samples are considered to represent urine from external genitalia rather than MSU, and to associate with mixed growth. Among the 39 035 MSU samples of this study, the SEC counts of different categories of growth were highly overlapping, resulting in a PPV of only 25% for SEC at 40 $\times$ 10^6/L or higher in predicting mixed growth, with a sensitivity of 6% (Fig. 3). The PPV did not improve at SEC 60 $\times$ 10^6/L. Thus, high SEC counts did not predict mixed culture results. A similar result has been obtained also in another study with retrospective data on 19 300 samples [25]. Samples with high SEC counts may still indicate that the observed growth is not representing bladder urine independently of the outcome.

Preanalytical information should be utilized to reduce the number of irrelevant clinical interpretations, despite challenges of high-quality evidence [26]. When the way of collection (MSU) and time of urine in bladder (with a cut-off of 4 h for absence of urgency) were reliably included in the classification of significant growth, the false negative rate at low CFB/L samples could be reduced. Consequently, the NPV at 10^6 CFB/L (without mixed cultures) improved from 96% to 98%, with the specificity remaining still at around 50% (Table 2, panel B). Iñigo *et al.* [12] demonstrated a sensitivity of 96% and NPV 98% with a specificity of 63% for SediMAX (UriSed) against growth of identified species at a cut-off of 10^5 CFB/L (10^2 CFU/mL) in their population of hospital patients, by using additional clinical data suggestive of UTI. As

a comparison, a sensitivity of 98% with a specificity of 52% was obtained with Sysmex UF-1000i flow cytometer, optimizing criteria for significant growth based on the European guidelines in a Swedish tertiary-level regional laboratory service in Umeå [7]. Age and sex-specific cut-off limits of counts have resulted in a sensitivity up to 97% with a specificity of 78% with a Sysmex 500i analyzer, using tailored definitions of positive culture in a second-level regional Finnish setting in Lahti [6]. The obtained specificity is highly dependent on the health care environment and is affected by patients and types of samples used in the assessments. Consequently, the Finnish criteria from Lahti provided a specificity of 78% in Lahti, but a specificity of 41% only, when applied in the Umeå laboratory [7].

4.2. Economical assessment

In process planning, a new screening step should be sensitive enough to be applicable for most samples, sufficiently specific to result in exclusion of most true negative cases, and cheap enough to justify additional costs associated with a new step. Furthermore, costs of the changes including those of computer interfaces and resources needed for customer training must be considered.

In urine cultures, samples from specific patient groups or those from specific ways of collection should be excluded from the screening process. After that, most clinicians probably accept a general sensitivity or NPV of 95% or higher for screening of urine samples from non-immunocompromised individuals. We reached this level of NPV with UriSed 3 PRO by ignoring samples with mixed growth at 10^7 CFB/L (Table 2, Panel B), but not against the current definition of significant growth (Table 2, Panel A). The following detail on economical outcomes remained then provisional, e.g., for other laboratories considering economy in their environments.

Specificity of screening offers savings that must be calculated against costs of a new screening step. If the screening test is already used for other purposes, the transitional costs for combining the screening test to urine cultures must be calculated. A diagnostic sensitivity of 90–95% with a specificity of 50–60% may work as a theoretical starting point for calculations.

With a specificity of about 50%, we estimated to save about 40% of samples from culture work (Table 2). If half of samples was saved from the culture, the limiting cost of rational screening is 50% of the cost of main test, to remain equal to the situation without a screening step, because the screening is applied to all samples. The cost of bacteriuria screening (either by particle counting or test strip measurement) should be less than 50% of the cost of the culture because of various added costs into the process, to be economically viable. The ratio becomes more favorable if a test for bacteriuria screening is already done for other purposes. At HUSLAB service, the 310 000 annual urine cultures are currently being requested directly, independently of the 150 000 particle counts, and about 300 000 urine test strip measurements requested from the laboratories. In our case, a systematic bacteriuria screening by particle counting with the provided specificity was deemed to create extra costs both because of the current test requisition practice and because screening cultures from urine are not twice as costly to us as a new particle counting step. This differs from the situation in Sweden, where the relative cost of culture was estimated to be 5 times higher than that of flow cytometric screening, thus supporting the adaptation of the screening process in the laboratory [7]. Similarly, a Turkish comparison estimated 3 times higher unit costs in culture than in flow cytometry [16].

5. Conclusions

Application of the eight UriSed 3 PRO particle analyzers provided a combined sensitivity of 87% and NPV of 90% only, with a specificity of 53% against the current definition of significant growth at 10^7 CFB/L, including mixed growth. The performance could be theoretically

improved to NPV of 96–98% with a specificity of about 50% by ignoring mixed growth at low colony counts, and some midstream samples based on provisional preanalytical information. Despite successful adaptation of UriSed 3 PRO in large-scale particle counting at HUSLAB laboratories, the bacteriuria screening was not deemed economically satisfactory because of direct costs of the tests in question and indirect costs related to the complex healthcare environment and specific logistical needs. Instead, saving in patient care remained to be anticipated from clinical advice to order urine cultures and other urine tests with proper clinical presentation only, because of the high prevalence of bacteriuria with mixed growth. High-quality particle counting with UriSed 3 PRO was installed and is already available for clinicians needing rapid diagnostics in emergency cases. Our conclusion on bacteria screening with UriSed 3 PRO is not applicable to other laboratories with less complex laboratory and health care environments, lower sample loads, different patient populations, or with different unit costs in their urine tests.

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CRediT authorship contribution statement

Timo Kouri: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Tanja Holma:** Validation, Formal analysis, Investigation, Writing - review & editing. **Vesa Kirjavainen:** Validation, Formal analysis, Data curation, Writing - review & editing. **Anna Lempiäinen:** Methodology, Investigation, Validation, Writing - review & editing. **Katariina Alagrund:** Methodology, Investigation, Validation, Resources, Writing - review & editing. **Niina Tohmola:** Investigation, Validation, Resources, Project administration, Writing - review & editing. **Tero Pihlajamaa:** Investigation, Validation, Resources, Writing - review & editing. **Vesa-Petteri Kouri:** Investigation, Validation, Formal analysis, Writing - review & editing. **Maaret Lehtonen:** Investigation, Methodology, Validation, Resources, Writing - review & editing. **Sirpa Friman:** Investigation, Validation, Resources, Writing - review & editing. **Anu Pätäri-Sampo:** Conceptualization, Methodology, Writing - review & editing, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. Supplementary data A-C

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