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Critically Appraised Topic**Performance of Sysmex UF-4000 research parameters in
predicting urine culture outcomes**

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CLINICAL BOTTOM LINE

This study evaluated the clinical and diagnostic value of Sysmex UF-4000 research parameters for predicting urine culture outcomes. The bacteria (BACT) count was evaluated for its added value within the existing white blood cells (WBC)-based workflow rather than as a standalone screening strategy. Among urine samples with pyuria ($n = 7,905$), defined as ≥ 25 WBC/ μL , the BACT count showed considerable discriminative ability for predicting culture-positive samples (area under the curve (AUC) = 0.845; 95% confidence interval (CI): 0.833–0.856). The optimal Youden index cut-off was 166 BACT/ μL . A BACT cut-off of 15 BACT/ μL achieved 95.2% sensitivity and could potentially reduce cultures by 8.2% among samples meeting the current WBC-based criterion. When only cultures with confirmed uropathogens were considered positive, excluding contamination and mixed flora, a cut-off of 19 BACT/ μL maintained 95.0% sensitivity and could potentially reduce cultures by 10.2%. However, among the samples that would have been excluded from culture at these cut-offs, 141 of 651 (21.7%) and 181 of 808 (22.4%), respectively, contained a confirmed uropathogen and would therefore have been missed. These missed samples represented 3.9% and 5.0% of all confirmed uropathogen-positive samples at the 15 and 19 BACT/ μL cut-offs, respectively.

The Sysmex UF-4000 BACT-Info flag demonstrated moderate agreement with culture-based bacterial classification (Cohen's $\kappa = 0.484$). Gram-negative (GN) classification yielded 69.3% sensitivity and 84.8% specificity, while Gram-positive (GP) classification yielded 88.1% sensitivity and 77.9% specificity. Although apparent GP classification performance was higher, this was strongly influenced by the heterogeneous composition of the GP culture group, consistent with findings reported in previous studies. When restricted to confirmed GP uropathogens, the positive predictive value (PPV) was only 18.9%.

Overall, incorporating a rule-out BACT count cut-off into the existing WBC-based screening algorithm may provide added value by potentially reducing culture workload but carries a risk of missing uropathogen-positive samples. The BACT-Info flag may provide early Gram orientation but cannot replace culture for definitive bacterial identification or therapeutic decision-making. Further prospective validation of both applications is required before implementation in routine clinical practice.

CLINICAL/DIAGNOSTIC SCENARIO

Urinary tract infections (UTIs) represent one of the most frequently encountered infectious diseases in both community and hospital settings. They account for a substantial proportion of antibiotic prescriptions and laboratory diagnostic requests. In routine clinical practice, urine culture is considered the gold standard method for confirming or excluding a UTI and identifying the causative pathogen. However, despite its central role, urine culture is associated with important limitations that increasingly challenge its efficiency and clinical utility (1–14).

A major challenge in daily practice is the high volume of urine samples submitted for culture, of which a large proportion ultimately yield no growth or findings considered clinically insignificant, such as contamination, rather than true infection. Nevertheless, each sample requires labour-intensive processing, incubation, interpretation, and reporting, resulting in substantial workload for microbiology laboratories and additional costs. Furthermore, the inherent time required for culture-based diagnostics leads to long turnaround times, delaying definitive microbiological results for clinicians. This may prompt the initiation of empirical antibiotic therapy, which may later prove unnecessary or suboptimal once culture results become available, thereby contributing to inappropriate antibiotic use and antimicrobial resistance (1–15).

To address these challenges, clinical laboratories have increasingly implemented automated urine flow cytometry (UFC) as a rapid screening tool. Modern UFC systems, such as the Sysmex UF-4000/5000 (Sysmex Corporation, Kobe, Japan), provide quantitative information on urinary particles, including BACT and WBC counts within minutes, allowing laboratories to rapidly differentiate between specimens with a high and low probability of infection and potentially reduce the number of unnecessary urine cultures. Numerous studies have proposed cut-off values for BACT and WBC counts, typically determined using receiver operating characteristic (ROC) curve analysis. These cut-offs generally provide a high negative predictive value (NPV), making them particularly useful for excluding UTIs and optimizing laboratory workflow. However, reported cut-offs vary considerably between studies, depending on patient population, definitions of significant bacteriuria, and local laboratory practices (1–16).

Beyond quantitative screening, the Sysmex UF-4000/5000 also provides qualitative information through the BACT-Info flag, which automatically classifies detected bacterial populations as GN, GP, mixed Gram-positive/Gram-negative (GP/GN), or unclassified based on differences in bacterial size, fluorescence intensity, and light-scattering characteristics measured by flow cytometry. This functionality was introduced to provide early etiological orientation, potentially supporting timely clinical decision-making and more targeted empirical antibiotic therapy while culture results are pending. From a clinical perspective, rapid discrimination between GN and GP pathogens could be highly valuable, as their empirical treatment strategies differ (1–18).

Despite these potential advantages, the clinical utility of the BACT-Info flag in routine practice remains uncertain. While UFC-based bacterial quantification generally provides good discrimination between culture-positive and culture-negative urine samples, the performance of the BACT-Info flag varies considerably between studies and bacterial classifications. Overall, the GN flag shows more consistent diagnostic performance, with several studies reporting high concordance with culture alongside high sensitivity and PPV. In contrast, GP classification is more variable and generally associated with lower PPVs, despite sometimes achieving high sensitivity (10). This lower PPV appears to be strongly influenced by contamination and mixed flora, as GP organisms may represent commensal skin or perineal flora frequently encountered in contaminated and mixed urine cultures. In addition, bacterial characteristics affecting fluorescence uptake and light scattering may contribute to difficulties in distinguishing GP organisms by flow cytometry. Therefore, uncertainty persists regarding the reliability and clinical interpretation of BACT-Info flag results, whether they should be reported to clinicians, and their added value in routine clinical decision-making (1,3,4,6,7,9,10,14).

Against this background, the current diagnostic strategy in many laboratories remains conservative. UFC BACT counts are generally used as a screening tool to decide whether to perform culture, whereas the BACT-Info flag classification is either not reported to clinicians or used only cautiously. Importantly, most published evaluations of the Sysmex UF-4000/5000 BACT-Info flag have been conducted in selected patient populations or controlled study settings, while fewer data are available from large cohorts of routine clinical specimens processed under real-world laboratory conditions. Furthermore, substantial variation in screening cut-offs, patient populations, culture definitions, and laboratory protocols limits the direct applicability of published findings to individual laboratories. Local validation is therefore essential to determine the diagnostic performance and practical value of UFC-based screening and bacterial classification of UTIs.

In this context, the present study addressed two key research questions in our local setting and evaluated the diagnostic performance and potential added value of the Sysmex UF-4000 using both a literature review and a retrospective analysis of routine urine samples processed in our clinical laboratory. The literature review focused on the clinical utility and diagnostic performance of the BACT-Info flag, while the retrospective analysis evaluated the diagnostic performance of both the BACT count and the BACT-Info flag classification. First, the diagnostic performance of the BACT count for predicting positive versus negative urine culture results was evaluated, with the aim of establishing an optimal locally validated cut-off. Second, the clinical and diagnostic value of the BACT-Info flag for bacterial classification was assessed. This included evaluating its clinical utility for early bacterial classification based on the available literature and its diagnostic performance in our routine clinical practice using culture-based bacterial classification as the reference standard. By combining evidence from the literature with real-world laboratory data, this study aims to clarify the role of the Sysmex UF-4000 as both a screening tool and a potential aid in bacterial classification. Ultimately, the results are intended to support evidence-based decisions regarding workflow optimization, clinical interpretation of UFC data, and result reporting in daily laboratory practice.

QUESTIONS

- 1) What is the added value of incorporating the Sysmex UF-4000 BACT count into the current WBC-based urine culture screening algorithm for the detection of culture-positive urine samples?
- 2) What is the clinical and analytical value of the UF-4000 BACT-Info flag for bacterial classification?
 - a. What is the clinical utility of the BACT-Info flag for early bacterial classification in suspected UTI, based on the available literature?
 - b. What is the diagnostic performance of the Sysmex UF-4000 BACT-Info flag in routine clinical practice, using culture-based results as the reference standard?

SEARCH TERMS

Question 2) What is the clinical and analytical value of the UF 4000 BACT-Info flag for bacterial classification?

- a. What is the clinical utility of the BACT-Info flag for early bacterial classification in suspected UTI, based on the available literature?

The literature search strategy was developed by combining three concepts: the UFC instrument, the BACT-Info flag, and the clinical application. Synonyms within each concept were combined using the Boolean operator OR, whereas the three concepts were combined using the Boolean operator AND.

Search concept	Search terms
UFC instrument	UF4000 OR "UF-4000" OR "UF 4000" OR UF4000i OR "UF-4000i" OR "UF 4000i" OR UF5000 OR "UF-5000" OR "UF 5000" OR UF5000i OR "UF-5000i" OR "UF 5000i" OR "Sysmex flow cytometer" OR "urine flow cytometry" OR "urinary flow cytometry"
BACT-Info flag	"BACT-info" OR "BACT flag" OR "bacterial information flag" OR "Gram flag" OR "Gram-flag" OR "Gram-negative?" OR "Gram negative?" OR "Gram-positive?" OR "Gram positive?" OR "Gram-negative flag" OR "Gram-positive flag" OR "Gram negative flag" OR "Gram positive flag" OR "bacterial morphology flag" OR "bacterial scatter" OR "bacterial discrimination" OR "Gram orientation"
Clinical application	Urine OR urinary OR urinalysis OR bacteriuria OR "urine culture" OR "urinary tract infection" OR UTI
Final search string	(UFC instrument) AND (BACT-Info flag) AND (clinical application)

Abbreviations: UFC, urine flow cytometry; UTI, urinary tract infection.

LIST OF ABBREVIATIONS

Abbreviation	Full term
Aspy	Antimicrobial susceptibility testing only if pyuria is present
AST	Antimicrobial susceptibility testing
AUC	Area under the curve
BACT	Bacteria
CFU	Colony-forming units
CI	Confidence interval
dAST	Direct antimicrobial susceptibility testing
ESBL	Extended-spectrum β -lactamase
FN	False-negative
FP	False-positive
FSC	Forward scattered light
GN	Gram-negative
GP	Gram-positive
GP/GN	Mixed Gram-positive/Gram-negative
ID	Identification
IQR	Interquartile range
IUC	Urine from an indwelling urinary catheter
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
Mid	Midstream urine
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NPV	Negative predictive value
NR	Not reported
OXA-48	Oxacillinase-48
PPV	Positive predictive value
REMIC	Référentiel en microbiologie médicale
ROC	Receiver operating characteristic
SE	Sensitivity
SFL	Side fluorescence light
SP	Specificity
SSC	Side scattered light
TN	True-negative
TP	True-positive
UFC	Urine flow cytometry
UGF	Urogenital flora
UP	Uropathogen(s)
UTI	Urinary tract infection
VIM	Verona integron-encoded metallo- β -lactamase
VRE	Vancomycin-resistant enterococci
WBC	White blood cells
YLC	Yeast-like cells

RELEVANT EVIDENCE/REFERENCES

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APPRAISAL**Question 1) What is the added value of incorporating the Sysmex UF-4000 BACT count into the current WBC-based urine culture screening algorithm for the detection of culture-positive urine samples?****Rationale for BACT count evaluation**

The current WBC-based screening approach (WBC count ≥ 25 WBC/ μ L) provides an initial triage step for urine culture requests, but WBC counts alone may not optimally discriminate between culture-positive and culture-negative samples. Bacterial growth may occur in the absence of pronounced pyuria, whereas pyuria may also be present without clinically relevant bacteriuria (9,13).

The Sysmex UF-4000 detects and quantifies bacteria using fluorescence flow cytometry in the dedicated CR-BACT channel. After staining with fluorescent dyes targeting nucleic acid components and bacterial surface structures, particles are hydrodynamically focused and passed individually through a 488 nm blue semiconductor laser beam. The resulting optical signals, including forward scattered light (FSC), side scattered light (SSC), and side fluorescence light (SFL), are measured for each particle. FSC mainly reflects particle size and refractive properties, SSC relates to internal complexity, and SFL reflects the degree of fluorescence staining. Based on these optical characteristics, bacterial particles are distinguished from debris and other urinary elements, allowing an accurate and quantitative BACT count to be generated. The bacterial population can be visualised in a dedicated scattergram, where classification algorithms analyse the distribution pattern of the detected events (1,11,17).

Previous studies have demonstrated promising diagnostic performance of the BACT count, measured by automated UFC, for discriminating between culture-positive and culture-negative urine specimens (10). As the BACT count provides a more direct measure of bacterial particles in urine, its incorporation into the current screening algorithm may provide additional diagnostic information beyond the WBC count alone.

The present study therefore aimed to determine an optimal BACT count threshold for predicting culture positivity and to assess whether incorporating a rule-out BACT count cut-off could further reduce the number of urine cultures while maintaining high sensitivity for culture-positive samples. The BACT count was evaluated as an additional screening parameter within the existing WBC-based triage approach, rather than as a standalone screening criterion for UTI.

Study population

During the study period, from 1 October 2025 until 31 May 2026, 26,343 urine sample records were identified in the laboratory database at Ziekenhuis Oost-Limburg, a supraregional hospital in Belgium. After removal of duplicate records corresponding to the same urine sample ($n = 5,547$), 20,796 unique urine samples remained. Samples without an available BACT count, for which no urine culture was performed, or with a WBC count < 25 WBC/ μ L were excluded (total $n = 12,891$). The final study population consisted of 7,905 urine samples with an available BACT count, urine culture results, and a WBC count ≥ 25 WBC/ μ L, which were included in the diagnostic performance analysis (see Figure 1).

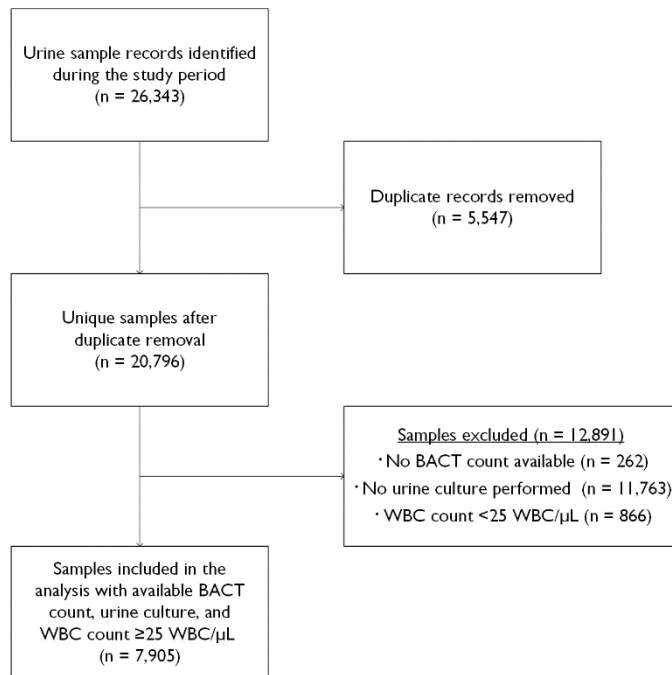


Figure 1: Flowchart of study sample selection.

Urine samples submitted for microbiological analysis were processed according to a standardized reading algorithm to ensure accurate detection and identification of urinary tract pathogens (see Attachment 1).

The study dataset included both urine specimens for which culture was requested irrespective of the WBC count ($n = 772$; 9.8%) and specimens that were selected for culture based on the predefined WBC-based screening criterion of ≥ 25 WBC/ μL ($n = 7,133$; 90.2%). This reflects the current real-world laboratory workflow, in which both types of culture requests occur.

The corresponding study population had a median age of 66 years (interquartile range (IQR): 35–79 years). Females accounted for the majority of the study population ($n = 5,242$; 66.3%). Most samples consisted of midstream urine ($n = 6,531$; 82.6%). The remaining samples originated from different collection methods, including urine from an indwelling urinary catheter ($n = 452$; 5.7%), urine after catheterization ($n = 384$; 4.9%), urine of unknown origin ($n = 294$; 3.7%), urine collected in a urine bag ($n = 230$; 2.9%), and urine obtained by suprapubic puncture ($n = 14$; 0.2%).

The study population comprised 7,905 urine samples, of which 1,006 (12.7%) yielded negative culture results, 3,299 (41.7%) were classified as contaminated, and 3,600 (45.5%) contained at least one uropathogen. Among culture-positive samples, all identified uropathogens were recorded, including multiple organisms from polymicrobial cultures, resulting in 4,551 uropathogen occurrences (see Attachment 2). *Escherichia coli* was the most frequently identified uropathogen ($n = 1,513$; 33.25%), followed by *Enterococcus faecalis* ($n = 752$; 16.52%), *Klebsiella pneumoniae/variicola* ($n = 313$; 6.88%), and *Streptococcus agalactiae* ($n = 300$; 6.59%). Antimicrobial-resistant organisms were relatively uncommon, with extended-spectrum β -lactamase-producing *E. coli* being the most frequently detected resistant organism ($n = 108$; 2.37%), whereas oxacillinase-48-producing Enterobacterales, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and Verona integron-encoded metallo- β -lactamase-producing *Pseudomonas aeruginosa* were rare.

ROC curve analysis

ROC curve analysis was performed to evaluate the diagnostic performance of the BACT count obtained by the Sysmex UF-4000 for predicting negative and positive urine culture results. Data management, statistical analyses, and graphical visualizations were performed using Microsoft Excel for Microsoft 365, version 16.112 (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS Statistics, version 30.0.0.0 (IBM Corporation, Armonk, NY, USA).

In the present study, the BACT count demonstrated considerable overall discriminative ability, with an AUC of 0.845 (95% CI: 0.833–0.856) (19). When contamination was classified as culture positive, the optimal cut-off identified by the Youden index was 166 BACT/ μ L. At this threshold, the UF-4000 classified 5,057 samples as positive and 2,848 as negative. The corresponding classification yielded 4,891 true positives (TP), 166 false positives (FP), 840 true negatives (TN), and 2,008 false negatives (FN), resulting in a sensitivity of 70.9%, specificity of 83.5%, PPV of 96.7%, and NPV of 29.5% (see Figure 2, Tables 1 and 2).

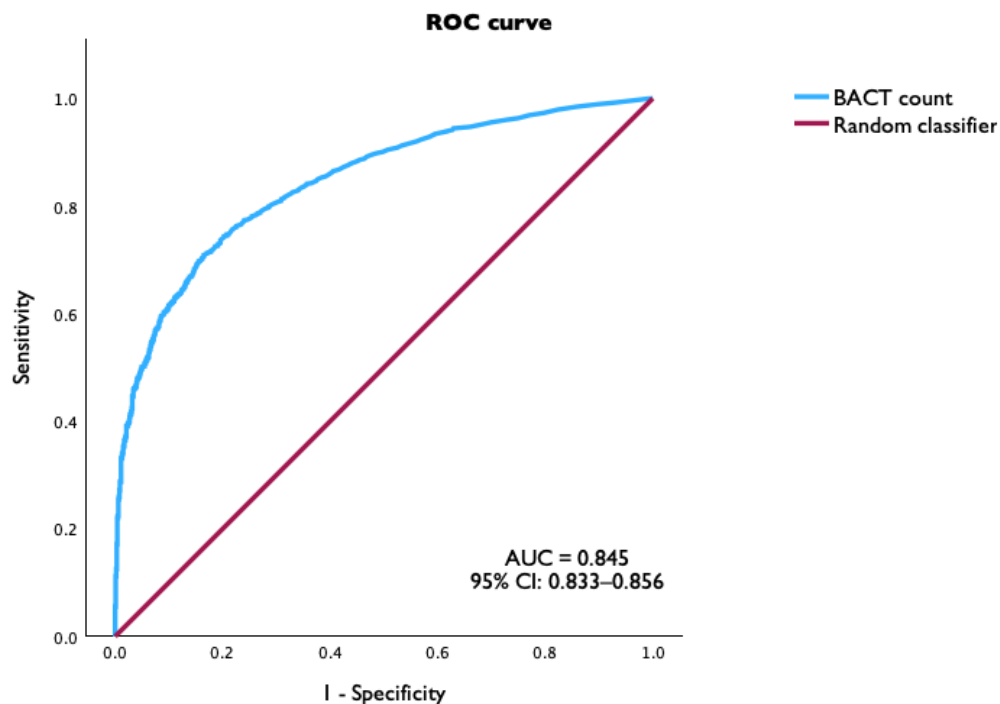


Figure 2: ROC curve analysis for the BACT count using Sysmex UF-4000.

Table 1: Diagnostic performance of the BACT cut-off (166/ μ L) compared to urine culture.

		Culture			Total
		Negative	Contamination	Positive	
Cut-off (166 BACT/ μ L)	Negative	840 (10.6%)	1,199 (15.2%)	809 (10.2%)	2,848 (36.0%)
	Positive	166 (2.1%)	2,100 (26.6%)	2,791 (35.3%)	5,057 (64.0%)
Total		1,006 (12.7%)	3,299 (41.7%)	3,600 (45.5%)	7,905 (100%)

Of the 2,008 FN samples identified using the predefined culture classification (culture-positive samples including both contamination/mixed flora and confirmed uropathogen-positive cultures), 1,199 samples (59.7%) were classified as contamination/mixed flora. This indicates that the relatively high number of FN results should be interpreted with caution, as a considerable proportion did not contain a confirmed uropathogen. Contamination/mixed bacterial growth may represent a less clinically relevant group, as it does not necessarily reflect a UTI requiring antimicrobial treatment. Consequently, when contamination/mixed flora was considered culture negative and only confirmed uropathogen-positive cultures were considered

culture positive, 1,199 of the 2,008 initially classified FN samples were reclassified as TN, while 2,100 samples previously classified as TP were reclassified as FP. Under this definition, the sensitivity, specificity, PPV, and NPV of the BACT count at the 166 BACT/ μ L cut-off were 77.5%, 47.4%, 55.2%, and 71.6%, respectively (see Table 2).

Given that a considerable proportion of FN samples consisted of contamination/mixed flora, additional analyses were performed to investigate whether alternative definitions of culture positivity would affect the diagnostic performance of the UF-4000 BACT count. Two definitions of the reference standard were considered: 1) contamination/mixed flora classified as culture positive and 2) contamination/mixed flora classified as culture negative. For each reference standard definition, three BACT count thresholds were evaluated: the cut-off corresponding to approximately 95% sensitivity, the optimal cut-off according to the Youden index, and the cut-off corresponding to approximately 95% specificity. The resulting diagnostic performance measures are summarized in Table 2.

Overall, these findings demonstrate that the diagnostic performance of the BACT count is strongly influenced by the definition of culture positivity, particularly the classification of contamination/mixed flora. The most appropriate cut-off therefore depends on the intended clinical application, with lower cut-offs favouring sensitive detection of culture-positive samples and higher cut-offs favouring specificity, which may allow a reduction in unnecessary culture testing.

Table 2: Diagnostic performance of different BACT count cut-offs using two reference standard definitions for urine culture positivity.

Contamination/mixed flora = positive		TP	TN	FP	FN	SE	SP	PPV	NPV
Cut-off (BACT/ μ L)	15	6,567	319	687	332	95.2%	31.7%	90.5%	49.0%
	166	4,891	840	166	2,008	70.9%	83.5%	96.7%	29.5%
	798	3,450	956	50	3,449	50.0%	95.0%	98.6%	21.7%
Contamination/mixed flora = negative		TP	TN	FP	FN	SE	SP	PPV	NPV
Cut-off (BACT/ μ L)	19	3,419	627	3,678	181	95.0%	14.6%	48.2%	77.6%
	166	2,791	2,039	2,266	809	77.5%	47.4%	55.2%	71.6%
	1,828	1,972	3,525	780	1,628	54.8%	81.9%	71.7%	68.4%
	12,638	1,198	4,088	217	2,402	33.3%	95.0%	84.7%	63.0%

Abbreviations: FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; SE, sensitivity; SP, specificity; TN, true negative; TP, true positive.

To assess the added value of the BACT count on top of the existing WBC-based screening criterion, a cut-off of 15 BACT/ μ L was selected based on a sensitivity of 95.2% when contamination/mixed flora was considered culture positive. At this cut-off, 651 samples (8.2%) had a BACT count below 15 BACT/ μ L and could potentially have been additionally excluded from urine culture testing. Of these, 319 samples (49.0%) were culture-negative, 191 (29.3%) showed contamination/mixed flora, and 141 (21.7%) yielded confirmed uropathogens. Overall, 332 of 6,899 culture-positive samples (4.8%) would have been missed at this cut-off. Of these 332 false-negative samples, 141 contained a confirmed uropathogen, representing 3.9% of all 3,600 confirmed uropathogen-positive samples. Thus, applying a cut-off of 15 BACT/ μ L could potentially reduce the number of cultures by 8.2% among samples already selected based on the WBC criterion, while maintaining a sensitivity of 95.2%.

When contamination/mixed flora was considered culture negative and only confirmed uropathogen-positive cultures were classified as culture positive, a cut-off of 19 BACT/ μ L corresponded to a sensitivity of 95.0%. At this cut-off, 808 samples (10.2%) had a BACT count below 19 BACT/ μ L. Of these, 386 (47.8%) were culture-negative, 241 (29.8%) showed

contamination/mixed flora, and 181 (22.4%) yielded confirmed uropathogens. These 181 false-negative samples represented 5.0% of all 3,600 confirmed uropathogen-positive samples. Therefore, applying a cut-off of 19 BACT/ μ L could potentially reduce the number of cultures by 10.2% among samples already selected based on the WBC criterion, while maintaining a sensitivity of 95.0%.

Overall, incorporation of the BACT count into the existing WBC-based screening approach showed potential to further reduce the number of urine cultures performed. However, 21.7% and 22.4% of the samples that would have been excluded from culture at the respective cut-offs contained a confirmed uropathogen, corresponding to 3.9% and 5.0% of all confirmed uropathogen-positive samples. A limitation of the present study is its single-center, retrospective design, which may limit the generalizability of the findings. In particular, the optimal BACT cut-off may depend on the patient population, culture practices, and local laboratory workflow and therefore may not be directly applicable to other clinical laboratories. In addition, the potential reduction in urine cultures was estimated retrospectively and was not prospectively validated in routine practice. Therefore, the BACT count may provide additional value beyond the current WBC-based screening criterion, but its potential use as an additional rule-out criterion requires careful consideration of the balance between culture reduction and missed uropathogen-positive samples, including their clinical relevance and characteristics. Further prospective validation is warranted before implementation in routine practice.

Question 2) What is the clinical and analytical value of the UF-4000 BACT-Info flag for bacterial classification?**a. What is the clinical utility of the BACT-Info flag for early bacterial classification in suspected UTI, based on the available literature?**

A structured literature search (see Figure 3, Attachment 3) was performed to identify published studies evaluating UFC-based bacterial classification, with particular focus on the Sysmex UF-4000/5000 BACT-Info flag. The search was conducted in PubMed (MEDLINE), and retrieved records were screened by title and abstract for relevance to UFC-based screening of UTIs and bacterial classification using the BACT-Info flag. Studies were eligible for inclusion if they evaluated the Sysmex UF-4000 and/or UF-5000 analyser and investigated the clinical utility or diagnostic performance of the BACT-Info flag, with full-text articles available for review. No restriction on publication year was applied to capture both early validation studies and recent evaluations of Sysmex UFC analysers. Studies published up to 31 May 2026 were included. Full-text articles were subsequently assessed for eligibility, and the reference lists of included studies were manually screened to identify additional relevant publications not retrieved by the initial search. The findings of the literature review were used to contextualize and interpret the results of the retrospective analysis of our routine laboratory data.

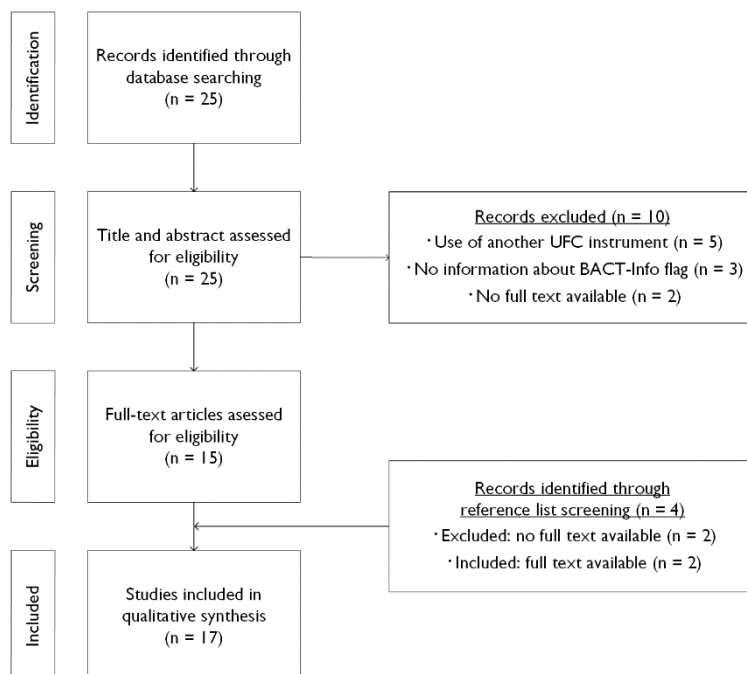


Figure 3: Flowchart of study selection for the literature review.

In addition to bacterial quantification, the Sysmex UF-4000 provides the BACT-Info flag, which predicts the Gram classification of the bacterial population in the sample. This prediction is based on differences in light scatter and fluorescence signals measured by flow cytometry, which reflect structural differences between bacterial cell types. GP bacteria possess a thicker peptidoglycan layer, which is associated with increased FSC and reduced dye penetration, leading to lower SFL. In contrast, GN bacteria have a thinner peptidoglycan layer and an additional outer membrane, which are associated with lower FSC and greater dye penetration, resulting in higher SFL and stronger SSC. These differences in light scatter and fluorescence characteristics are used by the instrument to distinguish between GP and GN bacterial populations. By evaluating the distribution of bacterial events within the scattergram, the analyser classifies the sample as “Gram negative?”, “Gram positive?”, “Gram pos/neg?”, or “unclassified”. Although not intended to replace culture, the BACT-Info flag provides rapid information that may support early clinical decision-making and potentially more targeted empirical antimicrobial therapy (7,11,14,17).

This literature review showed that, although UFC performs well as a screening tool for predicting urine culture positivity, the diagnostic performance of bacterial classification by the UF-4000/5000 differs substantially between GN and GP bacteria.

Overall, the GN flag showed more consistent diagnostic performance than the GP flag, with reported PPVs generally being high and several studies reporting values above 90% (1,3–8,10,14,15,20,21).

In contrast, the performance of the GP BACT-Info flag was considerably more variable and generally less specific. Although sensitivities were sometimes relatively high, PPVs were frequently substantially lower than those observed for GN classification. These findings indicate that GP classification is less consistent across study populations and methodological approaches than GN classification (3–5,7,8,10,14,15). The lower PPV of the GP flag appears to be strongly influenced by contamination and mixed flora. Several studies reported a substantial proportion of contamination or mixed flora among samples classified as GP or mixed by the BACT-Info flag. Contamination may frequently involve GP organisms, as skin and perineal flora such as *Lactobacillus* spp., coagulase-negative staphylococci, and streptococci are common contaminants in urine cultures. Gilboe et al., for example, found that more than half of GP-flagged samples showed contamination or mixed growth. Similarly, Zhang et al. found that contaminated cultures were significantly associated with GP and mixed BACT-Info flags, while no significant association was observed for GN flags. These findings suggest that the GP flag may frequently detect GP organisms that are present in the specimen but do not represent a clinically relevant GP uropathogen. In addition to contamination, intrinsic microbiological characteristics of GP bacteria may contribute to differences in flow cytometric discrimination. These include thicker peptidoglycan cell walls and altered fluorochrome uptake, both of which can affect light scatter and fluorescence signals (1,3,4,6,7,9,10,14).

In addition to diagnostic performance, agreement between BACT-Info flag classification and culture-based bacterial classification has been evaluated in several studies. Overall, agreement was generally higher for GN than for GP classification. Cohen's kappa values ranged from fair to almost perfect agreement across studies, with GP classification generally showing lower agreement than GN classification. For example, Enko et al. reported substantial agreement for GN classification ($\kappa = 0.78$) but only fair agreement for GP classification ($\kappa = 0.40$). In contrast, Liu et al. reported relatively high concordance for both GN and GP classification (90.3% and 86.8%, respectively), illustrating that performance may vary considerably depending on the study population, bacterial load threshold, culture definition, and handling of contamination/mixed cultures. The recent meta-analysis by Chang et al., including 18 studies and 25,337 urine samples, reported a pooled Cohen's kappa of 0.52, corresponding to moderate agreement, and supported better overall performance for GN than for GP classification (1,2,4,6–8,10–13,15,16,18,20,22,23).

Taken together, the literature supports the potential clinical utility of the BACT-Info flag primarily as a rapid orientation tool, particularly for identifying likely GN bacteria. The generally higher PPV and more consistent agreement observed for GN classification suggest that a GN flag may provide clinically useful information before culture results become available. In contrast, the GP flag should be interpreted more cautiously because of its lower and more variable PPV and its strong association with contamination and mixed flora. Therefore, the BACT-Info flag may complement, but cannot replace, conventional urine culture for definitive bacterial identification and antimicrobial susceptibility-guided treatment. The present retrospective study further evaluates whether these findings are reproducible under routine clinical laboratory conditions using the UF-4000 and examines the extent to which contamination and mixed flora contribute to apparent BACT-Info flag misclassification.

b. What is the diagnostic performance of the Sysmex UF-4000 BACT-Info flag in routine clinical practice, using culture-based results as the reference standard?

To assess the agreement between the BACT-Info flag classification and the culture-based classification, the analysis was restricted to samples with a BACT count ≥ 166 BACT/ μ L, corresponding to the optimal cut-off determined by the Youden index in the ROC analysis. Samples below this threshold were excluded from the concordance analysis, as the BACT-Info flag classification is primarily intended for samples with a sufficient bacterial load. Samples with a negative urine culture were retained, as the Sysmex UF-4000 generated a BACT-Info flag despite the absence of clinically significant bacterial growth. Consequently, these cases were considered discordant when evaluating agreement between the BACT-Info flag and the culture-based classification.

Among the 7,905 samples in the dataset, 5,057 (64.0%) had a BACT count ≥ 166 BACT/ μ L. These samples were classified according to the BACT-Info flag of the Sysmex UF-4000 as GN, GP, mixed, or unclassified, and these classifications were compared with the corresponding culture-based classifications (negative, GN, GP, or mixed). For culture-based classification, samples containing a confirmed uropathogen were classified according to the detected uropathogen(s), while the presence of contamination/mixed flora was disregarded when a uropathogen was identified. When only contamination/mixed flora was detected, the sample was categorized according to the bacterial species identified within the contamination/mixed flora.

Within this subset, based on culture, 1,617 (32.0%) were classified as GN, 2,230 (44.1%) were classified as GP, and 1,044 (20.6%) had a mixed culture result. An additional 166 samples (3.3%) had a negative culture (see Table 3). Importantly, the GP category did not exclusively consist of cultures containing a confirmed GP uropathogen. Of the 2,230 samples classified as GP, only 602 (27.0%) contained a confirmed GP uropathogen, whereas 1,628 (73.0%) contained contamination/mixed flora without a confirmed uropathogen. These samples were classified as GP because the organisms identified in the contamination/mixed flora were exclusively GP organisms. Thus, the relatively large GP culture category was predominantly composed of samples without a confirmed GP uropathogen.

Of the 5,057 included samples, 1,121 of 1,617 GN cultures (69.3%) were correctly classified as GN by the BACT-Info flag, while 1,964 of 2,230 GP cultures (88.1%) were correctly identified. Among mixed cultures, 305 of 1,044 samples (29.2%) were correctly classified as mixed, whereas 409 (39.2%) and 326 (31.2%) were classified as GN and GP, respectively. Forty-five samples (0.9%) received an unclassified BACT-Info flag result. Overall, 3,390 of 5,057 samples (67.0%) showed concordant classification between the BACT-Info flag and the culture-based classification, whereas 1,667 samples (33.0%) were discordantly classified (see Table 3). The agreement between the two classification methods was moderate, with a Cohen's kappa of 0.484 (standard error = 0.009, $p < 0.001$), indicating agreement beyond chance (24).

Table 3: Comparison of the BACT-Info flag classification with the culture-based classification for samples with a BACT count ≥ 166 BACT/ μ L.

		Culture				Total
		Negative	GN	GP	Mixed	
BACT-Info flag	Unclassified	2	7	32	4	45
	GN	31	1,121	83	409	1,644
	GP	120	179	1,964	326	2,589
	Mixed	13	310	151	305	779
Total		166	1,617	2,230	1,044	5,057

Abbreviations: GN, Gram-negative; GP, Gram-positive.

To further quantify the diagnostic performance of the BACT-Info flag, the GN and GP classifications were evaluated separately as binary outcomes. For the identification of GN cultures, the BACT-Info flag showed a sensitivity of 69.3% and a specificity of 84.8%, with a PPV of 68.2% and a NPV of 85.5%. For GP cultures, sensitivity was higher at 88.1%, while specificity was 77.9%. The corresponding PPV and NPV were 75.9% and 89.2%, respectively (see Table 4).

Table 4: Diagnostic performance of the BACT-Info flag.

BACT-Info flag	TP	TN	FP	FN	SE	SP	PPV	NPV
GN	1,121	2,917	523	496	69.3%	84.8%	68.2%	85.5%
GP (all GP cultures)	1,964	2,202	625	266	88.1%	77.9%	75.9%	89.2%
GP (confirmed GP uropathogens only)	489	2,355	2,100	113	81.2%	52.9%	18.9%	95.4%

Abbreviations: FN, false negative; FP, false positive; GN, Gram-negative; GP, Gram-positive; NPV, negative predictive value; PPV, positive predictive value; SE, sensitivity; SP, specificity; TN, true negative; TP, true positive.

Interestingly, the apparent classification performance was higher for GP than for GN bacteria, with a higher sensitivity for GP than for GN classification (88.1% vs. 69.3%). This contrasts with the generally better performance reported for GN classification in previous studies. However, the two culture-based groups differed substantially in composition. Of the 1,617 GN cultures, only 6 (0.4%) contained contamination/mixed flora without a confirmed GN uropathogen, indicating that the GN group predominantly represented cultures with a clinically relevant GN uropathogen. In contrast, among the 2,230 GP cultures, only 602 (27.0%) contained a confirmed GP uropathogen, whereas 1,628 (73.0%) contained contamination/mixed flora without a confirmed uropathogen. In addition, 129 (5.8%) of the GP-classified cultures contained at least one yeast species, including samples in which yeasts occurred in combination with GP bacteria. Thus, the higher apparent agreement for GP classification should be interpreted with caution, as it may partly reflect the composition of the culture-based GP category rather than superior classification performance for clinically relevant GP uropathogens.

When the analysis of the GP group was restricted to the 602 samples containing a confirmed GP uropathogen, 489 samples (81.2%) were correctly classified as GP, while 71 (11.8%) were classified as mixed, 34 (5.6%) as GN, and 8 (1.3%) as unclassified. The corresponding sensitivity, specificity, PPV, and NPV were 81.2%, 52.9%, 18.9%, and 95.4%, respectively (see Table 4). Restricting the analysis to confirmed GP uropathogens therefore resulted in a lower sensitivity (81.2% vs. 88.1%) and, particularly, a markedly lower PPV (18.9% vs. 75.9%) compared with the complete GP culture-based category. This finding indicates that the high apparent agreement observed for the overall GP culture-based classification was strongly influenced by samples containing GP contamination or mixed flora without a confirmed GP uropathogen. This is consistent with the literature, in which GP classification is generally more variable and more strongly affected by contamination and mixed flora.

Additional analyses were performed to further assess the relationship between the UF-4000 bacterial classifications and culture findings. To further characterize the discordant classifications, culture results were evaluated for the presence of contamination/mixed flora. Among the 1,667 discordant samples, 1,025 (61.5%) contained contamination/mixed flora, either alone or in combination with a uropathogen, whereas 642 samples (38.5%) showed no evidence of contamination/mixed flora. However, contamination/mixed flora was also common among concordantly classified samples, occurring in 2,517 of 3,390 cases (74.2%). Therefore, the presence of contamination/mixed flora alone is insufficient to explain all observed discordances.

Nevertheless, in cultures containing both a uropathogen and contaminating organisms, such as *E. coli* together with mixed urogenital flora, a UF-4000 classification as “mixed” may reflect the presence of multiple bacterial populations within the sample. Among the 310 samples classified as mixed by the UF-4000 but assigned to the GN culture-based classification, 176 samples (56.8%) also contained contamination/mixed flora. Similarly, among the 151 samples classified as mixed by the UF-4000 but assigned to the GP culture-based classification, 105 samples (69.5%) also contained contamination/mixed flora. These findings suggest that the UF-4000 may detect both clinically relevant uropathogens and contaminating organisms, resulting in a mixed BACT-Info flag, whereas contamination is disregarded in the culture-based classification when a clinically relevant uropathogen is present. Consequently, some classifications considered discordant relative to the culture-based reference standard may reflect the overall microbial composition of the specimen detected by the UF-4000 rather than true analytical misclassifications.

In the present study, the BACT-Info flag showed moderate overall agreement with culture-based bacterial classification, with better apparent performance for GP than for GN classification. However, the higher GP classification performance was strongly influenced by the composition of the culture-based GP category, which predominantly consisted of contamination/mixed flora without a confirmed GP uropathogen. This highlights the importance of distinguishing contamination/mixed flora from clinically relevant GP uropathogens when evaluating BACT-Info flag performance. Overall, these findings indicate that the BACT-Info flag provides useful information on the bacterial composition of urine samples, but its classification results should be interpreted with caution.

COMMENTS

/

TO DO/ACTIONS

- 1) Implement and evaluate a new clinical laboratory workflow for optimal triage of urine samples from patients with suspected UTI.
- 2) Evaluate the clinical utility of serial BACT count monitoring during antibiotic therapy in clinical practice, using the Sysmex UF-4000 to assess early changes in bacterial counts as an indicator of treatment response.

ATTACHMENTS

Attachment I: Urine sample workflow

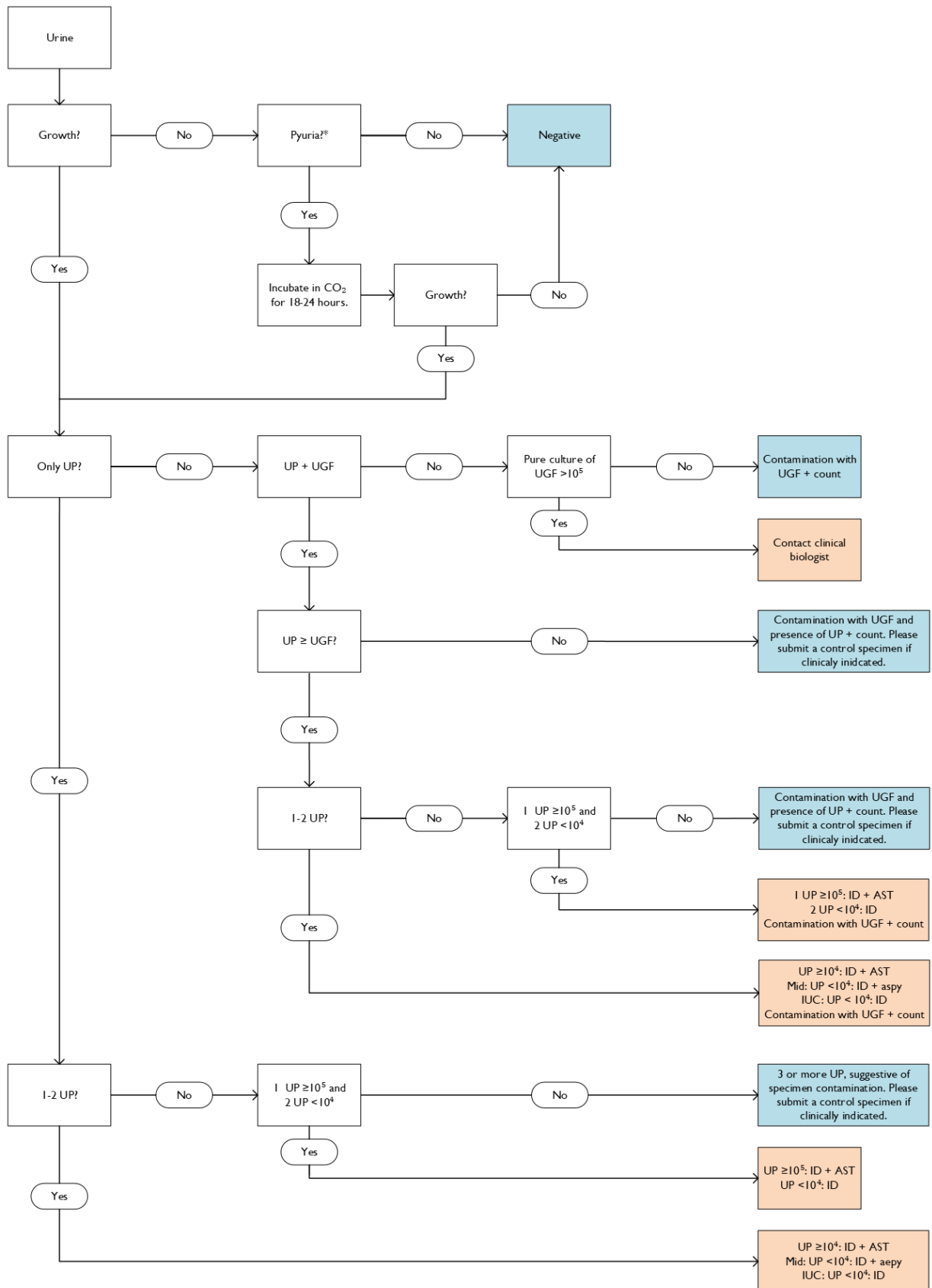
Accepted specimen types included midstream urine, urine collected from indwelling urinary catheters, catheterized urine, urine collected in a urine bag, urine obtained by suprapubic aspiration, and urine of unknown origin. Samples were transported to the laboratory under controlled conditions. Specimens collected at the Lanaken, Waterschei, and Maas & Kempen campuses were collected in two separate tubes: one containing boric acid preservative for urine culture and one without preservative for other analyses. Boric acid-preserved specimens could be stored at room temperature until processing. In contrast, specimens collected at Campus Sint-Jan were non-preserved and were processed within two hours of collection or stored refrigerated at 2–8°C for a maximum of 24 hours.

Upon arrival in the laboratory, specimens underwent a pre-analytical assessment. Urine cultures could be requested by clinicians either irrespective of the WBC count or based on the presence of pyuria, defined as ≥ 25 WBC/ μ L. For samples submitted through the latter pathway, specimens with < 25 WBC/ μ L were not routinely subjected to urine culture, whereas those with ≥ 25 WBC/ μ L proceeded to culture. Cytological examination was performed using the Sysmex UF-4000 to determine the WBC count and assess the presence of pyuria.

Urine cultures were inoculated using calibrated 1- μ L loops to enable quantitative enumeration. Samples were cultured on Columbia CNA agar and MacConkey agar for bacterial growth. Both plates were incubated at 36°C for 24 hours, with Columbia CNA agar incubated in a CO₂ atmosphere. In cases of pyuria without visible bacterial growth after 24 hours, bacterial cultures were re-incubated for an additional 24 hours and re-examined. When yeast culture was requested, samples were inoculated onto dedicated yeast culture medium using a 10- μ L loop and incubated at 30°C. Yeast culture plates were examined after 1, 2, and 7 days of incubation.

During the analytical phase, colony counts were used to estimate the bacterial load in colony-forming units (CFU)/mL. Isolated microorganisms were classified as uropathogens, urogenital flora, or organisms requiring clinical interpretation. Potential pathogens were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper®, Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Antimicrobial susceptibility testing was subsequently performed for clinically relevant isolates according to laboratory guidelines.

Culture results were interpreted based on the presence of pyuria, bacterial load expressed as CFU/mL, the balance between uropathogens and contaminating urogenital flora, and specimen type. Clinically significant isolates were reported together with their identification and antimicrobial susceptibility profile, whereas mixed cultures suggestive of contamination could require subculture and further purification. Finally, all culture results were reviewed and validated by a clinical microbiologist.



*Pyuria = ≥ 25 white blood cells/ μL .

In case of suprapubic puncture: always perform ID + AST, in case of an unexpected result (contamination or ≥ 3 UP), contact the clinical team to verify the specimen type.

Abbreviations: aspy = antimicrobial susceptibility testing only if pyuria is present, AST = antimicrobial susceptibility testing, ID = identification, IUC = urine from an indwelling urinary catheter, mid = midstream urine, UGF = urogenital flora, UP = uropathogen(s)

Attachment 2: Distribution of uropathogens identified in culture-positive urine samples (n = 4,551 uropathogen occurrences)

Microorganism	Number of occurrences (n)	Relative frequency (%)
<i>Escherichia coli</i>	1,513	33.25
<i>Enterococcus faecalis</i>	752	16.52
<i>Klebsiella pneumoniae/variicola</i>	313	6.88
<i>Streptococcus agalactiae</i>	300	6.59
<i>Candida albicans</i>	208	4.57
<i>Proteus mirabilis</i>	189	4.15
ESBL-producing <i>Escherichia coli</i>	108	2.37
<i>Pseudomonas aeruginosa</i>	106	2.33
<i>Enterococcus faecium</i>	95	2.09
<i>Klebsiella oxytoca/Raoultella</i> spp.	95	2.09
<i>Staphylococcus aureus</i>	94	2.07
<i>Candida glabrata</i>	82	1.80
<i>Aerococcus urinae</i>	78	1.71
<i>Gardnerella vaginalis</i>	50	1.10
<i>Enterobacter cloacae</i> complex	49	1.08
<i>Morganella morganii</i>	49	1.08
<i>Citrobacter</i> spp. (presumably <i>C. koseri</i>)	46	1.01
<i>Staphylococcus saprophyticus</i>	40	0.88
<i>Citrobacter</i> spp. (presumably <i>C. freundii</i>)	30	0.66
<i>Klebsiella aerogenes</i>	30	0.66
<i>Actinotignum sanguinis/schaalii</i>	28	0.62
<i>Candida tropicalis</i>	27	0.59
ESBL-producing <i>Klebsiella pneumoniae/variicola</i>	27	0.59
<i>Aerococcus sanguinicola</i>	22	0.48
<i>Candida parapsilosis</i>	18	0.40
<i>Serratia marcescens/ureilytica</i>	18	0.40
<i>Enterococcus avium</i>	13	0.29
<i>Staphylococcus epidermidis</i>	9	0.20
<i>Candida krusei</i>	8	0.18
ESBL-producing <i>Enterobacter cloacae</i> complex	8	0.18
<i>Streptococcus dysgalactiae</i>	8	0.18
MRSA	7	0.15
<i>Stenotrophomonas maltophilia</i> group	7	0.15
<i>Klebsiella pneumoniae</i>	6	0.13
<i>Providencia rettgeri</i>	6	0.13
<i>Serratia</i> spp.	6	0.13
<i>Streptococcus pyogenes/dysgalactiae/canis</i>	6	0.13
<i>Acinetobacter baumannii/calcoaceticus</i> complex	5	0.11
<i>Candida lusitanae</i>	5	0.11
<i>Proteus</i> spp. (presumably <i>P. vulgaris</i>)	5	0.11
<i>Actinomyces</i> spp.	4	0.09
<i>Candida kefyr</i>	4	0.09

<i>Citrobacter</i> spp.	4	0.09
OXA-48-producing <i>Escherichia coli</i>	4	0.09
<i>Pantoea</i> spp.	4	0.09
<i>Streptococcus anginosus</i>	4	0.09
<i>Staphylococcus lugdunensis</i>	4	0.09
<i>Candida dubliniensis</i>	3	0.07
<i>Enterococcus durans</i>	3	0.07
<i>Enterobacter</i> spp.	3	0.07
<i>Gardnerella leopoldii/swidsinskii</i>	3	0.07
VRE, <i>Enterococcus faecium</i>	3	0.07
<i>Acinetobacter</i> spp.	2	0.04
OXA-48-producing <i>Citrobacter freundii</i>	2	0.04
<i>Citrobacter</i> spp. (presumably <i>C. braakii</i>)	2	0.04
<i>Enterococcus casseliflavus</i>	2	0.04
<i>Enterococcus gallinarum</i>	2	0.04
<i>Escherichia marmotae</i>	2	0.04
VIM-producing <i>Pseudomonas aeruginosa</i>	2	0.04
ESBL-producing <i>Proteus mirabilis</i>	2	0.04
<i>Streptococcus pyogenes</i>	2	0.04
<i>Aerococcus viridans</i>	1	0.02
<i>Alloscardovia omnicolens</i>	1	0.02
<i>Actinomyces neuii</i>	1	0.02
<i>Bacteroides fragilis</i>	1	0.02
<i>Corynebacterium</i> spp.	1	0.02
<i>Enterococcus hirae</i>	1	0.02
<i>Enterococcus raffinosus</i>	1	0.02
<i>Hafnia alvei</i>	1	0.02
<i>Klebsiella pneumoniae</i>	1	0.02
<i>Lactobacillus</i> spp.	1	0.02
<i>Neisseria gonorrhoeae</i>	1	0.02
<i>Proteus</i> spp. (presumably <i>P. hauseri</i>)	1	0.02
<i>Proteus</i> spp. (presumably <i>P. penneri</i>)	1	0.02
Pseudomonadaceae	1	0.02
<i>Pseudomonas fluorescens</i> group	1	0.02
<i>Pseudomonas putida</i> group	1	0.02
<i>Pseudomonas</i> spp.	1	0.02
<i>Providencia stuartii</i>	1	0.02
<i>Saccharomyces cerevisiae</i>	1	0.02
<i>Salmonella</i> spp.	1	0.02
<i>Streptococcus pneumoniae</i>	1	0.02
<i>Staphylococcus haemolyticus</i>	1	0.02
<i>Staphylococcus hominis</i>	1	0.02
Total	4,551	100.00

Abbreviations: ESBL, extended-spectrum β -lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*; OXA-48, oxacillinase-48; VRE, vancomycin-resistant enterococci; VIM, Verona integron-encoded metallo- β -lactamase.

Attachment 3: literature review

	Author	Year	UFC device	Sample size (n)	UTI cut-off and ROC performance	Definition of positive urine culture	BACT-Info flag agreement (% concordance and κ)	BACT-Info flag diagnostic performance
1.	De Rosa et al. (1)	2018	UF-5000	2,719	<u>BACT:</u> 58 BACT/ μ L (AUC 0.973) <i>Male:</i> 25 BACT/ μ L (AUC 0.988) <i>Female:</i> 58 BACT/ μ L (AUC 0.959) <u>WBC:</u> 40 WBC/ μ L (AUC 0.844) <i>Male:</i> AUC 0.873 <i>Female:</i> AUC 0.816 <u>Combined BACT + YLC:</u> ≥ 58 BACT/ μ L and/or ≥ 150 YLC/ μ L	$\geq 10^5$ CFU/mL	<u>GN:</u> Full: 86.5% Partial: 13.3% Discordant: 0.2%	<u>GN:</u> SE = 81.6% SP = 95.9% PPV = 95.8% NPV = 81.7%
2.	Kim et al. (8)	2018	UF-5000	1,430	<u>BACT:</u> AUC 0.952 <u>WBC:</u> AUC 0.839 <u>Combined BACT + WBC:</u> BACT or >15 WBC/ μ L BACT or >50 WBC/ μ L	$\geq 10^3$ CFU/mL $\geq 10^5$ CFU/mL	<u>GN:</u> $\geq 10^3$ CFU/mL: 89.1% $\geq 10^5$ CFU/mL: 81.2% <u>GP:</u> $\geq 10^3$ CFU/mL: 66.5% $\geq 10^5$ CFU/mL: 80.4%	<u>GN:</u> $\geq 10^3$ CFU/mL: SE = 69.5% SP = 94.6% PPV = 78.4% NPV = 91.7%

								$\geq 10^5$ CFU/mL: SE = 91.7% SP = 90.0% PPV = 95.4% NPV = 82.8% <u>GP:</u> $\geq 10^3$ CFU/mL: SE = 24.2% SP = 92.3% PPV = 65.9% NPV = 66.5% $\geq 10^5$ CFU/mL: SE = 81.3% SP = 80.0% PPV = 64.4% NPV = 90.6%
3.	Allain et al. (18)	2019	UF-4000	<u>UTI cut-off:</u> 2,695 <u>BACT-Info flag:</u> 362	<u>BACT:</u> >100 BACT/ μ L >150 BACT/ μ L >200 BACT/ μ L	French REMIC guidelines; species-dependent threshold	<u>GN:</u> Gram stain: 91% Culture: 86% <u>GP:</u> Gram stain: 79% Culture: 20%	NR
4.	Gilboe et al. (9)	2021	UF-5000	1,000	<u>BACT:</u> $\geq 10,000$ BACT/mL $\geq 100,000$ BACT/mL	Norwegian guidelines; patient group-dependent threshold ($\geq 10^3$ – 10^5 CFU/mL)	NR°	

					<u>Combined BACT + WBC:</u> BACT and ≥ 10 WBC/ μ L BACT and ≥ 20 WBC/ μ L BACT and ≥ 30 WBC/ μ L BACT and ≥ 40 WBC/ μ L			
5.	Yang et al. (15)	2021	UF-5000	102	NR	$\geq 10^3$ CFU/mL	<u>Overall:</u> $\kappa = 0.46$ (moderate agreement)	<u>GN:</u> SE = 80.0% SP = 88.2% <u>GP:</u> SE = 70.0% SP = 86.5% <u>GP/GN:</u> SE = 4.5% SP = 94.9%
6.	Enko et al. (13)	2021	UF-5000	344	<u>BACT:</u> 135 BACT/ μ L Gram stain: AUC 0.983 Culture: AUC 0.939 <u>WBC:</u> 23 WBC/ μ L Gram stain: AUC 0.881 Culture: AUC 0.836	$\geq 10^5$ CFU/mL	<u>GN:</u> Gram stain: 60.0% Culture: 62.5%, $\kappa = 0.78$ (substantial agreement) <u>GP:</u> Gram stain: 73.7% Culture: 59.1%, $\kappa = 0.40$ (fair agreement) <u>GP/GN:</u> Gram stain: 33.3% Culture: 14.3%	NR
7.	Christy et al. (11)*	2022	UF-5000	100	<u>BACT:</u> 582.22 BACT/ μ L (AUC 0.873)	$\geq 10^5$ CFU/mL	<u>GN:</u> $\kappa = 0.876$ (excellent agreement)	NR

					<u>WBC:</u> 87.2 WBC/ μ L (AUC 0.995)		<u>Overall:</u> $\kappa = 0.212$ (fair agreement) <u>Overall after scattergram angle adjustment:</u> $\kappa = 0.876$ (excellent agreement)	
8.	Chun et al. (16)	2022	UF-5000	383	<u>BACT:</u> ≥ 196 BACT/ μ L (all patients: AUC 0.781; antibiotic-naïve patients: AUC 0.819) <u>WBC:</u> ≥ 14.7 WBC/ μ L (all patients: AUC 0.720; antibiotic-naïve patients: AUC 0.747) <u>Combined BACT + WBC:</u> BACT and WBC (all patients: AUC 0.783; antibiotic-naïve patients: AUC 0.821) BACT or WBC (all patients: AUC 0.719; antibiotic-naïve patients: AUC 0.748)	$> 10^5$ CFU/mL	<u>GP/GN:</u> All patients: 38.3% Antibiotic-naïve patients: 44.7% <u>Negative culture:</u> All patients: 95.1% Antibiotic-naïve patients: 96.2% <u>Overall:</u> All patients: 59.0% Antibiotic-naïve patients: 65.4%	NR
9.	Wang et al. (3)	2023	UF-5000	<u>UTI cut-off:</u> 671 <u>BACT-Info flag:</u> 158	<u>BACT:</u> 1,367 BACT/ μ L (AUC 0.86)	$\geq 10^4$ CFU/mL	NR	<u>GN:</u> SE = 81% SP = 70% PPV = 93%

					<u>WBC:</u> AUC 0.74			<u>GP:</u> SE = 70% SP = 78% PPV = 18%
10.	Chen et al. (12)*	2023	UF-5000	2,600	<u>BACT:</u> <i>Male:</i> 22.35/ μ L (AUC 0.856) <i>Female:</i> 127.25/ μ L (AUC 0.850) <u>WBC:</u> <i>Male:</i> 32.20/ μ L (AUC 0.942) <i>Female:</i> 39.15/ μ L (AUC 0.931)	$\geq 10^4$ CFU/mL	<u>Overall:</u> $\kappa = 0.339$ (fair agreement)	NR
11.	Zhang et al. (14)	2023	UF-5000	5,095	<u>BACT:</u> <i>Male:</i> >179.2 BACT/ μ L (AUC 0.958) <i>Female:</i> >325.8 BACT/ μ L (AUC 0.917) <u>WBC:</u> <i>Male:</i> >52.2 WBC/ μ L (AUC 0.874) <i>Female:</i> >67.9 WBC/ μ L (AUC 0.822)	$\geq 10^4$ CFU/mL	NR	<u>GN:</u> <i>Male:</i> SE = 54.1% SP = 99.3% PPV = 94.0% NPV = 91.0% <i>Female:</i> SE = 65.7% SP = 98.4% PPV = 92.5% NPV = 90.6%

					<u>Combined BACT + WBC:</u> <i>Male:</i> AUC 0.956 <i>Female:</i> AUC 0.925			<u>GP:</u> <i>Male:</i> SE = 68.8% SP = 96.2% PPV = 58.4% NPV = 97.5% <i>Female:</i> SE = 63.9% SP = 90.7% PPV = 26.5% NPV = 98.0%
12.	Angulo-López et al. (4)	2024	UF-5000	449	NR	$\geq 10^3$ CFU/mL	<u>GN:</u> $\kappa = 0.80$ <u>GP:</u> $\kappa = 0.49$	<u>GN:</u> SE = 95.6% SP = 85.6% PPV = 96.7% NPV = 81.6% <u>GP:</u> SE = 96.3% SP = 89.6% PPV = 37.1% NPV = 99.7%
13.	Lu et al. (6)	2024	UF-5000	1,522	<u>BACT:</u> $\geq 10^4$ CFU/mL: 42.2 BACT/ μ L (AUC 0.927) $\geq 10^5$ CFU/mL: 100.2 BACT/ μ L (AUC 0.946) <u>WBC:</u> $\geq 10^4$ CFU/mL: AUC 0.708 $\geq 10^5$ CFU/mL: AUC 0.732	$\geq 10^4$ CFU/mL $\geq 10^5$ CFU/mL	<u>GN:</u> 87.2% <u>GP:</u> 27.1% <u>Overall:</u> $\kappa = 0.227$ (fair agreement)	<u>GN:</u> SP = 94.0% PPV = 87.2%

					<u>Combined BACT + WBC:</u> ≥10 ⁴ CFU/mL: AUC 0.905 ≥10 ⁵ CFU/mL: AUC 0.933			
14.	Liu et al. (7)	2024	UF-5000	4,445	<u>BACT:</u> 92.2 BACT/μL (AUC 0.944) Rule-out: 7.8 BACT/μL <u>WBC:</u> 40.8 WBC/μL (AUC 0.863)	≥10 ⁴ CFU/mL	<u>GN:</u> 90.3% <u>GP:</u> 86.8% <u>GP/GN:</u> 85.2% <u>Overall:</u> 92.0%	<u>GN:</u> SE = 61.6% <u>GP:</u> SE = 38.1% <u>GP/GN:</u> SE = 11.8%
15.	Korsten et al. (5)	2024	UF-4000	<u>UTI cut-off:</u> 970 <u>BACT-Info flag:</u> 42,958	≥100 BACT/μL	≥10 ⁴ CFU/mL	NR	<u>GN:</u> SE = 95% SP = 65% PPV = 80% NPV = 90% <u>GP:</u> SE = 95% SP = 62% PPV = 20% NPV = 99% <u>GP/GN:</u> SE = 36% SP = 87% PPV = 17% NPV = 95%

16.	Sender et al. (2)	2025	UF-5000	4,005	<u>BACT:</u> Rule-out: <30 BACT/μL (AUC 0.921) Rule-in: >4000 BACT/μL (AUC 0.917)	≥10 ⁴ CFU/mL	<u>GN:</u> Full: 96% Partial: 3.3% Discordant: 0.37%	NR
17.	Chang et al. (10)†	2026	UF-5000	25,337	<u>BACT:</u> AUC 0.932 <u>WBC:</u> AUC 0.867	>10 ³ –10 ⁵ CFU/mL	<u>Overall:</u> Pooled κ = 0.52 (moderate agreement)	GN performance > GP performance; pooled SE/SP and PPV/NPV NR
No full text available: information extracted from cited source/meta-analysis.								
18.	Ren et al. (22)*	2018	UF-5000	566	<u>Combined BACT + WBC:</u> <30 BACT/μL and/or <200 WBC/μL	≥10 ⁴ CFU/mL	<u>Overall:</u> 67.7%, κ = 0.775	NR
19.	Zhang et al. (23)*	2019	UF-5000	1,021	NR	NR	<u>GN:</u> 94.0% <u>GP:</u> 82.2%	NR
20.	Kim et al. (21)	2022	UF-5000	1,063	<u>BACT:</u> >685.3 BACT/μL	≥10 ⁵ CFU/mL	NR	<u>Combined BACT count + BACT-Info flag:</u> SE = 91.5% SP = 90.5% <u>GN:</u> SE = 95.5% SP = 94.8%

21.	Karino et al. (20)	2026	UF-5000	1,529	<u>BACT:</u> 195.2 BACT/ μ L (AUC 0.940)	Any bacterial growth	<u>GN:</u> Accuracy: 90.4%, $\kappa = 0.76$ (substantial agreement) 17.2% incorrectly GP-flagged <u>GP:</u> Accuracy: 79.9%, $\kappa = 0.51$ (moderate agreement)	<u>GN:</u> SE = 73.9% SP = 97.4% PPV = 92.3% NPV = 89.8%
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*Studies identified through reference-list screening.

°The BACT-Info flag was evaluated as a triage tool for dAST rather than as an independent diagnostic test against urine culture. For GN samples, 92.9% (52/56) showed complete concordance between dAST and routine AST, with 99.1% (555/560) concordance across individual antimicrobial susceptibility test results. Performance for GP samples was insufficient for reliable dAST-based triage.

†Systematic review and meta-analysis including 18 individual studies.

NR: denotes data that were not reported in the original publication.

Abbreviations: AST, antimicrobial susceptibility testing; AUC, area under the receiver operating characteristic curve; BACT, bacteria; CFU, colony-forming units; dAST, direct antimicrobial susceptibility testing; GN, Gram-negative; GP, Gram-positive; GP/GN, mixed Gram-positive/Gram-negative; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; REMIC, Référentiel en microbiologie médicale; ROC, receiver operating characteristic; SE, sensitivity; SP, specificity; UFC, urine flow cytometry; UTI, urinary tract infection; WBC, white blood cells; YLC, yeast-like cells; κ , Cohen's kappa.