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Authors:	Dyah Ganis Kusumawardhani[1], Dwi Rahayuningsih[2,3], Ferdy Royland Marpaung[2,3], Aryati Aryati[2,*]
Affiliations:	[1]Department of Clinical Pathology Specialist Education Program, Faculty of Medicine, Universitas Airlangga, Surabaya; [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya; [3]Department of Clinical Pathology, Dr. Soetomo General Academic Hospital, Surabaya; Indonesia
Corresponding author:	Aryati Aryati; Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia Phone: +6281230570493; E-mail: aryati@fk.unair.ac.id
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ORIGINAL RESEARCH

Safety-focused triage of suspected bacterial urinary tract infection using urine flow cytometry outputs: prediction of culture positivity

Dyah Ganis Kusumawardhani[1], Dwi Rahayuningsih[2,3], Ferdy Royland Marpaung[2,3], Aryati Aryati[2,*]

[1]Department of Clinical Pathology Specialist Education Program, Faculty of Medicine, Universitas Airlangga, Surabaya; [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya; [3]Department of Clinical Pathology, Dr. Soetomo General Academic Hospital, Surabaya; Indonesia

Running title: Kusumawardhani et al. Urine flow cytometry for UTI triage

*** Correspondence:**

Aryati Aryati

Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Phone: +6281230570493

E-mail: aryati@fk.unair.ac.id

ORCID ID: 0000-0003-3959-0688

ABSTRACT

Aim: To evaluate the urine flow cytometry flagging feature (UF-5000) for predicting urine culture positivity and assess the agreement between BACT-info Gram outputs and culture Gram classification, with subgroup analyses in catheterised, immunosuppressed, and age-stratified patients.

Methods: This single-centre cross-sectional diagnostic accuracy study included 885 routine urine samples with paired UF-5000 analysis and culture results (440 culture-positive, 445 culture-negative). The diagnostic performance of the urinary tract infection (UTI)-flag (UTI vs. non-UTI) was calculated with Wilson 95% confidence intervals. Agreement between two BACT-info definitions and culture Gram category was also evaluated.

Results: UTI flagging demonstrated a sensitivity of 0.670, specificity of 0.921, positive predictive value (PPV) of 0.894, negative predictive value (NPV) of 0.739, and an accuracy of 0.797. The negative predictive value declined markedly in high-risk subgroups: catheterised patients (NPV, 0.612) and immunosuppressed patients (NPV, 0.500). Sensitivity was substantially lower in males (0.412) than in females (0.833). Indicator-derived Gram classification showed excellent agreement with culture Gram category ($\kappa = 0.826$), whereas categorical BACT-info label mapping yielded fair agreement ($\kappa = 0.294$). Bacterial count (BACT) discriminated culture positivity with an area under the curve (AUC) of 0.812 (optimal cut-off 487/μL, sensitivity 0.795, specificity 0.807), whereas white blood cell count (WBC) showed an AUC of 0.749 (cut-off 76.9/μL, sensitivity 0.735, specificity 0.773).

Conclusion: UF-5000 UTI flagging provides strong rule-in utility for bacterial UTI triage but should not be used as a universal rule-out test, particularly in catheterised and immunosuppressed patients. The BACT count improves discrimination and may support safety-oriented overriding rules.

Keywords: antimicrobial stewardship; catheter-related infections; predictive value of tests

INTRODUCTION

Urinary tract infection (UTI) is among the most common bacterial infections, driving substantial antibiotic use and antimicrobial resistance. Urine culture remains the reference standard for confirmation, but its 24–48-hour turnaround delays targeted therapy and encourages empirical prescribing (1). Automated urine flow cytometry platforms, such as the Sysmex UF-5000, provide rapid bacterial and leukocyte counts, an algorithm-derived UTI flag that predicts culture positivity, and Gram-classification outputs (BACT-info), offering a potential triage tool to reduce unnecessary cultures and support diagnostic stewardship (2–5). However, the reliability of UF-5000 flagging in high-risk populations, such as catheterised or immunosuppressed patients, remains unclear. These groups have higher rates of asymptomatic bacteriuria, potentially lowering the negative predictive value (NPV) and limiting rule-out safety. Furthermore, Gram-flag agreement with culture is inconsistent and depends on the output definition used (4,6). Local validation is needed to establish safe implementation thresholds.

The aim of this study was to evaluate the urine flow cytometry flagging feature (UF-5000) against urine culture categorisation in routine clinical specimens, to quantify diagnostic performance overall and in clinically important subgroups (catheterisation, immunosuppression, and age strata), and to assess agreement between BACT-info Gram outputs and the culture Gram category. We further examined the discrimination of quantitative white blood cell count (WBC) and bacterial count (BACT) measures and derived locally optimised thresholds to support a pragmatic, safety-first triage algorithm consistent with diagnostic stewardship principles.

MATERIAL AND METHODS

Patients and study design

This single-centre cross-sectional diagnostic accuracy study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, between June and October 2023. A total of 974 samples were screened for eligibility. Urine samples from patients with suspected UTI with paired UF-5000 analysis and routine culture were eligible. Samples were excluded for gross contamination, defined as "mixed flora" (≥3 morphologically distinct colony types) and/or >20 epithelial cells/low-power field (LPF), insufficient volume, or yeast/fungal growth. After exclusions, 885 bacterial-only samples were analysed, including 440 culture-positive and 445 culture-negative samples. For the primary analysis, "mixed bacterial growth" with two uropathogens meeting quantitative thresholds was classified as culture-positive; however, these mixed-growth samples were excluded from Gram-agreement analyses because they lack a single Gram category. Collected clinical variables included age, sex, UTI type (complicated/noncomplicated), catheter status, and immunosuppressant therapy. The study was approved by the hospital ethics committee (No. 1948/121/1/I/2023).

Methods

Urine was collected via midstream clean-catch or catheter. Catheter type was recorded (short-term <28 days, long-term ≥28 days, recurrent). Samples were processed within 1 hour. Automated analysis used the Sysmex UF-5000, providing UTI flag (positive/negative), white blood cell (WBC) count (/µL), bacterial count (BACT) (/µL), and BACT-info Gram outputs. The UTI flag was the primary index test. Two Gram-output definitions were evaluated: (i) indicator-derived Gram class (from binary Gram-positive and Gram-negative indicators, deriving Gram-positive only, Gram-negative only, Mixed, or None), and (ii) categorical BACT-info label (Gram-Pos, Gram-Neg, Mixed, Non-classified).

The reference standard was routine urine culture. Culture positivity was defined as $\geq 10^5$ colony-forming units (CFU)/mL for midstream and $\geq 10^3$ CFU/mL for catheter specimens, with a single uropathogen; mixed growths (≥2 organisms) were considered positive for primary analysis but excluded from Gram-agreement analyses. For Gram-agreement analyses, only samples with a single Gram-type organism were included. Clinical symptom data were not systematically collected; thus, "UTI" in the context of the flag refers to prediction of microbiological positivity, not clinical infection (7).

Statistical analysis

Diagnostic accuracy measures, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive likelihood ratio (LR+), and negative likelihood ratio (LR−), were computed with Wilson 95% confidence intervals (CIs) for the UTI flag against culture. Subgroup analyses were performed for catheterisation status, immunosuppressant use, sex, and age strata (18–<65 years and ≥65 years). Agreement between BACT-info Gram outputs and culture Gram category was assessed using Cohen's kappa (κ) with bootstrap 95% CIs. Receiver operating characteristic (ROC) curves were generated for quantitative WBC and BACT counts, and Youden index-optimised cut-offs were derived. Internal validation of cut-off stability was performed using 1,000 bootstrap resamples with replacement; optimism-corrected sensitivity and specificity were estimated from out-of-bag samples. Fisher's exact test was used to assess the association between UTI flagging and culture results. Categorical variables were compared using the chi-squared test and continuous variables using the independent samples t-test. Statistical significance was set at $p < 0.05$.

RESULTS

Among 974 screened samples, 925 had UF-5000 and culture results. After excluding 40 yeast or fungal growths, 885 were analysed (Figure 1, Table S1). Demographics and baseline

characteristics are in Table 1; the mean age was 41.1 ± 23.4 years overall, with no significant difference between culture-positive (42.1 ± 23.4 years) and culture-negative groups (40.1 ± 23.4 years; p = 0.20), and the majority of participants were female (485/885, 54.8%). Culture positivity was significantly associated with female sex (270/440, 61.4% vs. 215/445, 48.3%; p < 0.001), catheterisation (250/440, 56.8% vs. 170/445, 38.2%; p < 0.001), immunosuppression (110/440, 25.0% vs. 55/445, 12.4%; p < 0.001), and complicated UTI type (140/440, 31.8% vs. 80/330, 24.2%; p = 0.021).

Sensitivity was 0.670 (95% CI 0.624–0.714), specificity 0.921 (0.892–0.945), PPV 0.894, NPV 0.739, accuracy 0.797, and OR 23.83 (p<0.0001) (Table 2). Subgroup analyses (Table S2) revealed reduced NPV in catheterised (0.612) and immunosuppressed patients (0.500), while non-catheterised and non-immunosuppressed groups had NPVs of 0.839 and 0.785, respectively. Sensitivity was markedly lower in males (0.412) than in females (0.833).

Agreement between BACT-info Gram outputs and culture Gram category varied by definition. Indicator-derived Gram class showed excellent agreement (κ=0.826) (Table S3), whereas the categorical label had only fair agreement (κ = 0.294) (Table S4).

ROC analyses (Figure 2) gave AUCs of 0.812 (95% CI 0.792–0.853) for BACT and 0.749 (0.731–0.802) for WBC. Youden-optimised cut-offs were 487/μL for BACT (sensitivity 0.795, specificity 0.807) and 76.9/μL for WBC (sensitivity 0.735, specificity 0.773). Bootstrap validation results are presented in Table S5 and confirmed minimal optimism, cut-off stability, and an optimism-corrected AUC of 0.817 for BACT.

DISCUSSION

This study demonstrated that UF-5000 UTI flagging had high specificity but only moderate sensitivity for predicting urine culture positivity. Diagnostic performance varied substantially

across clinical subgroups, particularly among catheterised and immunosuppressed patients, while BACT showed better discriminatory ability than WBC.

In this tertiary-care cohort with a high prevalence of catheterisation and immunosuppression, the UF-5000 UTI flag showed high specificity but only moderate sensitivity, confirming its strength as a rule-in tool while cautioning against its use as a stand-alone rule-out test. The NPV dropped dramatically in high-risk groups—catheterised patients and immunosuppressed patients, indicating that a negative flag cannot safely exclude significant bacteriuria. Gram-output reliability depended heavily on the definition: indicator-derived classification achieved excellent agreement, while the categorical label performed poorly. BACT count outperformed WBC, reinforcing BACT as the more discriminatory parameter.

Our results align with prior studies showing that UF-5000 flagging can achieve high NPVs in low-risk outpatient populations (3,8,9), but performance deteriorates when prevalence of colonization is high (10). The high specificity we observed is comparable to decision-tree models in mixed populations (11), while others have achieved higher sensitivity by using lower BACT thresholds and multiparameter rules to exclude infection (7). These differences reflect distinct operational objectives "rule-out versus rule-in" and underlying prevalence.

The subgroup findings have direct safety implications. In catheterised and immunosuppressed patients, a negative flag would miss approximately 38% and 41% of culture-positive samples, respectively, potentially delaying treatment of true infections. Catheter biofilms, blunted inflammatory responses, and low-burden organisms likely contribute to this reduced sensitivity (12–14). The observed difference in sensitivity between females and males requires cautious interpretation and external validation (15,16). Notably, culture positivity in this cohort was significantly associated with catheterisation, immunosuppression, and complicated UTI type

(all $p \leq 0.021$; Table 1), consistent with a higher pre-test probability of true infection in these groups and reinforcing the requirement for mandatory culture irrespective of flag status.

Gram-flag disagreement, particularly with the categorical label, is consistent with previous reports (7,11). The strong performance of the indicator-derived Gram classification suggests that Gram flagging can be informative when properly defined, but it should not guide empirical therapy without culture confirmation. Our locally optimised BACT cutoff of 487/µL, although internally validated with bootstrap, requires external validation given population-specific factors.

Diagnostic stewardship efforts must avoid both undertesting high-risk patients and overtreating asymptomatic bacteriuria (17,18). We propose a safety-first algorithm (Figure 3): UTI flag-positive samples are prioritized for culture; flag-negative samples from low-risk, non-catheterised, immunocompetent adults with low clinical suspicion may safely defer culture, but if WBC ≥76.9/µL or BACT ≥487/µL, culture should proceed. For all high-risk patients, culture is mandatory regardless of flag status.

Limitations include the single-centre design, use of culture rather than clinical UTI as the reference standard, and the absence of symptom data. No external validation cohort was available, limiting the generalisability of the proposed thresholds. Subgroup point estimates from smaller strata, such as the perfect specificity in patients ≥65 years (due to no false positives), should be interpreted cautiously. In addition, baseline characteristics differed significantly across culture-status groups, and the clinical covariates examined (catheterisation, immunosuppression, complicated UTI, and sex) are mutually correlated; subgroup performance estimates were unadjusted and may therefore be confounded by case-mix, underscoring the need for multivariable modelling and external validation.

CONCLUSION

UF-5000 UTI flagging provides strong rule-in value for predicting positive urine cultures and may support diagnostic stewardship when implemented with subgroup-aware safeguards, particularly given that culture positivity was significantly patterned by catheterisation, immunosuppression, and UTI complexity. These results should not be interpreted as predicting clinical UTI, as symptom data were not incorporated.

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Conflicts of interest: None to declare.

Author contributions (CRediT): Conceptualization—D.G.K, D.R, F.R.M, and A.A.; methodology—D.G.K, D.R., F.R.M, and A.A.; formal analysis—D.G.K, D.R., F.R.M., and A.A.; investigation—D.G.K, F.R.M, and A.A.; data curation—D.G.K and F.R.M; writing—original draft— D.G.K, D.R, F.R.M, and A.A.; writing—review and editing—D.G.K and F.R.M; supervision—D.R, F.R.M, and A.A.; project administration—D.G.K and F.R.M. All authors have read and agreed to the published version of the manuscript.

Ethics statement: This study was approved by the Hospital Ethics Committee (Dr. Soetomo General Hospital), Surabaya (Approval No. 1948/121/1/I/2023). Informed consent was obtained according to institutional ethics guidance.

Data availability statement: All relevant data are within the article and its supplementary materials.

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TABLES AND FIGURES

Table 1. Baseline characteristics overall and by urine culture status

Characteristic	Overall (N=885)	Positive culture (N=440)	Negative culture (N=445)	p
		Mean ± SD		
Age, years	41.1 ± 23.4	42.1 ± 23.4	40.1 ± 23.4	0.20
		N (%)		
Sex				<0.001
Female	485 (54.8)	270 (61.4)	215 (48.3)	
Male	400 (45.2)	170 (38.6)	230 (51.7)	
Catheter				<0.001
No	465 (52.5)	190 (43.2)	275 (61.8)	
Yes	420 (47.5)	250 (56.8)	170 (38.2)	
Immunosuppressant				<0.001
No	720 (81.4)	330 (75.0)	390 (87.6)	
Yes	165 (18.6)	110 (25.0)	55 (12.4)	
UTI†				0.021
Uncomplicated	550 (71.4)	300 (68.2)	250 (75.8)	
Complicated	220 (28.6)	140 (31.8)	80 (24.2)	

SD, standard deviation; UTI, urinary tract infection; †, UTI type data were missing for 115 patients (13.0%) of overall cohort and percentages shown reflect available data only.

Table 2. Diagnostic performance of urinary tract infection (UTI) flagging

Measure	Value (95% CI)
Sensitivity	0.670 (0.624–0.714)
Specificity	0.921 (0.892–0.945)
Accuracy	0.797 (0.770–0.822)
PPV	0.894 (0.856–0.924)
NPV	0.739 (0.700–0.775)
LR+	8.52 (6.12–11.86)
LR−	0.36 (0.31–0.41)
Odds ratio	23.83 (15.92–35.66)
p (Fisher's exact)	<0.0001

PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio;

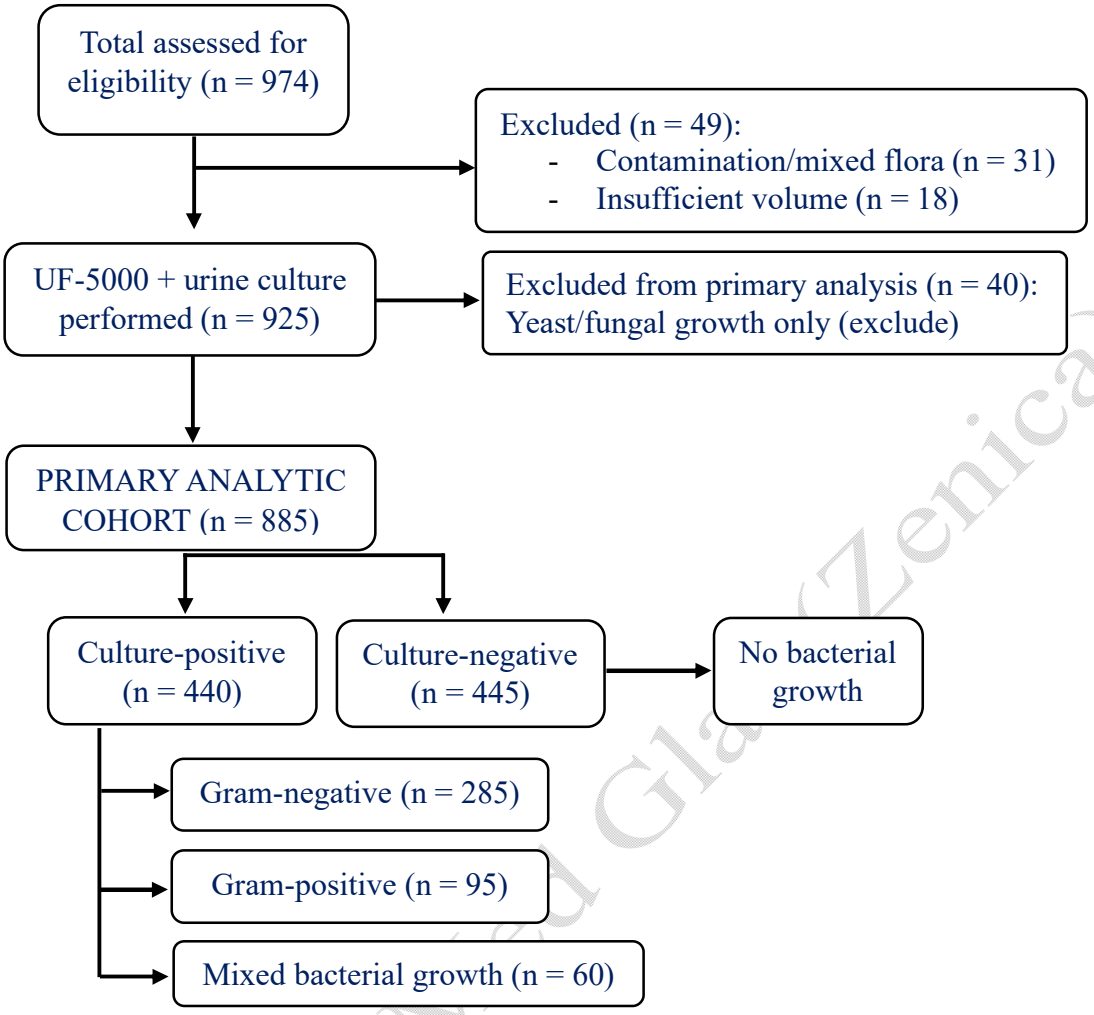

Figure 1. Study flowchart

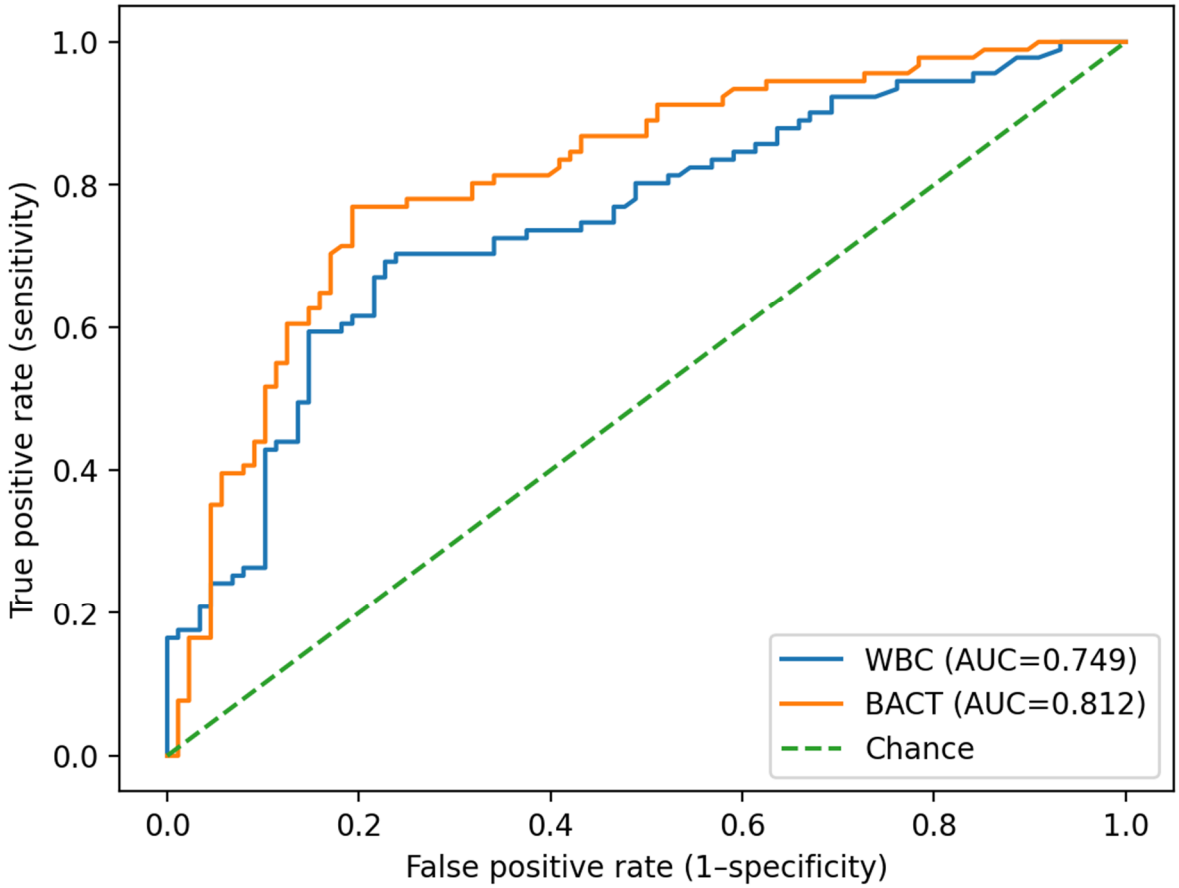

Figure 2. Receiver operating characteristic (ROC) curves for white blood cell count (WBC) and bacterial count (BACT) versus culture positivity

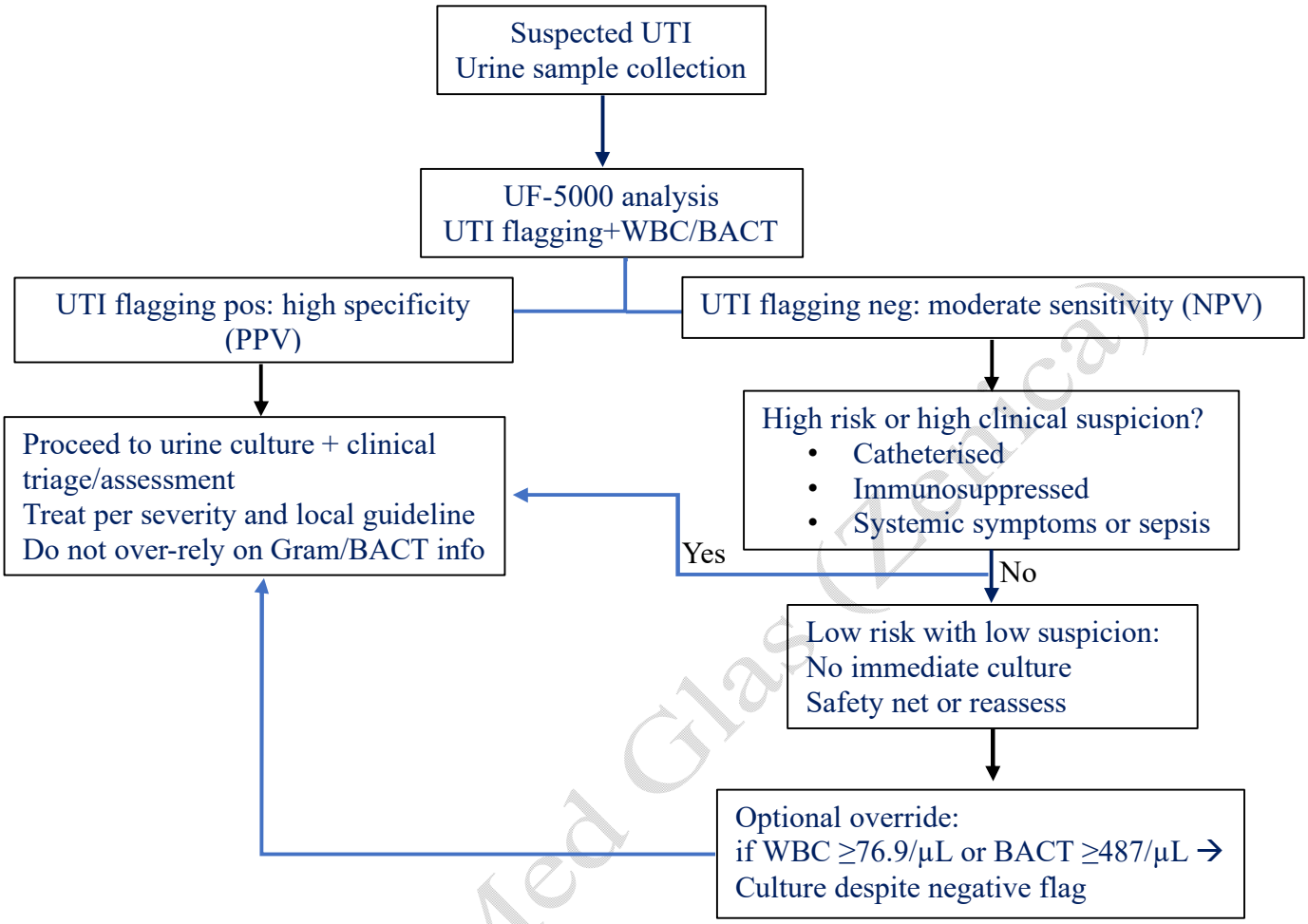

Figure 3. Clinical algorithm for urinary tract infection (UTI) using UF-5000 analysis, UTI flagging, and white blood cell (WBC) and bacterial count (BACT)

SUPPLEMENTAL DATA

Table S1. Table of urinary tract infection (UTI) flagging vs culture

Index test result	Positive culture (N = 440)	Negative culture (N = 445)	Total
UTI flagging positive (UTI)	295	35	330
UTI flagging negative (non-UTI)	145	410	555
Total	440	445	885

Table S2. Subgroup diagnostic performance of urinary tract infection (UTI) flagging with 2x2 contingency data

Subgroup	N	Positive culture prevalence	2×2 table (Flag+/flag- vs culture+/culture-)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
With urinary catheter	420	59.5% (250/420)	TP=155, FN=95, FP=20, TN=150	0.620 (0.558–0.679)	0.882 (0.821–0.927)	0.886 (0.830–0.927)	0.612 (0.545–0.676)
Without urinary catheter	465	40.9% (190/465)	TP=140, FN=50, FP=15, TN=260	0.737 (0.668–0.798)	0.945 (0.911–0.968)	0.903 (0.845–0.943)	0.839 (0.791–0.879)
Immunosuppressant: Yes	165	66.7% (110/165)	TP=65, FN=45, FP=10, TN=45	0.591 (0.495–0.682)	0.818 (0.689–0.909)	0.867 (0.772–0.930)	0.500 (0.395–0.605)
Immunosuppressant: No	720	45.8% (330/720)	TP=230, FN=100, FP=25, TN=365	0.697 (0.645–0.745)	0.936 (0.906–0.958)	0.902 (0.857–0.935)	0.785 (0.745–0.821)
Female sex	485	55.7% (270/485)	TP=225, FN=45, FP=20, TN=195	0.833 (0.783–0.875)	0.907 (0.861–0.941)	0.918 (0.875–0.949)	0.812 (0.756–0.860)
Male sex	400	42.5% (170/400)	TP=70, FN=100, FP=15, TN=215	0.412 (0.340–0.487)	0.935 (0.895–0.962)	0.824 (0.731–0.893)	0.683 (0.628–0.734)
Age: 18–<65	695	48.9% (340/695)	TP=210, FN=130, FP=30, TN=325	0.618 (0.565–0.668)	0.915 (0.879–0.943)	0.875 (0.826–0.914)	0.714 (0.670–0.755)
Age: ≥65	145	51.7% (75/145)	TP=65, FN=10, FP=0, TN=70	0.867 (0.769–0.931)	1.000 (0.949–1.000)*	1.000 (0.945–1.000)*	0.875 (0.786–0.934)

Note: The perfect specificity and PPV in the ≥65 age group should be interpreted with caution given the small sample size (n = 145) and the absence of false positives in this stratum. The confidence interval lower bound for specificity is 0.949, reflecting uncertainty around the point estimate of 1.000. Age-stratified analyses were restricted to adults; 45 patients aged <18 years (25 culture-positive, 20 culture-negative) were retained in the overall cohort (N = 885) and in all other analyses but were not assigned to an adult stratum, so the two age strata sum to N = 840.

Table S3. Gram agreement of BACT-info Gram (derived from Gram positivity or Gram-negative indicators) vs culture Gram

Culture Gram	Gram-negative	Gram-positive	Total
Gram negative	235	15	250
Gram positive	5	65	70
Total	240	80	320

Note: Denominator (n = 320) represents bacterial culture samples with unambiguous Gram classification (Gram-negative or Gram-positive) after excluding: mixed bacterial growth (n = 60), no growth (n = 445), and samples with indeterminate indicator outputs (both positive or both negative; n=60). Cohen's κ = 0.826 (bootstrap 95% CI 0.746–0.893).

Table S4. Gram agreement of BACT-info category label (flagging BACT-info mapped to Gram positive only) vs culture Gram

Culture Gram	Gram-negative	Gram-positive	Total
Gram-negative	115	90	205
Gram-positive	0	40	40
Total	115	130	245

Note: The denominator (n = 245) comprises 380 single-Gram culture-positive samples (Figure 1: 285 Gram-negative and 95 Gram-positive) after excluding 135 samples who's categorical BACT-info label was non-binary ('Mixed', 'Non-classified', or 'No flag'). These non-binary labels affected both Gram classes — 80 Gram-negative samples (285 reduced to 205) and 55 Gram-positive samples (95 reduced to 40) — rather than Gram-negative samples alone. Culture-negative (no-growth, n = 445) and mixed-growth (n=60) samples were excluded by definition. Cohen's κ = 0.294 (bootstrap 95% CI 0.201–0.397).

Table S5. Bootstrap validation of Youden-optimized cutoffs

Parameter	Training set cutoff	Bootstrap median cutoff	Bootstrap 95% CI	Optimism-corrected sensitivity	Optimism-corrected specificity
WBC (/μL)	76.9	78.2	52.3–101.5	0.728	0.768
BACT (/μL)	487	491	382–612	0.788	0.796

Note: The narrow confidence intervals and minimal optimism (≤0.007 difference) suggest that the derived cutoffs are reasonably stable within this dataset. However, external validation in an independent cohort remains necessary before these thresholds can be recommended for clinical use.