Final Technical Report

for

Research Project

Incidences of Urinary Tract Infection among pregnant women visiting Jetsun Pema Mother and Child Hospital, Jigme Dorji Wangchuk National Referral Hospital, Bhutan: A cross -sectional study.

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Disclaimer

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Final summary report on the completion of the study

April 24, 2026

1. Title:

Incidences of Urinary Tract Infection among pregnant women visiting Gyaltsuen Jetsun Pema Mother and Child Hospital, Jigme Dorji Wangchuck National Referral Hospital, Bhutan: A Cross-Sectional Study.

2. Purpose:

To determine the incidence of UTI among the pregnant women visiting the Gyaltsuen Jetsun Pema Mother and Child Hospital for antenatal checkup using fully automated urine particle analyzer (Sysmex UF 5000). This study will also attempt to assess overall effectiveness and usefulness of Sysmex UF 5000 in rapid diagnosis of UTI in healthcare setting of Bhutan.

3. Study Period

Research approval date: 2025/05/01
Contract signing date: 2025/05/08
Research Completion date: 2026/03/23

4. Introduction

Urinary tract infections (UTIs) are one of the leading causes of higher morbidity and mortality in the developing countries[1,2]. UTIs are commonly caused bacterial infections worldwide and represent the most frequent infectious complication during pregnancy. The global prevalence of UTIs (both symptomatic and asymptomatic) in pregnant women is 23.9%[3]. Both symptomatic and asymptomatic UTIs are prevalent during pregnancy due to physiological and anatomical changes such as urinary stasis, ureteral dilatation, and hormonal effects that predispose women to bacterial colonization and infection of the urinary tract. UTIs during pregnancy have been associated with adverse outcomes, including preterm delivery and low birth weight, for which prompt recognition and treatment are strongly recommended. Asymptomatic bacteriuria occurs in a substantial minority of pregnancies, and many guidelines emphasize early screening [4-6]. Therefore, early detection and treatment of UTI during pregnancy is crucial to prevent complications and ensure the well-being of both the mother and fetus.

Urine culture is the gold standard method to identify and quantify the presence or absence of bacteria in urine. However, with rapid technological advancements in the medical laboratory sciences, automatic flow cytometers for urine analysis have evolved as a rapid UTI screening method. Screening for urine samples by flow cytometry has reduced the need for culture, significantly diminishing the amount of manual labor required in the microbiology laboratory while expediting the turn-around time for clinicians to initiate an appropriate treatment[7-9].

The Fully Automated Urine Particle Analyzer UF-5000 (Sysmex Corporation, Kobe, Japan, hereafter; UF-5000) is a third generation fully automated urine particle analyzer using measurement principle based on flow cytometry. It can differentiate 17 clinical parameters in human urine along with other human body fluids[10]. Of the 17 parameters, many studies and a meta-

analysis have shown that the parameters for bacterial count (BACT) and white blood cells (WBC) could be detected with high sensitivity (SE) by automated urine sediment analyzers and that both are key for UTI screening[11]. UF-5000 can quantify BACT and WBC within minutes and has shown good accuracy for UTI screening in prior studies[12, 13]. With a novel function to discriminate gram-positive and gram-negative bacteria in urine specimens, the UF-5000 has shown high diagnostic accuracy in bacterial quantification and stainability, as well as in bacteriuria screening with a low rate of false negatives[14-16]. UF 5000 which provides bacteria information (BACT Info.) flags for more accurate bacterial discrimination of urinary tract infections (UTIs), is a reliable diagnostic tool for UTI screening, allowing a quick diagnostic orientation for early clinical empirical medication[17, 18].

In settings where culture turnaround time and laboratory capacity are constrained, rapid screening tools could help identify women at higher risk who require confirmatory testing and timely treatment. Understanding the epidemiology of UTIs is crucial for health care providers to guide effective management and minimize UTI-associated complications among pregnant women as well as in other vulnerable population. In light of this, Bhutan has been selected for this study as, the information about UTIs in pregnant women in Bhutan is limited and has not been investigated comprehensively. The primary objective of this study is to determine the prevalence of UTIs in pregnant women in the JDWNRH hospital using UF-5000. For the secondary outcome, to evaluate the diagnostic performance of Sysmex UF-5000 to screen out for UTI during pregnancy in Bhutan.

5. Materials and Methods

Study design and study population

A cross-sectional study was conducted among pregnant women attending routine antenatal checkups at the Community Health Department, GJPMCH, JDWNRH. A total of 2,000 pregnant women participated in the study between June 30, 2025, and November 18, 2025.

Demographic information:

Demographic information, including age, occupation, residential area, term of pregnancy, and the presence of any UTI related symptoms such as fever, backache, dysuria, or other symptoms was collected from the participants.

Inclusion and exclusion criteria

Pregnant women attending routine antenatal checkups were included in the study. Those with malignancies, autoimmune disorders or with urine sample volume less than 10 ml were excluded from the study.

Ethical consideration

Ethical approval for this study was obtained from the Research Ethics Board of Health (REBH), Bhutan and the Institutional Review Committee, Sysmex Corporation, Japan. A written informed

consent was obtained from each participant by the clinicians /laboratory personnel after a thorough explanation of the purpose of the study.

Specimen collection and transportation

The pregnant women were educated on how to collect clean catch mid-stream urine samples (at least 10 ml) into a sterile container. These containers were then barcoded, properly labelled and subjected to dipstick testing by Semi-Automated Urine Chemistry Analyzer UC-1000 (Sysmex Corporation, Kobe, Japan, hereafter; UC-1000), followed by UF-5000 in the CHD laboratory within 1 hour of collection. Routine quality control and equipment maintenance were performed according to the manufacturer's instructions. The remaining samples were then transported within 2 hours to the main microbiology laboratory in JDWNRH for culture and bacterial identification.

Sysmex UF-5000

Urine samples were analyzed using the UF-5000, a fully automated urine particle analyzer based on flow cytometry. The instrument uses a 488-nm blue semiconductor laser and hydrodynamic focusing in two analytical channels to detect and classify urine particles with high precision. In the analytical process, particles are stained with fluorescent dyes and characterized using optical signals generated from forward scattered light and side scattered light, side fluorescence, and depolarized side scattered light, enabling automated counting and classification of urinary particles. The analyzer has a maximum throughput of up to 105 samples per hour. The required sample volume is 2.0 mL in sampler mode and 0.6 mL in STAT mode, with an aspiration volume of approximately 0.45 mL per measurement.

Microbiology laboratory testing:

Urine specimens were received in sterile, leak-proof containers and stored at 2–8 °C if processing was delayed. Urine specimens were gently mixed and cultured on cystine-lactose-electrolyte-deficient (CLED) agar using a calibrated 1 µL loop. Plates were incubated aerobically at 35–37 °C and bacterial growth was quantified at 24h and 48h intervals. Cultures were assessed for colony count, colony morphology, lactose fermentation, mucoid appearance, and the presence of pure or mixed growth according to laboratory standard operating procedures. Representative colonies were identified by MALDI-TOF MS (VITEK MS, bioMérieux) in accordance with the manufacturer's instructions. Routine quality control was performed for culture media, and instrument calibration.

Statistical Analysis

The results of the UF-5000 BACT and WBC were compared with the urine culture results using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) were calculated for both parameters against the reference standard urine culture. Diagnostic accuracy of bacterial and white blood cells counts for UTIs was assessed by the area under the ROC curve (AUC). The Youden`s Index (J = Sensitivity + Specificity – 1) was applied to determine the optimal cut-off values for discriminating culture-positive from culture-negative samples. Group comparisons between cultures were performed using the Mann-Whitney U test (Wilcoxon rank-sum test). Statistical analysis was performed with Microsoft Excel and JMP software (version 18), supplemented by Python (version 3.10.5 with Scikit-learn 1.6.1 and SciPy 1.15.1). Statistical significance was considered at $p<0.001$.

6. RESULTS

Characteristics of the urine samples and sample selection for analysis

The final analysis was performed on 570 complete urine samples from pregnant women (26 - 33 years; median, 29 years), derived from an initial set of 2,000 samples after exclusion of contaminated and incomplete samples (Figure 1). A total of 2,000 urine specimens were screened during the study period between June 30, 2025, to November 18, 2025. After exclusion of 1,407 contaminated samples, 593 specimens with valid urine culture results were identified. Among these, 23 specimens were excluded because of missing feature data, leaving 570 complete samples for final analysis. Of the samples analyzed, 149 (26.1%) were classified as culture-positive (>10^4 CFU/mL) and 421 (73.9%) as culture-negative (<10^4 CFU/mL). This final cohort was used for subsequent evaluation of the diagnostic performance of the UF-5000.

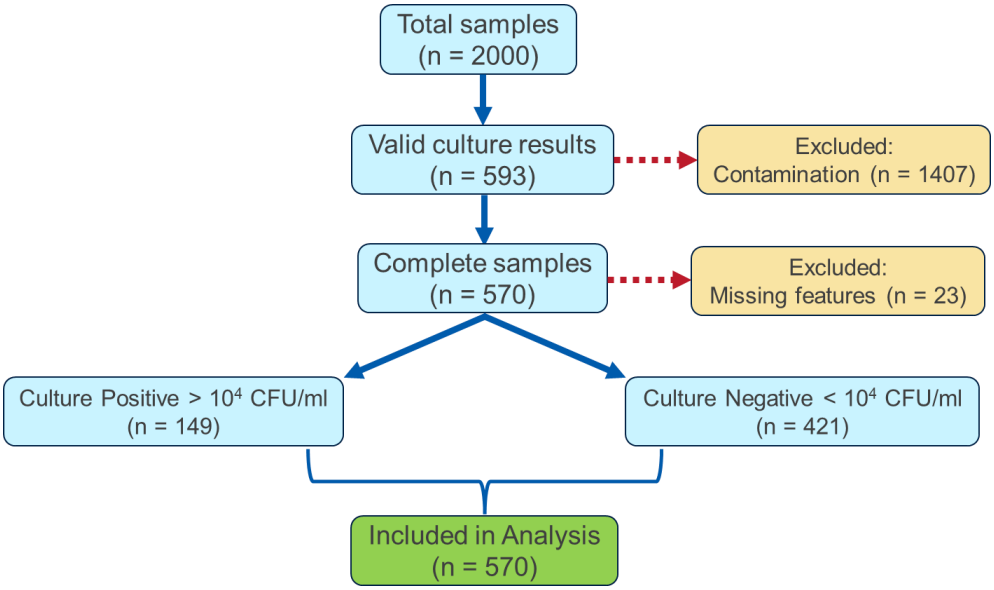

Figure 1. Flow diagram of sample selection for analysis.
Of 2,000 total urine samples, 593 had valid culture results after exclusion of 1,407 contaminated samples. Among these, 23 samples with missing features were excluded, leaving 570 complete samples for analysis. The final dataset comprised 149 culture-positive samples (>10^4 CFU/mL) and 421 culture-negative samples (<10^4 CFU/mL).

Prevalence of UTI during pregnancy

Among the 570 evaluable urine samples, 149 were culture-positive, corresponding to an overall UTI prevalence of 26.1%. Among culture-positive cases, 125 (83.9%) were asymptomatic and 24 (16.1%) were symptomatic, corresponding to 21.9% and 4.2% of the overall evaluable cohort, respectively.

The prevalence of UTI across different trimesters of pregnancy was assessed, with symptomatic and asymptomatic cases. The overall prevalence was highest in the third trimester (42.9%), followed by the second trimester (38.9%), and lowest in the first trimester (18.1%). In the third trimester, 12.5% of cases were symptomatic (n=8), while 87.5% were asymptomatic (n=56).

During the second trimester, 18.9% of cases were symptomatic (n=11), and 81.0% were asymptomatic (n=47). In the first trimester, 18.5% of cases were symptomatic (n=5), whereas 81.48% were asymptomatic (n=22). These findings indicate that the majority of UTI cases in all trimesters were asymptomatic, with the highest overall prevalence observed in the third trimester. With respect to microbiological etiology, *Escherichia coli* was the most frequently isolated organism, accounting for 129 isolates (86.6%), followed by *Staphylococcus saprophyticus* with 11 isolates (7.4%) and *Klebsiella pneumoniae* with 4 isolates (2.7%) (Table 1). These findings indicate that asymptomatic bacteriuria constituted the major burden of UTI during pregnancy, with *E. coli* as the predominant pathogen.

Uropathogens in urine culture	**Count**	**Total %**
Escherichia coli	129	86.6
Staphylococcus saprophyticus	11	7.4
Klebsiella pneumoniae	4	2.7
Acinetobacter baumannii	1	0.7
Citrobacter koseri	1	0.7
Enterococcus faecalis	1	0.7
Klebsiella oxytoca	1	0.7
Proteus mirabilis	1	0.7
Total	**149**	**100**

Table 1. Microbiological Profile of Urinary Isolates in Pregnant Women

UF-5000 bacterial count (BACT) for prediction of culture-positive UTI

ROC curve analysis demonstrated that UF-5000 BACT had good discriminatory ability for identifying culture-positive urine samples, with an area under the curve (AUC) of 0.834 (Figure 2A). Using the optimal cut-off value of 4501/μL, determined by Youden's Index, BACT yielded a SE of 63.8% and a SP of 90.7%. The corresponding PPV and NPV were 70.9% and 87.6%, respectively. At a cut-off value of 2075/μL, BACT showed a SE of 75.8%, SP of 77.9%, PPV of 54.9%, and NVP of 90.1% (Table 2). These findings indicate that BACT has strong rule-out capability and high specificity, although its sensitivity was more moderate. BACT values and distributions were significantly higher in culture-positive samples than in culture-negative samples ($p < 0.001$), supporting the diagnostic utility of BACT as a screening marker for urinary tract infection during pregnancy (Figure 2B).

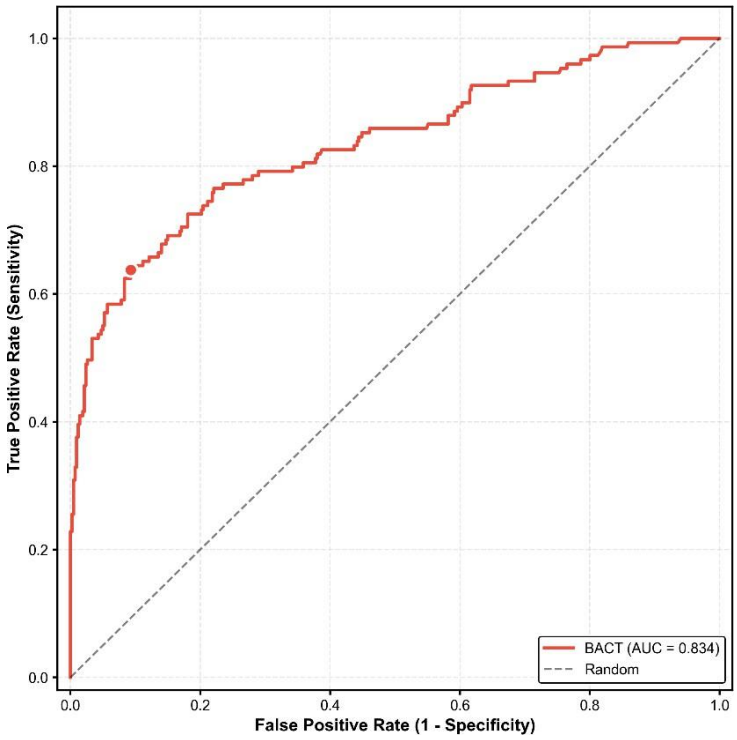

Figure 2A: Receiver Operating Characteristic Curve for Bacteria Count (BACT)
ROC curve demonstrating diagnostic accuracy of BACT for predicting culture-positive results (n = 570 samples). Optimal operating point (circle) determined using Youden's Index, AUC with 95% CI shown with a diagonal dashed line representing random classification (AUC = 0.50).

Variable	BACT count cut-off (/µL)							
	500	1,000	2,075	4,501 †	5,000	10,000	25,000	50,000
Sensitivity (%)	85.9	80.5	75.8	63.8	58.4	46.3	29.5	20.8
Specificity (%)	47.7	63.4	77.9	90.7	92.4	97.6	99.5	100
PPV (%)	36.8	43.8	54.9	70.9	73.1	87.3	95.7	100
NPV (%)	90.5	90.2	90.1	87.6	86.3	83.7	80	78.1
TP	128	120	113	94	87	69	44	31
FN	21	29	36	55	62	80	105	118
TN	201	267	328	382	389	411	419	421
FP	220	154	93	39	32	10	2	0

Table 2. Different cut-off values for BACT counts in UF-5000. Sample n = 570 | AUC = 0.834 | † = Youden's Index optimal cut-off

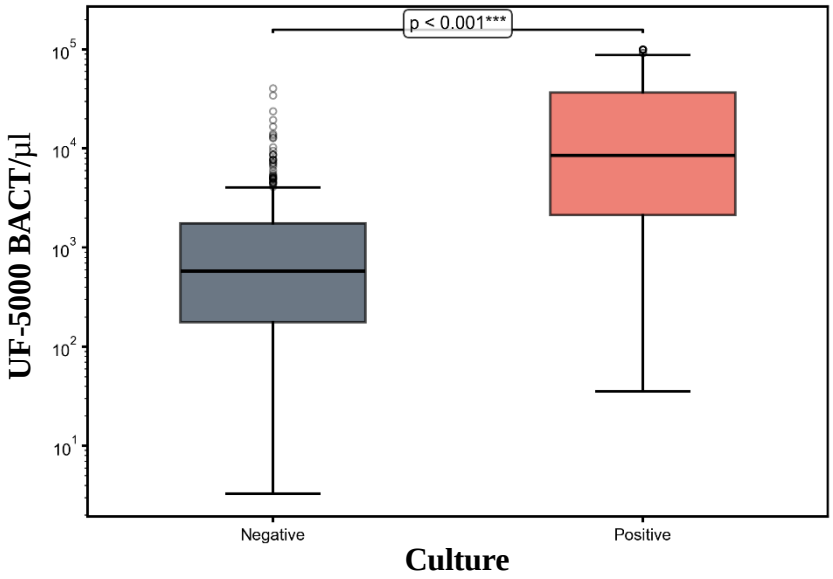

Figure 2B: Sample distribution between Culture-Negative and Culture-Positive samples
The box plot displays logarithmic transformation of the BACT readings across both groups: Negative (n = 421) and Positive (n = 149) with their respective p-values from Wilcoxon rank-sum tests for pairwise comparisons.

UF-5000 White blood cell (WBC) for prediction of culture-positive UTI
UF-5000 WBC count provided moderate discriminatory performance for predicting culture-positive urine samples, with an AUC of 0.726 (Figure 3A). Using the optimal threshold of 6 cells/µL, identified by Youden's Index, the test yielded 74.5% sensitivity and 59.9% specificity with a PPV of 39.6% and a NPV of 86.9%. The distribution of WBC counts differed significantly between culture-positive and culture-negative groups ($p < 0.001$), with higher values observed in culture-positive samples supporting the association between pyuria and bacteriuria (Figure 3B). Collectively, these results indicate that UF-5000 WBC count has clinical utility as a rule-out screening marker, but the limited specificity reduces its effectiveness as an independent predictor of culture-positive UTIs.

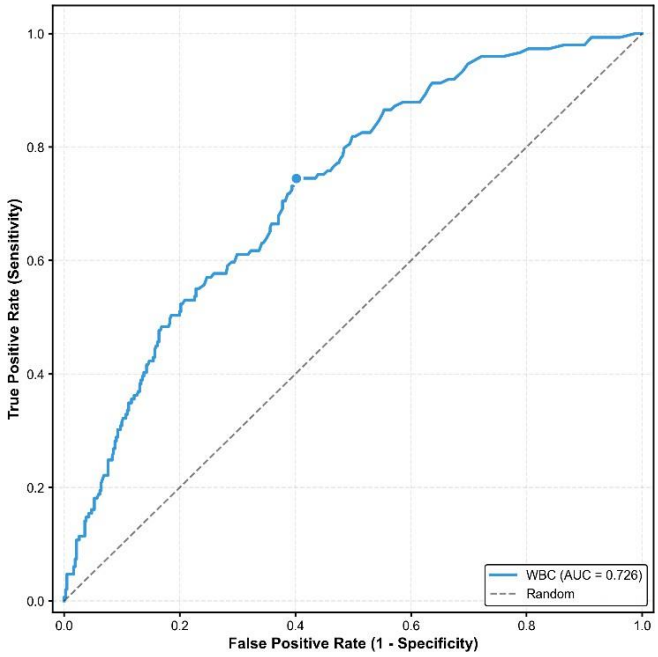

Figure 3A: Receiver Operating Characteristic Curve for White Blood Cells Count (WBC) ROC curve demonstrating diagnostic accuracy of WBC for predicting culture-positive results (n = 570 samples). Optimal operating point (circle) determined using Youden's Index, AUC with 95% CI shown with a diagonal dashed line representing random classification (AUC = 0.50).

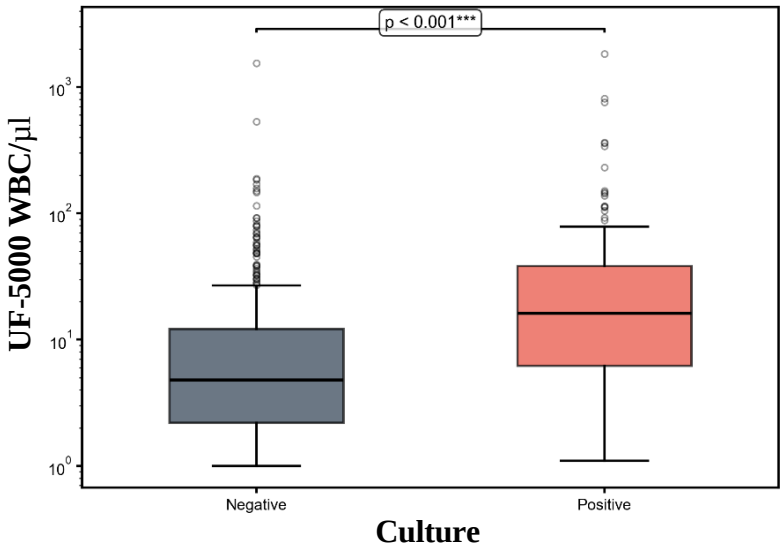

Figure 3B: Sample distribution between Culture-Negative and Culture-Positive samples The box plot displays logarithmic transformation of the WBC readings across both groups: Negative (n = 421) and Positive (n = 149) with their respective p-values from Wilcoxon rank-sum tests for pairwise comparisons.

Diagnostic performance of combined UF-5000 WBC and BACT

The combination of UF-5000 WBC and BACT displayed clinically meaningful diagnostic accuracy for predicting culture-positive UTI, with an AUC of 0.833. Using the optimal decision threshold, the combined model achieved 74.5% sensitivity and 91.7% specificity, with a PPV of 72.6% and an NPV of 87.3%. Notably, combining WBC with BACT improved specificity relative to WBC alone, while preserving sensitivity. Nevertheless, the AUC of the combined model (AUC 0.833) was nearly identical to that of BACT alone (AUC 0.834), indicating that the addition of WBC provided only limited incremental improvement over BACT as a standalone predictor (Figure 4).

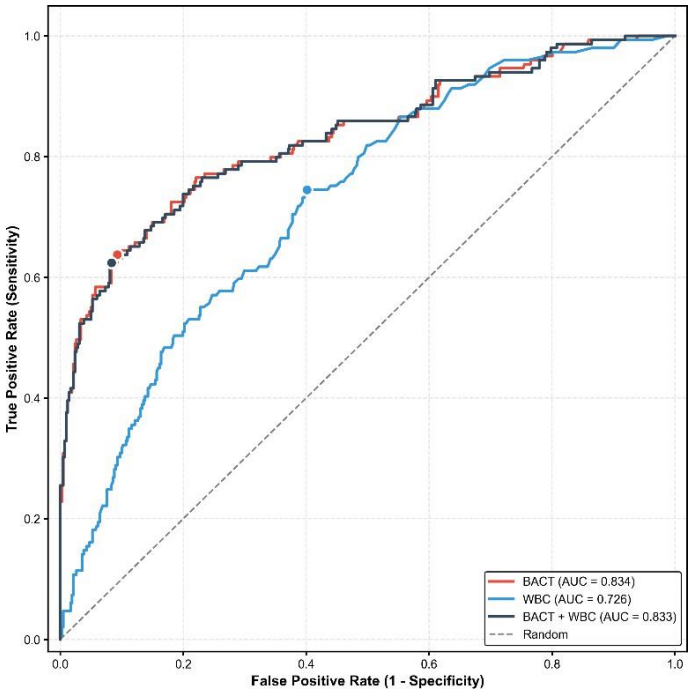

Figure 4: Comparison of Individual and Combined Biomarkers

ROC curves comparing BACT, WBC, and combined logistic regression models. The combined model (BACT+WBC) uses standardized predictors with maximum likelihood estimation. Optimal operating point (circle) determined using Youden's Index, AUC with 95% CI shown with a diagonal dashed line representing random classification (AUC = 0.50)

UF-5000 showed better performance than dipstick analysis

Compared with urine culture, dipstick nitrite positivity showed the highest specificity (98.8%) and positive predictive value (90.2%), but low sensitivity (30.9%), identifying 46 true-positive cases and missing 103 culture-positive samples. Dipstick leukocyte positivity showed higher sensitivity (66.4%), but lower specificity (56.1%) and positive predictive value (34.9%), with 99 true-positives, 185 false-positives, and 50 false-negatives (Table 3). UF-5000 BACT demonstrated a more balanced diagnostic performance than the dipstick parameters, with higher sensitivity (63.8%) than nitrite and greater specificity (90.7%) than leukocyte esterase for detecting culture-positive UTIs in this cohort.

Test	SE (%)	SP (%)	PPV (%)	NPV (%)	TP	FP	TN	FN
Dipstick NIT	30.9	98.8	90.2	80.2	46	5	416	103
Dipstick LEU	66.4	56.1	34.9	82.5	99	185	236	50

Table 3. Diagnostic performance of dipstick nitrite (NIT) and leukocyte esterase (LEU) compared with urine culture.
Total samples (n = 570 | Culture Positive = 149 | Culture Negative = 421)
NIT positive = + /LEU positive = (1+, 2+, 3+)
TP = True Positive; FP = False Positive; TN = True Negative; FN = False Negative
PPV = Positive Predictive Value; NPV = Negative Predictive Value

7. Discussion

This cross-sectional study demonstrated that urinary tract infection remains a substantial burden among pregnant women in Bhutan, with an overall culture-positive prevalence of 26.1%. Notably, the majority of infections were asymptomatic, accounting for 83.9% of all culture-positive cases, whereas only 16.1% were symptomatic. These findings underscore the clinical importance of routine screening during pregnancy, as reliance on symptoms alone would fail to identify a large proportion of infected women. The predominance of asymptomatic bacteriuria observed in this study is particularly important in antenatal care, because untreated bacteriuria during pregnancy may progress to symptomatic infection and contribute to adverse maternal and fetal outcomes if left untreated[19]. In the present cohort, UTI prevalence was highest in the third trimester, followed by the second and first trimesters, suggesting that the burden of infection may increase with advancing gestation. This trend may reflect physiological and anatomical changes during pregnancy that promote urinary stasis and facilitate bacterial colonization.

The microbiological profile was dominated by *Escherichia coli* (86.6%), consistent with the established etiological pattern of UTIs during pregnancy[19]. This distribution indicates that the epidemiology of UTIs in this pregnant population is largely driven by classical enteric pathogens, with *E. coli* as the predominant causative organism. These findings support the continued relevance of screening strategies that can rapidly detect bacteriuria in antenatal settings. In addition, the ability of UF-5000 which provides Bacteria Gram dye-affinity Information (BACT-Info.) flags for more accurate bacterial discrimination either Gram positive or Gram-negative, enables a quick diagnostic guidance for early clinical empirical medication[10,16].

We also evaluated the clinical utility of the UF-5000 as a screening tool for UTI during pregnancy. Among the UF-5000 parameters, bacterial count (BACT) showed the strongest diagnostic performance, with an AUC of 0.834, indicating good discrimination between culture-positive and culture-negative samples. At the Youden`s Index-derived cut-off (4501/µL), BACT achieved 63.8% sensitivity and 90.7% specificity, with an NPV of 87.6%. At the lower threshold (2075/µL), sensitivity increased to 75.8%, although specificity decreased to 77.9%. These findings suggest that BACT has practical value as a screening marker, particularly when the aim is to reduce unnecessary urine cultures while maintaining acceptable rule-out performance.

The WBC count also showed significant discrimination between culture-positive and culture-negative samples, but its overall diagnostic performance was lower than that of BACT (AUC 0.726). At the optimal cut-off of 6 cells/µL, WBC provided 74.5% sensitivity but only 59.9% specificity, indicating that pyuria alone is less specific for culture-confirmed infection in this population. When BACT and WBC were combined, specificity improved to 91.7% while sensitivity remained at 74.5%; however, the combined model did not meaningfully improve the AUC over BACT alone (0.833 vs 0.834). This suggests that bacterial quantification is the principal contributor to diagnostic accuracy, while the additional value of WBC is limited. Consistent with previous reports, the AUC for bacterial count was higher than that for WBC count, suggesting that bacterial quantification may provide better diagnostic discrimination for culture-positive UTI[17].
In the present study, dipstick nitrite and leukocyte esterase showed contrasting diagnostic profiles compared with urine culture, consistent with the known limitations of dipstick testing [10,11]. Although nitrite demonstrated excellent specificity, the underlying low sensitivity indicates that many culture-positive infections would be missed, making it unsuitable as a standalone screening tool. Leukocyte esterase improved detection rates but had a high false-positive rate, limiting its ability to distinguish true infection from noninfectious pyuria or other causes of leukocyturia. In contrast, UF-5000 BACT showed a more balanced diagnostic profile, with greater sensitivity than nitrite and higher specificity than leukocyte esterase. These findings suggest that UF-5000 BACT may provide better overall screening utility than dipstick parameters in pregnant women, supporting its potential role as a rapid and objective screening method in antenatal care, particularly where rapid identification of patients requiring confirmatory urine culture is important. Nevertheless, urine culture remains essential for definitive confirmation. Prior studies have also shown that UF-based bacterial parameters can outperform conventional dipstick testing, supporting the clinical utility of UF-5000 as an early screening tool[10,11].

This study also highlights an important operational challenge in routine antenatal screening; of the 2,000 initially screened urine samples, 1,407 (71.2%) were excluded because of contamination, which markedly reduced the number of interpretable cultures. This high contamination rate likely reflects the practical difficulty of obtaining clean-catch midstream urine specimens in routine clinical settings, particularly during pregnancy, and may have affected the generalizability of the UTIs prevalence estimate. Nevertheless, this is the first study to provide real-world evidence from Bhutan on the epidemiology of UTIs during pregnancy and demonstrates the feasibility of using UF-5000 to support antenatal UTIs screening in settings where laboratory workload and culture turnaround time may limit timely diagnosis. Urine specimens collected during pregnancy are particularly vulnerable to contamination because of anatomical and collection-related factors. Therefore, improving patient instruction for midstream urine collection and optimizing specimen handling are likely to be essential aspects to strengthen the reliability of future screening programs[6].

Overall, the findings indicate that UTIs, particularly asymptomatic bacteriuria is common among pregnant women in this setting, and that Sysmex UF-5000, especially the BACT parameter, can serve as a useful rapid screening tool to prioritize samples for culture. Even though urine culture remains the reference standard, integration of automated screening into antenatal workflows may improve efficiency, reduce unnecessary cultures, and support earlier identification of women who require microbiological confirmation and clinical follow-up.

8. Conclusion

UTIs were common among pregnant women in this Bhutanese cohort, with a prevalence of 26.1% among evaluable samples, and were predominantly asymptomatic, emphasizing the need for effective routine screening during antenatal care. UF-5000 BACT showed the best diagnostic performance. These findings support the clinical utility of UF-5000 as a practical rapid screening tool for UTIs during pregnancy, particularly for identifying women who should undergo confirmatory urine culture and improving early detection in antenatal care settings.

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