ACCURACY OF THE URINE DIPSTICK VERSUS CULTURE TEST FOR DETECTING ASYMPTOMATIC BACTERIURIA IN PREGNANCY IN KENYA; A RETHINK OF THE TESTING STRATEGY?

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Key words; Asymptomatic bacteriuria, urine culture, dipstick urinalysis, nitrite, leucocyte esterase.

ABSTRACT

Background: The quantitative urine culture, the "gold standard" test for detection of asymptomatic bacteriuria (ASB), is time consuming, expensive, requires special equipment and trained personnel thus limiting its routine use in low-resource settings. Although the dipstick test is cheaper, easier to perform and interpret, its accuracy and role in detecting ASB in pregnancy in this setting has not been evaluated.

Methodology: This was a cross-sectional study of pregnant women without symptoms of urinary tract infection, who were receiving routine antenatal care at Kenyatta National Hospital, in Nairobi Kenya. Clean catch, mid-stream urine specimens from 132 eligible participants were subjected to concurrent dipstick urinalysis and bacteriologic culture. Markers of ASB in urine dipstick (presence of either or both leucocyte esterase (LE) and nitrites) were compared with culture. Accuracy of urine dipstick, as measured from the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) in detecting ASB was estimated using culture as the "gold standard."

Results: Out of 320 women screened, 132 (41%) were eligible. Prevalence of ASB was 6.9%. Sensitivity, specificity, PPV and NPV were 66.7%, 74.4%, 16.2% and 96.8% respectively for LE; 44.4%, 97.5%, 57.1% and 95.9% respectively for nitrite; 22.2%, 100%, 100% and 94.5% for either LE or nitrite; and 88.9%, 71.9%, 19% and 98.9% for both LE and nitrite respectively. The PLR and NLR for LE was 2.61 and 0.45 whereas that of nitrite was 17.76 and 0.37 respectively.

Conclusion: A negative urine dipstick test rules out ASB in pregnancy and the need for routine culture. Positive dipstick test has low accuracy in detecting ASB in pregnancy and requires confirmatory testing with culture. This would avoid exposing pregnant women to unnecessary antibiotics and their side effects.

INTRODUCTION

Asymptomatic bacteriuria (ASB) is defined as the presence of live bacteria in the urinary tract in counts of ≥ 105 colony forming units (CFU) of a single bacterial species per millilitre of urine in an individual without symptoms of urinary tract infection(s) (UTI)(1). Although there is no benefit in treating ASB in the general population, anatomic and physiologic changes in pregnancy increase the risk of progression of ASB to symptomatic UTI. Both ASB and symptomatic UTI are associated with increased risk of adverse obstetric outcomes such as preterm deliveries, low birth weight infants, foetal growth restriction, preterm premature rupture of membranes and pre-eclampsia(2)(3)(4).

Routine screen-and-treat policies for ASB have been incorporated in most professional and national antenatal care (ANC) guidelines, because treatment of ASB in pregnancy significantly reduces the incidence of adverse obstetric outcomes(5). For example, the American College of Obstetricians and Gynaecologists and National Institute of Clinical Excellence guidelines recommend urine culture in early pregnancy or at the first antenatal visit if later(6)(7). The quantitative urine culture, the "gold standard" test for detecting ASB is costly, time-consuming, requires special equipment and trained personnel, thereby limiting its routine use in low-resource settings. The Kenyan Ministry of Health (MOH) guidelines recommend detection of ASB using a urine dipstick test because it is cheaper, easier to perform and interpret compared to quantitative urine culture(8). Positive urine dipstick tests are reported as either leucocyte esterase (LE) positive or nitrite positive or both. The LE tests for the presence of LE enzyme released from degraded white blood cells (WBC), depicting presence of WBC in urine. Nitrite tests for the presence of nitrites derived from conversion of dietary nitrate in urine to nitrite by bacteria present in the urine. In the MOH protocol, pregnant women with positive urine dipstick are usually presumptively treated with antibiotics. However, wide variability in sensitivity and specificity of the dipstick test in detecting ASB has been reported for both LE and nitrites. These varying reports have resulted in a lack of consensus in recommendation of the most appropriate diagnostic test for ASB in pregnancy in low-resource settings(9).

The aim of this study was to determine the accuracy of urine dipstick in detecting ASB by estimating the sensitivity, specificity, positive predictive values, negative predictive values, positive likelihood ratios and negative likelihood ratios in this low resource setting. The markers of likely ASB in the dipstick test were LE and nitrites and urine culture, the gold-standard for diagnosing ASB in pregnancy.

METHODOLOGY.

Study design: This was a cross-sectional study of pregnant women receiving routine ANC. They had no symptoms or history of treatment for UTI in the current pregnancy. Consenting pregnant women provided clean-catch midstream urine specimens which were concurrently subjected to dipstick (for LE and nitrite) and culture tests.

Study setting: The study was conducted at the ANC clinics at Kenyatta National Hospital (KNH) in Nairobi, Kenya from November 2017 to January 2018. KNH is Kenya's largest referral and teaching hospital, forming the apex of Kenya's public healthcare system. The clinic handles approximately 600 pregnant women per week. In this clinic, ASB diagnosis and treatment is based on positive urine dipstick for LE, nitrites or both. Cultures are not routinely performed for detection of ASB in pregnancy.

Study population: Pregnant women of legal consenting age were eligible if they had no symptoms suggestive of UTI (lower abdominal pain, fever, dysuria and frequency of micturition), vaginal bleeding, vaginal discharge, drainage of liquor, antibiotic treatment in the preceding month.

Sample size: Using the Buderer et al formula(10), assuming a two tailed $Z_{(\alpha/2)}$ of 0.05, $Z\beta = 1.96$, 95% confidence interval and an estimated prevalence of 40% ASB in pregnancy, a minimum sample size of 118 patients was calculated.

Study procedures: Eligible women were briefed about the study and those who provided informed consent enrolled. A structured questionnaire on socio-demographic and reproductive health characteristics

was administered. Participants then provided clean-catch mid-stream urine specimens into sterile containers supervised by a trained nurse research assistant. No antiseptics were used prior to collection

Box 1: Reading of results of dipstick and culture tests.

Nitrite component of the dipstick test was read as positive if the reagent pad turned pink, otherwise it was negative. The LE component was read as positive if the reagent pad matched a colour code of trace, +, ++ or +++. A positive dipstick test was defined by the presence of nitrites or a reaction greater than or equal to a trace of leukocytes. Combination of LE and nitrite test was interpreted as positive if any one or both LE or nitrite tests were positive and as negative only when both LE and nitrite tests were negative. The microbiologist was blinded to the results of dipstick test. The second urine sample was kept in a cool box at 4 degrees Celsius and delivered to the laboratory within one hour of collection for culture testing. Urine samples for culture were classified as "sterile" if no growth was obtained, as "significant growth" if growth of the pathogen(s) was at a count $\geq 10^5$cfu/ml of urine and as "insignificant growth" if growth of $<10^5$cfu/mL was obtained.

of specimen. Each sample from each participant were divided into two parts, one part for the dipstick test, to test for the presence of nitrite and LE in the ANC clinic and a second part for urine culture in the microbiology laboratory.

Data were entered into an Excel spreadsheet and analysed using the Statistical Package for Social Science SPSS® version 21. Chi-square goodness-of-fit test was used to test for association between positive culture results and the socio-demographic and reproductive health characteristics. The diagnostic value of the LE and nitrite components of the dipstick test method in terms of sensitivity, specificity, predictive values and likelihood ratios were evaluated against urine culture as the gold standard.

Ethics: This study was approved by the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee and KNH administration (Protocol number P684/09/2016). Informed consent was also obtained from every willing eligible participant.

RESULTS

Three hundred and twenty pregnant women were screened (Figure 1). Eligible women 132(41%) were enrolled and gave urine samples. Two urine samples spilled and the remaining130 were subjected to both dipstick and culture tests.

Nine out of 130 urine samples were culture-positive,

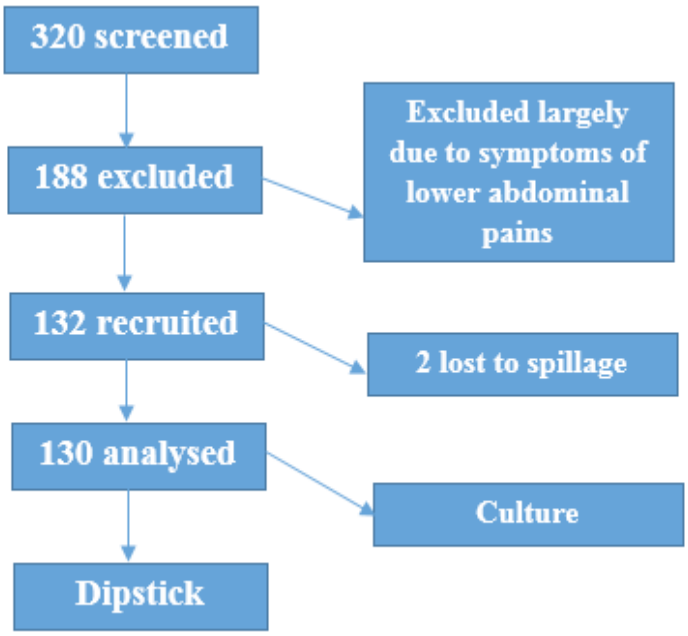
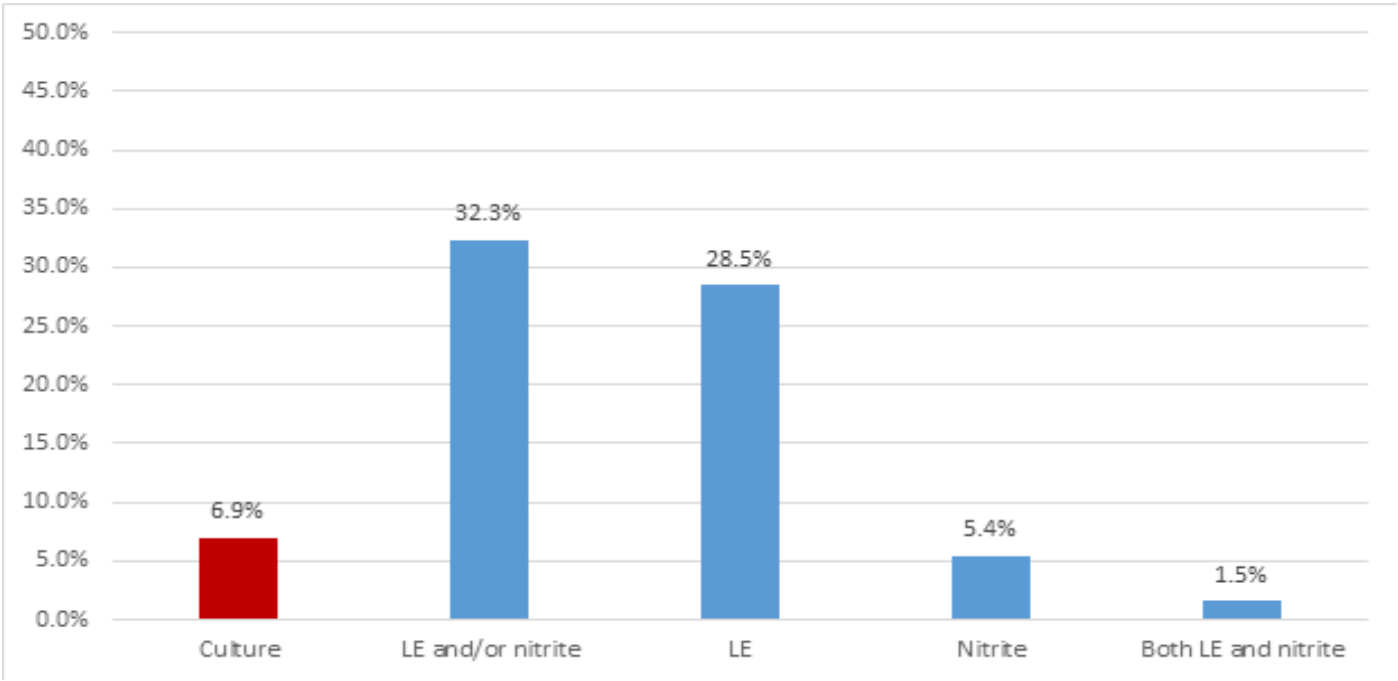


Figure 1: Flow chart of study participants

giving an overall prevalence of asymptomatic bacteriuria (ASB) of 6.9% (figure 2). In the urine dipstick test, 37 (28.5 %) samples were positive for leucocyte esterase (LE) while 7 (5.4 %) samples were positive for nitrite. A total of 42 (32.3 %) urine samples were dipstick positive for either LE and/ or nitrite and 2 (1.5 %) samples had both dipstick positive LE and nitrite. Negative urine dipstick for both LE and nitrite occurred in 88 (67.7 %) of the samples.

The baseline socio-demographic and reproductive characteristics are summarized in Table 1. There were no significant associations between socio-demographic, reproductive health characteristics and positive urine cultures.

The mean age of the participants was 30 years (SD= 5.5) and more than half (57.7%) were aged 26 –



*LE – Leucocyte esterase

Figure 2: Positive urine culture and dipstick results (LE and nitrite) of study participants.

Table 1: Socio-demographic and reproductive characteristics stratified by culture-diagnosed asymptomatic bacteriuria among the study population

Characteristic	Number (%)	Culture Positive No. (%)	Culture Negative No. (%)	p value
	(Mean 30, SD 5.5)			
Age (years)				
18 – 25	29(22.3)	2 (22.2)	27 (22.3)	
26 – 35	75(57.7)	5 (55.6)	70 (57.9)	0.984
>35	26(20.0)	2 (22.2)	24 (19.8)	
Occupation				
Unemployed	29(22.3)	1 (11.1)	28 (23.1)	
Self employed	63(48.5)	5 (55.6)	58 (47.9)	0.705
Formal employment	38(29.2)	3 (33.3)	35 (28.9)	
Marital status				
Single	19(14.6)	1 (11.1)	18 (14.9)	0.758
Married	111(85.4)	8 (88.9)	103 (85.1)	
Highest education level				
None	1(0.8)	0 (0.0)	1 (0.8)	
Primary	16(11.6)	1 (11.1)	13 (10.7)	
Secondary	39(30.0)	4 (44.4)	35 (28.9)	0.556
Tertiary	76(58.5)	4 (44.4)	72 (59.5)	
Parity				
0	54(41.5)	1 (11.1)	53 (43.8)	
1	37(28.5)	5 (55.6)	32 (26.4)	0.172
2	27(20.8)	2 (22.2)	25 (20.7)	
≥3	12(9.2)	1 (11.1)	11 (9.1)	
Gestational age (weeks)				
<13	12(9.2)	1 (11.1)	11 (9.1)	
13 – 28	41(31.5)	4 (44.4)	37 (30.6)	0.484
>28	77(59.2)	4 (44.4)	73 (60.3)	
History of UTI in current pregnancy				
Yes	33(25.4)	4(44.4)	29(24.0)	0.166
No	97(74.6)	5(55.6)	92(76.0)	

*SD – Standard deviation, UTI – Urinary tract infection.

Table 2: Sensitivity and specificity of urine dipstick in detection of asymptomatic bacteriuria in the study population.

Dipstick test	Urine culture		Sensitivity (%)	Specificity (%)
	Positive n=9	Negative n=121		
Nitrite test				
Positive	4	3	44.4	97.5
Negative	5	118		
Leucocyte esterase				
Positive	6	31		
Negative	3	90	66.7	74.4
Leucocyte esterase and/or nitrite				
positive	8	34	88.9	71.9
negative	1	87		
Both leucocyte esterase and nitrite				
positive	2	0	22.2	
negative	7	121		100

Table 3; Predictive values and likelihood ratios of the dipstick test for detecting asymptomatic bacteriuria in pregnant Kenyan women

Characteristic	PPV	NPV	Positive LR	Negative LR
LE	16.2%	96.8%	2.61	0.45
Nitrite	57.1%	95.9%	17.76	0.37
Combined LE and nitrite				
Either LE or nitrite	19%	98.9%	2.3	0.46
Both LE and nitrite	100%	94.5%	8.6	0.30

Key: LE – leucocyte esterase, PPV – positive predictive value, NPV – negative predictive value, LR – likelihood ratio.

35 years. About half were self-employed and had attained tertiary level of education (48.5% and 58.5% respectively). Most of them (85.4%) were married. Over half (58.5%) were multigravidae at >28 weeks gestational age (59.2%). One quarter (25.4%) of the study participants had a past history of symptoms or treatment for urinary tract infection at least one month prior to recruitment.

The sensitivity and specificity of dipstick urinalysis in detecting asymptomatic bacteriuria are shown in Table 2. There were 34 false positive cases for either LE or nitrite tests, majority (n=31, 91.1 %) for LE. There were 3 false negative cases for LE and 5 false negative cases for nitrites. No false negative cases occurred when both LE and nitrite were negative. For both components of dipstick, the sensitivities were low 44.4% for the nitrite and 66.7% for LE respectively. However the nitrite test had a high specificity of 97.5% while that of LE was lower at 74.4%. With the combined test (any one or both tests positive) the sensitivity improved to 88.9% and the specificity to 100% (both LE and nitrite).

The positive predictive values (PPV) for both tests were low 57.1% for the nitrite and 16.2% for the LE respectively as shown in Table 3. However, the negative predictive values (NPV) were high at 96.8% for the LE and 97.5% for nitrite. The combined test also gave high NPV; 98.9% for either LE or nitrite and 94.5% for both LE and nitrite. All the positive likelihood ratios were greater than 1, indicating that the positive test results were associated with the presence of ASB. However, a positive nitrite test

was strongly predictive of a diagnosis of ASB as the likelihood ratio was substantially higher than 10.

DISCUSSION

In this study, a negative dipstick effectively ruled out ASB in pregnancy while a positive urine dipstick had low sensitivity, low PPV and low Positive LR for detection of ASB in pregnancy. Thus, a positive dipstick should be subjected to urine culture before diagnosing ASB in pregnancy. This high false positive rate for dipstick implies that treatment based on the presence of LE and nitrites alone would expose 23. 6% (n=31) of pregnant women and their foetuses to unnecessary antibiotic treatment, with its associated adverse effects and costs. Similar findings have been suggested by other studies (9)(11). In India, a 25% false positive rate was reported with the use of LE (11). The high false positive rate could be due to the fact that presence of LE, an enzyme produced by neutrophils, signifies pyuria and not an absolute indicator of bacteriuria. Pyuria may occur with other inflammatory disorders of the urinary tract or may continue for a while after bacteriuria has been cleared(12). These studies suggest that overall, the sensitivity of the LE and nitrite urine dipstick test is low, and therefore it is poor in accurately diagnosing ASB in pregnancy.

The other important finding in this study was the high specificity of urine dipstick nitrite test suggesting the usefulness of the nitrite test in excluding ASB in pregnancy. Specificities upwards of 98% have been found in Northwest Ethiopia (13) and India (14). We found low sensitivity for the nitrite test compared to 35.7% in Northwest Ethiopia (13) and 82.5% in Delhi, India(14). This variation may be due to differences in prevalence and type of uropathogens that possess nitrate reductase, the enzyme that reduces nitrates to nitrites and thus allow for detection of the nitrite by the urine dipstick test kit(15). The high negative predictive values for urine dipstick LE (96.8%), nitrite test (95.9%), combined LE and nitrite test (98.9%) and high specificity if both LE and tests are negative in our study is comparable to that reported in other studies and reviews(9)(14)(16). Thus, a negative LE and nitrite test effectively rules out ASB in pregnancy and eliminates the need for culture.

This study had several strengths. It was the first study in this setting to test the routine MOH policy of urine dipstick against the gold standard of urine culture as recommended elsewhere (6)(7). Therefore, these findings provide critical data to inform local policy and practice. Secondly, the collection of mid-stream urine specimen was supervised to minimize contamination. Also, strict laboratory quality assurance and control procedures were adhered to. The main study limitation was inability to collect strictly early morning urine sample or urine that had stayed in the bladder for at least four hours. However, this did not adversely affect the interpretation of our study findings since in routine practice, urine is collected as soon as the patients present irrespective of duration of stay or time of the day.

CONCLUSION AND RECOMMENDATION

At the point of care, negative urine dipstick tests effectively exclude ASB in pregnancy. The sensitivity of either test alone or in combination is insufficient for detecting ASB due to the high false positive rate, hence a positive test requires confirmation with culture rather than empirical treatment.

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POLICY BRIEF

URINE DIPSTICK SCREEN AND TREAT APPROACH LEADS TO OVERTREATMENT OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY; A RETHINK OF TESTING STRATEGY?

BACKGROUND

Asymptomatic bacteriuria (ASB) in pregnancy is associated with adverse obstetric outcomes if left untreated. ASB can only be detected via testing as it occurs in absence of symptoms. Quantitative urine culture being the gold standard test for the detection of ASB is time consuming, expensive, requires special equipment and trained personnel limiting its routine use in low resource settings. The Kenyan ministry of health (MOH) guidelines recommend urine dipstick testing at every antenatal visit for detection of ASB because it is cheaper, widely available and easier to perform and interpret (1). Pregnant women with positive urine dipstick are usually treated with antibiotics for presumptive diagnosis of ASB. However variable accuracy of the dipstick test has been reported in various systematic reviews leading to lack of consensus on its reliability in detecting ASB (1) (2).

PRIORITY ACTIONS

1. All dipstick positive cases should be subjected to confirmatory culture testing. An initial dipstick test is still useful in excluding those without asymptomatic bacteriuria.
2. Ensure appropriate funding and resources to enable culture testing.

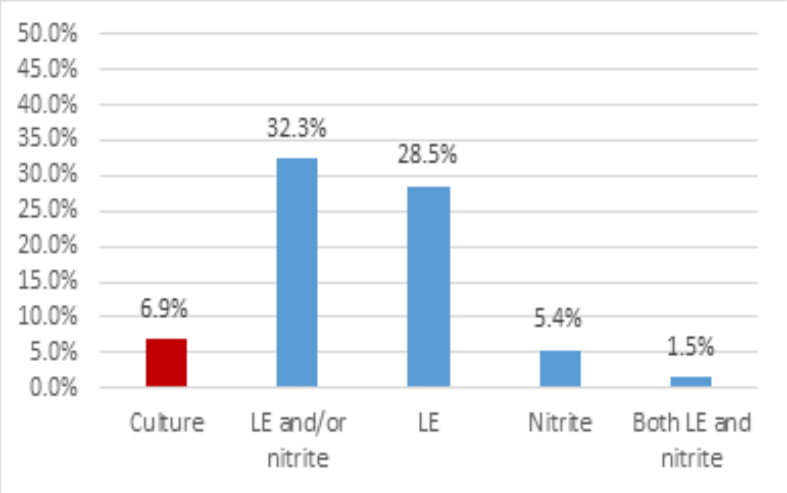
Start by having appropriate laboratory staff and supplies in facilities already offering culture, later equip laboratories in low level facilities.

DIAGNOSTIC VALUE OF THE DIPSTICK TEST.

False positive results can lead to unnecessary prescription of antibiotics to patients while false negative result lead to failure of treatment hence the bacteriuria can progress to cause ASB related complications.

Sensitivity of the dipstick test has been found to be insufficient (high false positive rate) to allow use for detection of ASB hence a positive test would require confirmation with culture (3) (4). Instituting treatment based on LE positive would expose 25% of the mothers and unborn babies to unnecessary antibiotics (4).

Percent of women with positive results on culture and dipstick test (LE and nitrite)



KEY FINDINGS

1. High number of false positives noted with use of dipstick test (LE) compared to culture. (32.3% versus 6.9%)
2. Low sensitivity of the dipstick test in accurately detecting those with ASB.
3. High specificities of the dipstick test (nitrite and the combined test) in excluding those without ASB.

Acknowledgement: The research leading to this publication was conducted through an adaptation of the Structured Operational Research and Training Initiative (SORT IT), a global partnership led by the UNICEF/UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR). The model is based on a course developed jointly by the International Union against Tuberculosis and Lung Disease (The Union) and Médecins sans Frontières. The specific SORT IT programme which resulted in this publication was developed and implemented by the University of Nairobi, Department of Obstetrics and Gynaecology, Nairobi, Kenya with financial support from WHO/TDR.

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IMPLICATIONS

Having an initial dipstick test for all women to exclude those without ASB, and subjecting only the positive dipstick cases for confirmatory testing (culture) instead of giving empirical treatment, will ensure high diagnostic performance, reduction in laboratory costs and workload as not all tests are send for culture analysis.

This will also ensure that pregnant women are not subjected to unnecessary antibiotics which have cost implications, increase the risk of drug resistance and side effects to both mother and foetus.

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