## Research Article

# USE OF REAGENT STRIPS IN THE DETECTION OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY, SHOULD IT BE EMPLOYED?

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## ARTICLE INFO

## ABSTRACT

**BACKGROUND AND AIMS**: Asymptomatic bacteriuria is the major risk factor for developing symptomatic urinary tract infection during pregnancy and untreated urinary tract infection can have adverse foeto-maternal effects. This study was carried out to evaluate the diagnostic competence of reagent strip in detecting asymptomatic bacteriuria of pregnancy.

**METHODS**: The study utilized 600 pregnant women between the gestational ages of 12 to 20 weeks. Mid-stream clean catch urine of about 10 to 15 milliliters was collected; half was tested with reagent strip for nitrite and leucocyte esterase. The other half was sent for culture and bacterial growth of $10^5$ cfu/ml of a single or more species was significant.

The main outcome measures were; sensitivity, specificity, positive and negative predictive values of the reagent strips in diagnosing asymptomatic bacteriuria defined as $10^5$ cfu/ml using urine microscopy, culture and sensitivity as the standard.

**RESULT**: The prevalence of asymptomatic bacteriuria in this study was 17.3%. The sensitivity of the combined reagent strip testing at 47.7% was low. Leucocyte esterase had a better sensitivity and but a lower specificity when compared to nitrite (68.3% vs 53.8% and 92.7% vs 94.0%) but when used in combination, there was a decrease in the validity and reliability when both nitrite and Leucocyte esterase are positive. Due the high specificity when used singly they can be employed to rule out asymptomatic bacteriuria.

**CONCLUSION**: Reagent strip testing incorporating nitrite and leucocyte esterase use singly or in combination is not a sensitive enough screening test to detect asymptomatic bacteriuria in the obstetric patient.

**INTRODUCTION:**

A myriad of anatomic and physiological alterations in the urinary system occurs in the course of pregnancy. Some of such changes which include; dilatation of the ureters with consequent reduced tone which results in stasis of urine and increase vesico-ureteral reflux[1-3], differences in urine pH and osmolality, pregnancy induced glycosuria and aminoaciduria all create an enabling milieu for bacterial multiplication in the urinary tract[4]. These changes begin as early as 6 weeks and peaks at 20 weeks[5].

Asymptomatic bacteriuria refers to actively multiplying bacteria within the urinary tract but with no manifest symptoms of urinary tract infection. It is quite common with prevalence varying from 4-7% depending on the studied population[6-8]. In Nigeria, prevalence of 9.6-21% asymptomatic bacteriuria has been reported in pregnant women[9-11].

Asymptomatic bacteriuria is the major risk factor for developing symptomatic urinary tract infection during pregnancy[12] and about 25% of women with asymptomatic

bacteriuria during pregnancy will develop symptoms subsequently[4]. Other contributing factors recognized as an additional risk for bacteriuria include recurrent urinary tract infection, sexual intercourse, diabetes and anatomical abnormalities of the urinary tract[13,14].

In non-pregnant women, asymptomatic bacteriuria infrequently causes severe illness or significant long-term consequences because in half of such cases, even without antimicrobial therapy the bacteriuria is cleared within 72 hours[12]. In contrast, in pregnancy, it is associated with the risk of intra-uterine growth restriction, low birth weight, preterm labour and the increased cost of medicare[6,10]. While several screening tests for asymptomatic bacteriuria has been proposed, none have been recommended as the ideal screening tool. This study was undertaken to assess the diagnostic competence of reagent strip incorporating leucocyte esterase and nitrite in asymptomatic bacteriuria.

**1.MATERIALS AND METHODS**

**2.STUDY DESIGN**

This was a prospective cohort study conducted from 16th February to 27th November 2013, at the Department of Obstetrics and Gynaecology of the University of Benin Teaching Hospital, Benin City, Edo state, Nigeria. The hospital serves as a major referral centre for four states in Nigeria viz; Delta, Edo, Kogi and Ondo states. The department has 84 beds and undertakes about 2,600 deliveries annually.

**1.STUDY POPULATION**

The subjects in the study were booked pregnant women between 12th and 20th weeks of gestation, presenting at the antenatal booking clinic of the Department of Obstetrics and Gynaecology of the University of Benin Teaching Hospital, Benin City. Excluded from the study were women with diabetes mellitus, sickle cell disease, HIV positive patients, patients with fever, patients with symptoms of urinary tract infection or who had taken antibiotics in the

preceding 48 hours, patient with gestational age equal to or greater than 21 weeks and patients who declined to participate in the study.

The study was fully explained to the patients and their informed consent obtained before recruitment into the study. Ten to fifteen millilitres of freshly voided urine was collected by "midstream clean catch" technique as elucidated by the South Central Association for microbiology (south central association for clinical microbiology)[15]

Each collected urine sample was divided into two sterile containers and labelled (with the patient's name, serial number and hospital number). Dipstick test (life-screen URS -10T LIFE-SAVE BIOTECH UK) was performed within 15 minutes of voiding, using the Multistix, observing the manufacturer's instructions and examining for leukocyte esterase and nitrite in the first set of sealed containers.

The second set of sealed containers was sent to the laboratory within 1 hour for microscopy, culture and sensitivity. Culture was done using the calibrated loop technique. With this technique, a loopful of well mixed uncentrifuged 0.001 ml ($1\mu$ ) of urine was streaked onto the surface of cysteine lactose electrolyte deficient (CLED) agar. This was incubated at 35 to 37°C for at least 24 hours. The number of colonies on the agar multiplied by 1,000 determined the colony forming units. A positive culture was considered as one that grew more than $10^5$ colony forming unit/ml of a single species, while a negative culture was a sample with no growth or growth $<10^5$ colony forming unit/ml or with obvious contamination. The microbiologist was unaware to the result of the dipstick test done in the antenatal clinic. Ethical clearance was given by the ethical and research committee of the hospital and consent was obtained from the pregnant women.

All data were coded and analysis was done using SPSS version 16. The diagnostic competence of leucocyte esterase and nitrite (singly and in combination) were qualified in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using urine culture as the gold standard.

The proportion of agreement between urine culture and the urine dipstick incorporating leucocyte esterase and nitrite used singly and in combination was calculated using the kappa's statistical test

**2.RESULTS**

A total of 600 eligible patients had urine dipstick done between 10th November 2013 and 21st April, 2014. Culture was also done for all the patients as control. Out of the six hundred (600) urine samples that were cultured, one hundred and four (104) grew microorganism giving a prevalence of 17.3%. The age range of the study population was 16 to 43 years. The mean age of the patients was $30.6 \pm 5.3$ and the mean estimated gestational age was $16.4 \pm 2.8$. The highest number of positive cultures occurred in patients with estimated gestational age of 18 weeks accounting for 32 (30.7%) out of the 104.

*Table I:  Test Results Of Urine Samples Collected*

| Indicator | Test Result | | Total No. |
| | Positive (%) | Negative (%) | |
| --- | --- | --- | --- |
| Urine samples | – | – | 600 |
| Culture | 104 (17.3) | 496 (82.7) | 600 |
| Nitrite | 86 (14.3) | 514 (85.7) | 600 |
| Leucocyte esterase | 107 (17.8) | 493 (82.8) | 600 |

The sensitivity of leucocytes esterase for detecting asymptomatic bacteriuria was 68.3% and that of nitrite was 53.8%. When leucocyte esterase and nitrite are both positive the sensitivity dropped to 47.7%, but when either nitrite or leucocyte esterase or both are positive the sensitivity was 84.6% (Table2)

*TABLE 2:Test Of Validity And Reliability Of Nitrite, Leukocyte Esterase, Used Singly And In Combination, With Urine Culture As Control.*

| Screening test | Sensitivity % | Specificity % | PPV % | NPV % | FPR % | FNR % |
| --- | --- | --- | --- | --- | --- | --- |
| Nit | 53.8 | 94.0 | 65.1 | 90.7 | 6.0 | 46.2 |
| LE | 68.3 | 92.7 | 66.4 | 93.3 | 7.3 | 31.7 |
| Nit & LE both pos | 47.7 | 87.2 | 38.3 | 90.9 | 12.8 | 52.3 |
| Either Nit or LE pos or both | 84.6 | 86.6 | 53.3 | 98.6 | 13.3 | 15.4 |

| | | | |
| --- | --- | --- | --- |
| LE | = | Leucocyte esterase | PPV = Positive predictive value |
| NPV | = | Negative predictive value | FPR = False positive rate |
| FNR | = | False negative rate | Nit  =  Nitrite |
| pos | = | positive. | |

The specificity of nitrite was 94.0% and leucocyte esterase was 92.7%. When both leucocyte esterase and nitrite are positive, the specificity dropped to 87.2%, and it dropped further to 86.7% when either nitrite or leucocyte esterase or both are positive

When the tests were evaluated using their predictive values, the positive predictive value for nitrite was 65.1% and leucocyte esterase was 66.4%. When leucocyte esterase and nitrite are both positive, the positive predictive value was 38.3%. The negative predictive value for nitrite was 90.7% and for

leucocyte esterase it was 93.3%. With positive leucocyte esterase and nitrite it was 90.9% but when either nitrite or leucocyte esterase or both are positive it was 98.6% (Table 2).

The false positive rate for nitrite was 6.0% and 7.3% for leucocyte esterase. The false positive rate when nitrite and leucocyte esterase are both positive was 12.8%. When either nitrite or leucocyte esterase or both are positive it increased to 13.3%. The false negative rate for nitrite was 46.2% and for leucocyte esterase 31.7%. With positive nitrite and leucocyte esterase, the false

negative rate was 52.3%, but when either nitrite or leucocyte esterase or both are positive it dropped to 15.4%.

The urine microscopy, culture and sensitivity, in the study yielded growth in 95 (15.8%) pregnant women. Of this; 72 (69.2%) grew *Escherichia coli*, 3(2.9%) grew *Staphylococcus,* 11(10.6%) was *Klebsiella* and 18 (17.3%) grew *Proteus*. All the patients who had positive culture were counselled to take the appropriate antibiotics (based on the sensitivity result) for 7 days, after which repeat urine culture done yielded no growth.

*TABLE 3:  Agreement Between Components Of The Dipstick With Culture (Kappa's Test).*

| Reagent | Kappa  agreement |
| --- | --- |
| Nitrite | 0.51 |
| Leucocyte esterase | 0.60 |
| Nit & LE both pos | 0.32 |
| Either Nit or LE pos or both | 0.61 |

*Kappa Agreement*
*< 0 Less than chance agreement*        *0.01–0.20 Slight agreement*
*0.21– 0.40 Fair agreement*        *0.41–0.60 Moderate agreement*
*0.61–0.80 Substantial agreement*        *0.81–0.99 Almost perfect agreement*
*There was moderate amount of agreement when leucocyte esterase and nitrite were used alone but when combined, it was fair agreement.*

## DISCUSSION

Most studies assessed the performance of reagent strips in detecting asymptomatic bacteriuria in pregnancy in the first trimester[16,17] or across all trimesters[5,6,18]. This study looked at it at between 12 to 20 weeks. The rationale for the upper limit of 20 weeks was to assess the performance of the strips before the physiologic/anatomic changes that occur in the urinary system, reaches its peak[5]. The prevalence of asymptomatic bacteriuria in this study was 17.8%. This was similar to

the finding reported by McCormick et al[14,] but less than that reported by other authors[5,16-19].

A positive nitrite test is interpreted as a definite sign of urinary tract infection. In our study nitrite only correctly identified patient with asymptomatic bacteriuria in only half of the cases. It was able to rule out the disease in 9 out of every 10 patients with asymptomatic bacteriuria. The ascribed reasons for this low sensitivity and high false negative rate are; (i) nitrites in the urine

resides in the bladder for about 4 hours, before returning a positive test, hence the most reliable result is obtained using the first morning urine[20]. However, in this study this was not feasible due to the nature of our antenatal clinic. (ii) Some of the organisms identified reduced nitrate to nitrite or ammonia. Therefore, a negative test implies absence of nitrate due to absence of infection, absence of nitrate substrate in spite of infection or when the nitrite level is reduced below its detection. The culture result yielded no growth in 9 out of every 10 cases with a negative test strip when used singly. However, when used in combination this dropped to 8 in every 10 cases. This implies that in resource limited setting, the reagent strips can be used to rule out asymptomatic bacteriuria. This is also reflected in the low false negative rate which ranges from 6 to 13.3%.

As reported by other authors[5,17] the diagnostic competence of leucocyte esterase (68.3%) was better than nitrite, however, when combined, an improvement was only seen when either or both strips are positive (84.8%). This is also reflected in the high false negative rate which ranges from 31.7 to 46.2% when used singly. This reflects that when used singly, a positive culture result was identified in only 5 to 6 patients with asymptomatic bacteriuria using the test strips. When the complications of asymptomatic bacteriuria are considered against the backdrop of this low sensitivity, they can be considered as inadequate.

There was generally moderate degree of agreement between the individual components used singly with urine culture. This implies that leucocyte esterase and nitrate have fairly good diagnostic competence.

## CONCLUSION

The dipstick test incorporating nitrite and leukocyte esterase when used singly reduced by half the number of patients that it will require urine microscopy and culture. In resource limited setting this can be employed in commencing prophylactic antibiotics. The excellent specificity of nitrite and leucocyte esterase can be utilized to rule out asymptomatic bacteriuria. However, their low sensitivity when used singly or in combination means they cannot be employed as screening test for asymptomatic bacteriuria of pregnancy.

## REFERENCES

1. Cietak KA, Newton JB. Serial quantitative maternal nephrosonography in pregnancy. Br J nephrol 1984:4;198-207.
2. Peake SL, Roxburg HB, Langlois S. Ultrasonic assessment of hydronephrosis: Radiology 1983;78:55-61.
3. Fainstat T. Ureteral dilatation in pregnancy: A review; Obstet Gynecol Surv. 1983;18:845 - 9.
4. Patterson TF, Andriole VT. Bacteriuria in pregnancy. Infect Dis Clin North Am. 1987;1:807-22.
5. Eigbefoh JO, Isabu P, Okpere E, Abebe J. The diagnostic accuracy of the rapid dipstick test to predict asymptomatic urinary tract infection of pregnancy. J Obstet Gynaecol 2008;28:490–495.
6. Gratacos E. Torres PJ, Vila J, Alonso PL, Cararach V. Screening and treatment of asymptomatic bacteriuria in pregnancy in preventing pyelonephritis. J Infect Dis.1994:169:1390–2.

7. Fatima N, Ishrat S. Frequency and risk factors of asymptomatic bacteriuria during pregnancy. J Coll Phys and Surg. Pakistan 2006;16(4):273–5.

8. McIsaac W, Carroll JC, Biringer A, Bernstein P, Lyons E, Low DE. Screening for asymptomatic bacteriuria in pregnancy. J Obst Gynaecol Canada JOGC 2005;27(1):20–4.

9. Nnatu S, Essien EE, Akinkugbe A, Odum CU. Asymptomatic bacteriuria in pregnant Nigerian patients. Clin Exper Obstet Gynaecol 1989;16:126–9.

10. Ojo OA, Akinkugbe OO. The significance of asymptomatic bacteriuria in pregnancy in Ibadan, Nigeria. W Afri J Surg 1976; I-3.

11. Akinloye O, Ogbolu DO, Akinloye OM, Terry Alli OA. Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: a re-assessment. Br J Biomed Sci. 2006;63(3):109-12

12. Christiaens TC, De Meyere M, Verschraegen G. Randomised controlled trial of nitrofurantoin versus placebo in the treatment of uncomplicated urinary tract infection in adult women. Br J Gen Pract 2002;52:729-34.

13. Ronald A. Sex and urinary tract infections. N Engl J Med 1996;335:511–2. doi:10.1056/NEJM199608153350711

14. McCormick T, Ashe RG, Kearney PM. Urinary tract infections in pregnancy. Obs Gynae 2008;10:3:156-62

15. South Central Association for clinical microbiology. (nd). How to provide clean catch urine specimen, retrieved 2 November 2015 from http:/www.scsnm.org/free/

16. Etherington IJ, James DK. Reagent infection strip testing of antenatal urine specimens for infection Br J Obstet Gynaecol 1993;9:(100):806-8.

17. Tincello D, Richmond D. Evaluation of reagent strips in detecting asymptomatic bacteriuria in early pregnancy: prospective case series. Br Med J 1998; 316(7):435-7.

18. Turpin CA, Minkah B, Danso KA, Frimpong EH. Asymptomatic Bacteriuria in Pregnant Women Attending Antenatal Clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. Ghana Med J. 2007;4(1):26-9.

19. Gabre-Selassie S. Asymptomatic bacteriuria in pregnancy; epidemiological clinical and microbiological approach. *Ethiop Med J.* 1998;36:185–192.

20. Pollock HM. Laboratory techniques for detection of urinary tract infection and assessment of value. Am J Med 1983;75:79–84.

21. Jayalakshmi J, Jayaram VS. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian J Pathol Microbiol 2008;51:379-81.