

Evaluation of acridine orange staining of uncentrifuged urine as a screening method for diagnosis of urinary tract infection

Abiroo Jan, M.D.,¹ Akeela Fatima, M.D.,¹ Nighat Akhter, M.Sc.²

¹Senior Resident, Department of Microbiology, SKIMS, Srinagar.

²Potgraduate student of Clinical Biochemistry, University of Kashmir

The term urinary tract infection (UTI) encompasses a variety of clinical entities, including asymptomatic bacteriuria (ASB), cystitis, prostatitis, and pyelonephritis.¹ UTIs are the leading cause of gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters.² UTI is associated with considerable cost in terms of morbidity and economic and research expenditure.³

Evaluation of suspected UTI includes history, physical examination and laboratory investigations. Urine culture and urine analysis for presence of pus cells and bacteria are important in the adequate management of UTI.⁴ Gold standard for diagnosis of UTI is quantitative urine culture for specific bacteria. However, this procedure is costly, and takes at least 24 hours; whereas an ideal test should be cheap, quick and should require only limited technical skill.⁵ Several rapid screening tests are used commonly to make a presumptive diagnosis of UTI, like dipstick biochemical analysis of urine for nitrites or leukocyte esterase (LE), catalase test, glucose oxidase test, automated assays and microscopic examination of urine for white blood cells by wet mount or for bacteria by Gram's stain.⁶

Rapid diagnostic tests can rule out negative samples, are economical, save valuable time and thus are useful in high-end laboratories. Screening is also required in special circumstances where it is difficult to identify UTI on the basis of clinical criteria alone but where early diagnosis and prevention of complications affords significant benefit (e.g.

children, and post renal transplant patients).⁷

In the present study, we evaluated the accuracy, cost and turnaround time of acridine orange staining of quantitative unspun urine for screening of urine samples keeping semiquantitative urine culture as the gold standard for diagnosis of UTI. The first ten consecutive urine samples that were received in the microbiology laboratory daily from patients with suspected urinary tract infection were included in the study. A total of 618 samples were examined in the study. These comprised clean catch mid-stream, catheterized and suprapubic urine samples received in sterile containers.

Acridine orange staining: Hundred microlitre of well mixed uncentrifuged urine was smeared on a slide, air dried, fixed in 96% methanol for 2 minutes, flooded with acridine orange for 2 minutes, washed with distilled water, dried and examined under a fluorescent microscope. Presence of ≥ 1 morphologically similar orange coloured organism / 12 high power fields (40X) was considered significant. Presence of ≥ 2 morphologically different organisms indicated presence of mixed flora.⁸

Urine culture and antimicrobial susceptibility testing were routinely put up in the laboratory and identification of the organisms was done as per the standard procedure.² Time taken by each test for one sample was calculated and compared. *The cost of chemical constituents, consumables and other overhead charges was calculated for acridine orange staining test and for culture and then compared.*

Out of 618 samples, 332 (53.7%) were sterile, 198 (32%) showed significant microbial growth on culture, 22 (3.6%) yielded mixed growth and 66 (10.7%) showed growth of no significance. The results of 88 samples which showed

Correspondence

Dr. Abiroo Jan

Department of Microbiology, SKIMS, Srinagar.

e-mail: abroo_naqash@yahoo.com

mixed growth and growth of no significance were excluded from the study. Among 198 culture positive urine samples, acridine orange stain (Fig. 1) was positive in 192 samples. Sensitivity, specificity, PPV and NPV of the test were calculated taking urine culture as gold standard. Acridine orange has Sensitivity, specificity, PPV and NPV of 96.96, 93.37, 89.71 and 98.10 respectively. Time taken by acridine orange staining test for processing of one sample was 0.25 h which was significantly rapid as compared to culture test. Cost of acridine orange staining was 0.90 INR which compared to urine culture was 15-40 times less.

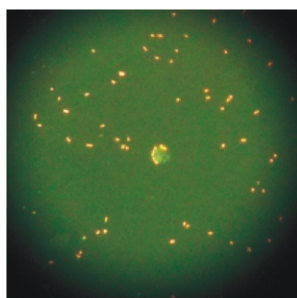


Figure 1: Acridine orange staining of a urine sample showing cocci.

Acridine orange stain as a urinary screening test has not been evaluated as much as Gram's stain. Various studies have reported sensitivity from 92 to 98% and specificity from 59 to 87%.¹² In the present study, we found that this test had sensitivity same as that of Gram's stain i.e. 96.96% but a better PPV (89.71%), specificity (93.37%) and NPV (98.10%). Our results were in concordance with a study by Taneja N et al. who reported this test with a sensitivity of 90% and NPV of 98.8% but he reported lower specificity of 76.6%.⁸ Hoff et al. reported that this test had NPV of 99%.⁹ Based on the results of above studies and ours, it is inferred that when counts of $>10^4$ cfu/ml are taken as significant on culture, this test will eliminate the need for cultures in majority of the specimens. Thus, this technique can be recommended as a routine negative screening test especially in large laboratories.

Negative predictive value is the probability that subjects with a negative test truly don't have the disease. Our study indicated that acridine orange stain had NPV of 98%; that is strong for a screening test, and brings up property of this test to rule out UTI. This is important for a tertiary health care where a large number (more than 50% in our study) of cultures ordered routinely are negative. More than half of the urine samples routinely received in our laboratory during the study period (1400 out of 2880) showed no

significant growth on culture, this results in unnecessary expenditure and delay in patient care. This could be attributed to prior use of antibiotics. In our patient population, 342 patients were on antibiotics at the time of collection of sample and out of these only 102 were culture positive.

Acridine orange staining test was found to be significantly rapid than the culture test. As urine culture takes a minimum of 24 h to show any significant growth, microscopic tests serve as quite good screening tests for UTIs because of their rapidity; particularly in laboratories where a large percentage of urine cultures prove to be negative. The average cost of the screening test was INR 1 as against INR 20 for the urine culture, amounting to a cost saving of 97.5%. Thus, *the financial benefits of screening cannot be overemphasized. This cost factor makes these screening tests a viable and attractive option in peripheral centers where facilities of culture are not available and also in hospitals where majority of urine cultures prove to be negative.*

Acridine orange stain is recommended as a single screening test as it is an accurate, rapid and inexpensive method to rule out UTI but the need of a fluorescent microscope is a limitation to this test in resource poor settings.

REFERENCES

1. Kasper DL, Fauci AS, Hauser SL, Longo DLL, Jameson JL and Loscalzo J. Urinary Tract Infections, Pyelonephritis, and Prostatitis. In: Kalpana Gupta, Barbara W. Trautner. *Harrisons principles of internal medicine*. 19th edition. McGraw-Hill Education publications; 2005: 861.
2. Betty AF, Sahm DF, Weissfeld AS. *Bailey and Scott's Diagnostic Microbiology*, 11th edition. Philadelphia, Mosby publications; 2004: 927-38.
3. Parajuli KP, Chaulagain DD, Singh G, Shrestha M, Chaudhary B, Surendra Chaurasia et al. Diagnosis of urinary tract infection: fresh urine microscopy or culture. *Journal of Biomedical and Pharmaceutical Research*. 2013; 2 (6): 63-64.
4. Ninama AB and Shah PD. Comparison of various screening methods for presumptive diagnosis of significant bacteriuria. *Int J Med Sci Public Health* 2014; 5.
5. Chenari MR, Gooran AS, Zarghami A and Faramarz

- Fazeli. Assessment of Urine Analysis Diagnostic Role: A Cross-Sectional Study in South Eastern of Iran *Open Journal of Urology*. 2012; (2): 227-231.
6. Pezzlo M. Detection of Urinary Tract Infections by Rapid Methods. *Clinical microbiology reviews*. 1988; 268-280.
 7. Taneja N, Chatterjee SS, Singh M, Sivapriya S, Sharma M and Sharma SK. Validity of quantitative unspun urine microscopy, dipstick test, Leucocyte Esterase and nitrate tests in rapidly diagnosing Urinary Tract Infection. *JAPI*. 2010; 58.
 8. Neelam T, Kavya M , Rungmei M, Nandita D and Meera S. Evaluation of three screening methods for detection of urinary tract infection in antenatal women. *J Obstet Gynecol Ind*. 2004; 54(3): 267-70.
 9. Hoff RG, Newman DE and Staneck JL. Bacteriuria screening by use of acridine orange-stained smears. *J.Clin. Microbiol* 1985; 21: 513-16.