

# Validity of Quantitative Unspun Urine Microscopy, Dipstick Test Leucocyte Esterase and Nitrite Tests in Rapidly Diagnosing Urinary Tract Infections

N Taneja<sup>1</sup>, SS Chatterjee<sup>1</sup>, M Singh<sup>1</sup>, S Sivapriya<sup>1</sup>, M Sharma<sup>1</sup>, SK Sharma<sup>2</sup>

## Abstract

**Objectives:** Rapid diagnostic tests can screen out negative samples and can save valuable time and money. The study was conducted to assess the usefulness of leukocyte esterase, nitrate reductase and quantitative microscopic unspun urine wet mount examination in rapidly diagnosing urinary tract infections (UTI).

**Methods:** Four hundred and fifty samples were tested by semi-quantitative culture on cysteine lactose electrolyte deficient medium, microscopic examination of unspun urine for significant pyuria, dipstick leucocyte esterase test (LET) and nitrite test (NT). Culture was used as gold standard to evaluate the performance of direct microscopy and dipstick tests.

**Results:** Urine culture examination revealed significant bacteriuria ( $>10^5$  cfu/ml) 98 (21.8%), in urine samples. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the diagnostic odds ratio (DOR) of the dip-stick LET were 73.5%, 58.5%, 33.0%, 88.8%, and 3.9 respectively; those of the dip-stick NT were 57.1%, 78.7%, 42.7%, 86.8%, and 4.9 respectively; and those for microscopic significant pyuria detection were 68.4%, 60.8%, 32.7%, 87.3%, and 3.4 respectively. Highest sensitivity (95.9%), NPV (97.9%) and DOR (25.7) was obtained on combining microscopy and dip-stick LET and NT (either of them positive). This can potentially cut costs by 79 %.

**Conclusion:** Quantitative unspun urine wet mount microscopy and dipstick tests for leucocyte esterase and nitrite test should be added into routine laboratory practices for faster diagnosis of UTI.

## Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections encountered in clinical practice both in community and hospital settings. Clean-catch, mid stream urine for culture and sensitivity testing is considered the best method to diagnose UTI. At our tertiary care hospital, approximately 150 urine specimens are submitted daily for culture examination, and 80% of these turn out to be sterile.

Rapid diagnostic tests can rule out negative samples, are economical, save valuable time and thus useful in high-end laboratories.<sup>1</sup> Screening is also required in special circumstances where it is difficult to identify UTI on basis of clinical criteria alone but where early diagnosis and prevention of complications affords significant benefit (e.g. children, and post renal transplant patients).<sup>1</sup> Many rapid diagnostic methods are available which include wet mount microscopy, gram stain, dipstick and automated assays.<sup>1,2</sup> Screening tests are useful adjuncts to culture in diagnosing complicated UTI. Few studies have evaluated their role as a single test in complicated UTI.<sup>3,4</sup> Moreover, there is no such study from India.

In the present study, we evaluated quantitative unspun wet mount microscopy for significant pyuria and dipstick leucocyte esterase test and nitrite tests for rapid screening of urine samples, keeping semiquantitative culture as the gold standard for diagnosis of UTI. We further compared the results in complicated and uncomplicated UTI.

## Methods

**Place of study:** The study was conducted at the Post Graduate Institute of Medical Education & Research, Chandigarh, a 1406 bed tertiary care referral centre located in northwest India.

**Sample collection:** Clean-catch, mid-stream urine (MSU) samples were collected in sterile, wide mouthed, universal containers from 450 cases of suspected UTI. These were transported to the laboratory and processed within one hour of collection.

**Patient Profile:** Demographic details, clinical features, and underlying illnesses of the patients were recorded. Complicated UTI was defined as UTI developing in anatomically, physiologically or functionally compromised urinary tract.

**Culture:** Semi-quantitative culture on cysteine lactose electrolyte deficient (CLED, Hi-Media, Mumbai) media was used as the gold standard for diagnosing UTI. Semi-quantitative culture is done by plating 1 µl urine using a calibrated bacteriological loop on CLED agar, and colonies are counted after overnight incubation at 37°C. Number of colonies obtained is multiplied by 1000 to obtain the colony forming units (cfu) / ml. Cut off for significant bacteriuria was taken as  $10^5$  cfu/ml.<sup>5</sup> Samples showing growth of 2 or more bacterial species ( $\geq 10^3$  cfu/ml) of doubtful significance were noted and repeat cultures asked for. MSU samples showing scanty bacterial growth ( $<10^3$  cfu/ml) were reported as bacterial growth of no significance.

**Quantitative unspun wet mount microscopy:** 50 µl of well mixed uncentrifuged urine sample was placed on a clean, grease free, glass slide and covered with a 20 mm X 20 mm coverslip. The wet mount preparation was then examined under a high power (40X) microscope for presence of pus cells. The cut off for a significant finding was  $> 1$  pus cell / 7 high power fields

<sup>1</sup>Department of Medical Microbiology, <sup>2</sup>Department of Urology, PGIMER, Chandigarh.

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**Table 1 : Results of wet mount microscopy and strip leukocyte esterase and nitrite tests in 450 clinical samples**

		Culture					Culture		
		+ ve	- ve				+ ve	- ve	
Microscopic Pyuria	+ ve	67	138	205	LET or NT	+ve	78	153	231
	- ve	31	214	245		-ve	20	199	219
		98	352	450			98	352	450
Leucocyte esterase test (LET)	+ ve	72	146	218	LET or NT or Microscopy	+ ve	94	168	262
	- ve	26	206	232		- ve	4	184	188
		98	352	450			98	352	450
Nitrite test (NT)	+ ve	56	75	131	Microscopy + (either of LET or NT)	+ ve	70	86	156
	- ve	42	277	319		- ve	28	266	294
		98	352	450			98	352	450

**Table 2: Usefulness of wet mount microscopy and strip leukocyte esterase and nitrite tests in 450 clinical samples**

	Microscopic pyuria	Leukocyte Esterase (LET)	Nitrite (NT)	Either LET or NT positive	Microscopy positive + Either of LET or NT positive	Any one of three tests positive
Positives <sup>§</sup> (n=98)	67	72	56	78	70	94
Sensitivity (%)	68.4	73.5	57.1	79.6	71.4	95.9
Specificity (%)	60.8	58.5	78.7	56.5	75.6	52.3
Positive predictive value (%)	32.7	33.0	42.7	33.8	44.9	35.9
Negative predictive value (%)	87.3	88.8	86.8	90.9	90.5	97.9
Diagnostic Odds Ratio	3.4	3.9	4.9	5.1	7.7	25.7

<sup>§</sup> Positives – among the culture positives only (n=98).

(hpfs) for pyuria.<sup>5</sup>

**Dipstick test:** Dip-stick leucocyte esterase test (LET) and nitrite test (NT) were evaluated using the BM Line-10 test strips (Roche Diagnostics, India). Manufacture methodology was followed to perform the test and read the results. Briefly, the test strip was dipped in the urine sample and taken out immediately. The strips were blotted with a blotting paper to remove excess urine. Comparative reading was taken at 1 minute and 2 minute intervals for the LET and NT respectively.

**Data analysis:** Data including patient profile, microscopy and culture results were recorded and analyzed using Microsoft Excel software. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each of the methods (microscopic pyuria detection, microscopic pyuria / bacteriuria detection, dip-stick LET and NT) using standard formulae. Diagnostic odds ratio (DOR) was calculated as per the following formulae<sup>1</sup>:  $DOR = \frac{\text{sensitivity} / (1 - \text{sensitivity})}{(1 - \text{specificity}) / \text{specificity}}$ . This ratio reflects the ratio of the odds of a positive test result in true UTI patients to the odds of a positive test result in non UTI affected patients.<sup>1</sup>

The same indices (sensitivity, specificity, PPV, NPV, and DOR) were calculated for the following three combination of the screening methods a) either of two dipstick tests (LET or NT) positive, b) any one positive (microscopic pyuria, dip-stick LET and NT), and c) either of two dipstick tests positive plus positive microscopy.

**Cost Analysis:** Cost of each dip-stick was Rs. 10.00. Cost of wet mount microscopy and culture are 50 paise and Rs. 50.00 respectively in our laboratory. The cost benefit was estimated as the difference between cost of culture sensitivity examination and the cost of the dip-stick.

## Results

Four hundred and fifty samples from same number of patients were tested. The ward: Out Patient Department ratio of submitted samples is 1.1:0.9. The samples came from urology (34%), gynecology and maternity (20%), pediatric (16%), medicine (7%), surgery (8%), emergency (6%), ICU units (6%) and other departments (3%). Ninety eight (21.8%) samples showed significant growth (>10<sup>5</sup> cfu/ml) of single type of microorganism. Organisms implicated in culture positive UTI were *E. coli* (61, 62.2%), *Klebsiella pneumoniae* (9, 9.2%), *Enterobacter aerogenes* (5, 5.1%), *Enterobacter cloacae* (1, 1%), *Citrobacter diversus* (2, 2%), *Pseudomonas aeruginosa* (5, 5.1%), and *Candida* spp. (15, 15.3%). Urine culture was reported sterile, bacterial growth of no significance, and bacterial growth of doubtful significance in 266, 67, and 19 samples, respectively. Number of samples positive by LET, NT, either of the LET or NT, and microscopic pyuria were 218, 131, 231, and 205 respectively (Table 1). Of the 98 culture positive samples, LET, NT, either of LET or NT, and microscopic pyuria of the various tests and combinations are shown in table 1. The dipstick NT test showed the highest specificity (78.6%) and the combination of either of three tests positive had the highest sensitivity (95.9%), NPV (97.9%) and DOR (25.7) (Table 2).

Among 177 samples from patients with suspected complicated UTI, 39 were positive by culture. Of these, 33 were positive by dipstick test (either LET or NT) and 35 by microscopy (Table 3). A combination of either of three tests positive could diagnose 100% of complicated UTI.

Of the 67 samples showing growth of no significance, LET, NT, microscopic pyuria, and either of three tests were negative in 42, 67, 38, and 67 samples respectively. Among 19 samples which showed growth of doubtful significance, LET, NT, and

**Table 3: Usefulness of Wet mount microscopy and Strip Leukocyte esterase and Nitrite tests in 177 clinical samples from patients with suspected complicated UTI**

	Either LET or NT positive	Microscopic pyuria	Any one of three tests positive
Positives <sup>‡</sup> (n=39)	33	35	39
Sensitivity (%)	84.6	89.7	100.0
Specificity (%)	45.7	49.6	40.9
Positive predictive value (%)	30.6	33.7	32.5
Negative predictive value (%)	91.3	94.4	100.0
Diagnostic Odds Ratio	4.6	8.6	-

<sup>‡</sup>Positives – among the culture positives only (n=39).

microscopic pyuria were negative in 15, 16, 11, and 16. Only 3 specimens were repeated in the follow up period of which 2 turned out sterile and 1 showed significant growth.

The total cost of screening tests was Rs. 10.50 as against Rs.50.00 for the culture sensitivity examination, amounting to a cost saving of 79%.

## Discussion

The dip-stick LET was more sensitive (73.5% vs. 57.1%) and had higher NPV (88.7% vs. 86.8%) than the dip-stick NT. On the other hand dipstick NT was the most specific of all tests used. On combining the two dipstick tests sensitivity, NPV, and DOR increased to 79.6%, 90.9 % and 5.1 respectively. So a combination of the LET and NT taken as either positive is a better way to rule out UTI. This is indeed simple as both the tests are located on the same strip.

The dipstick LET in the present study performed better than a study carried out by Al Daghistani *et al.*, 2002.<sup>6</sup> The LET in our study had higher DOR than those of a meta-analysis (3.9 vs. 2).<sup>1</sup> Deville *et al.* have suggested that LET results read by family physicians result in high sensitivity, low specificity and poor DOR.<sup>1</sup> Our LET results were read by a clinical microbiologist, and expectedly the results were better. Similarly the specificity of the NT in our study (79.6% vs. 80%) is comparable to that of the meta-analysis.<sup>1</sup> Other groups working on the NT have however, found this test to be less sensitive.<sup>7,8</sup> A problem we faced during the study was the difficulty in reading of the nitrite test in a few cases when the colour change on the strip did not match that on the interpretation chart. In our view the nitrite test on this strip needs further refinement to achieve better results.

Best results were obtained by combining both microscopy and strip test. Highest sensitivity (95.9%) and DOR (25) was obtained when a positive result in either was taken as significant, similarly negative samples could be screened out with a very high degree of confidence (NPV - 97.9% overall and 100% for complicated cases). A combination of screening tests was also able to detect correctly majority of samples showing growth of no significance and of doubtful significance. However, semi-quantitative culture still remains the gold standard, as 4 cases of culture positive UTI were missed on a combination of screening tests. Most importantly, the combination of three screening tests could diagnose all culture positive complicated UTI cases. This coupled with the 100% NPV in such a scenario makes screening tests extremely useful. While reviewing literature on the

pubmed, we found few such studies on the utility of screening tests in complicated UTI.<sup>3,4</sup> Further investigation in this group of patients is necessary before rational use of these tests can be recommended in them.

The financial benefits (79% cost saving) of screening cannot be overemphasized, as have been noted by Fowlis GA *et al.*, 1994.<sup>9</sup> The majority of samples turned out to be negative (352 of 450, 78.2%); thus screening in a high-end laboratory like ours will definitely lead to enormous cost saving.

So, a combination of three methods with either of them positive can rule out UTI in nearly 98% of cases. A positive screening test will require culture and sensitivity examination so that the right antibiotic can be prescribed. There will be few cases (4 out of 98 in our study) which could be missed by screening assays. This situation can be tackled by doing a culture in symptomatic cases with a negative screening test. Further screening tests can be reliably used to rule out UTI in patients with suspected complicated UTI.

## Conclusion

Using the screening methods (wet mount microscopy, leukocyte esterase test and nitrite test), with either of them being positive, appears to be a rational and cost effective approach towards ruling out UTI, even in cases of complicated UTI.

## Legends

UTI – Urinary Tract Infection; LET – Leucocyte esterase test; NT – Nitrite test; MSU – Mid-stream urine;  $\mu$ l - micro liter; PPV – Positive predictive value; NPV - Negative predictive value; DOR - Diagnostic Odds Ratio

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