

# Laboratory Evaluation of Urinalysis Parameters to Predict Urinary Tract Infection

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We evaluated the performance of urinalysis dipsticks and microscopic urine sediment analysis as predictors of urinary tract infection (UTI) in patient visiting hospital. The samples were processed for macroscopic examination to observe Leukocyte esterase (LE) and Nitrite (NIT) by dipstick method, microscopic examination to observe pus cells, culture of urine sample on Blood agar and MacConkey agar to identify the potential pathogen and Colony count was evaluated.

The positive predictive values was significantly greater for the NIT test alone than for either LE alone and LE and NIT in combination: 68.00% at  $\geq 105$  CFU/ml. The LE and NIT combination had a significantly higher predictive value of a negative test than either test alone at all two level of bacteriuria. Microscopic examination for White blood cells (WBCs) and bacteria, found that of the 178 cases were positive for LE, NIT or both by the dipstick method, Among them 115 cases were positive for pyuria, 45 cases were positive for bacteriuria and the rest (28 cases) negative for pyuria or bacteriuria. We concluded that though it is laborious, microscopic urinalysis is a good analytical tool. Taken together with dipsticks, we obtained a clinically-acceptable prediction of urinary-tract infection.

**Keywords:** Leukocyte esterase, Nitrite, UTI, uropathogens.

## INTRODUCTION

UTI is defined as the persistent presence and proliferation of active microorganisms within the urinary tract. UTI implies both microbial colonization of the urine and invasion of the lower or upper urinary tract by microorganisms. UTI is the most prevalent disease of the urinary tract that has a high morbidity in both hospital and the community (2, 5, 6).

The epidemiology and Prevalence rates of UTI are grouped by age, sex, race, and circumcision status of the patient. The Incidence of UTI is bimodal; highest during the first year of life and peaking again during adolescence (13). UTI is the most prevalent infectious diseases affecting approximately 150 million people worldwide annually which result in more than 6 billion US dollars loss to the global economy (15). In Nepal,

UTI account for approximately 15.9 % among pregnant women whereas it was only 5.0% in non-pregnant women (7).

The gold standard for UTI diagnosis is bacterial culture, which is based on bacterial counts and identification. Culturing of the samples is fairly time- and labor consuming, and most of the samples yield no growth or insignificant growth (14). In most cases, rapid tests are used for initial treatment. Urinalysis is one of the most important tests used in clinical laboratories in the diagnosis and follow-up of UTI (2, 11). In order to improve the efficiency of handling of the urine samples, methods for screening out the culture-negative samples from the culture-positive samples have been developed. Chemical screening with strips for NIT, LE is widely used (8), in this rapid and inexpensive screening test, both a test for LE activity ( a host

response specific test) and a test for NIT ( a bacteria specific test ) are use to predict bacteriuria ( i.e., colony count  $\geq 10^5$  CFU/ml) (10). when both NIT and LE are negative. Cells, particles, and microorganisms in urine can be examined by microscopic-urine-sediment analysis, but this method is time-consuming, labor-intensive, and sensitive to inter-observer variability (8).

## **MATERIALS AND METHODS**

### **Urine Specimens**

We evaluated 329 urine specimens submitted to our laboratory for diagnostic urinalysis during August 2006. Only samples for which cultures were solicited were included in our study. Most of the urine samples were obtained by the midstream technique (recommended). All samples were submitted to Microscopic, chemical dipstick tests and culture.

### **Dipstick Urinalysis**

Dipstick urinalysis was done using Multisticks of NOVA test TEST STRIPS FOR URINALYSIS (Roche Diagnostics, São Paulo, Brazil). The strips had reagent pads for semiquantitative assessment of density, pH, LE , NIT, protein, glucose, ketones, urobilogen, bilirubin, and hemoglobin/myoglobin. As a predictive parameter for UTI, we evaluated LE(3+) and NIT reaction. A strip was dipped into urine for 1 s and then withdrawn, and the excess fluid was removed. After the prescribed period of incubation at room temperature (1 min for LE, and 30 s for NIT), color reactions of each test pad were

compared with a color chart provided by the manufacturer.

### **Detection of Piuria by urine Microscopy:**

10 ml of urine sample was taken in a clean sterile centrifuge tube and was centrifuged at 3000 rpm for 10 min. The supernant was discarded and the sediment was examined by wet mount preparation method (3, 16). Wet mount preparation of urinary sediments was observed through microscope for the presence of WBC, pus cells and RBC. Number of WBC and RBC was estimated as number per HPF that is 40X objective of microscope (3, 16).

**Gram staining:** Gram staining technique was used urine specimen and to detect the presence of bacteriuria by standard methods (4).

### **Urine culture**

The urine samples were cultured onto the MacConkey agar and Blood agar plates by the semi-quantitative culture technique using a standard calibrated loop have internal diameter of 3 mm. The protocol was followed as recommended by WHO (16).

### **Identification of isolates**

The isolates were identified by standard diagnostic procedure.

The identification of bacterial isolates was done biological techniques as using standard micro described in Bergey's Manual of systemic bacteriology which comprises of studying the staining reactions and various ,colonial morphology Isolated colonies from the .biochemical properties

pure culture were identified by standard conventional biochemical tests

**RESULT**

Comparative analysis of the LEand NIT screening test alone and in combination at  $\geq 10^5$  and  $\leq 10^4$  CFU of potential pathogen per ml. The positive predictive values was significantly greater for the NIT test alone than for either LEalone and LE and NIT in combination: 68.00% at  $\geq 10^5$  CFU/ml. The LEand NIT combination had a significantly higher

predictive value of a negative test then either test alone at all two level of bacteriuria.

Microscopic examination for WBCs and bacteria, found that of the 178 cases were positive for Leukocyte, NIT or both by the dipstick method on the dipstick screening test, Among them 115 cases were positive for pyuria, 45 cases were positive for bacteriuria and the rest (28 cases) negative for pyuria or bacteriuria.

**DISCUSSION**



TABLE 1  
 COMPARATIVE ANALYSIS OF MICROSCOPY AND MACROSCOPIC RESULTS

Screening test	Sensitivity (%)		Specificity (%)		Predictive value (%) of test			
					Positive		Negative	
	$\geq 10^5$ cfu/ml	$\leq 10^4$ cfu/ml	$\geq 10^5$ cfu/ml	$\leq 10^4$ cfu/ml	$\geq 10^5$ cfu/ml	$\leq 10^4$ cfu/ml	$\geq 10^5$ cfu/ml	$\leq 10^4$ cfu/ml
<b>Nitrite</b>	43.00	40.00	83.00	84.50	68.00	50.00	79.71	76.61
<b>Leukocyte esterase</b>	68.00	63.00	91.00	88.00	62.20	65.00	85.96	83.00
<b>Nitrite- Leukocyte esterase</b>	84.00	91.30	90.00	91.00	60.00	63.50	91.66	95.00

TABLE 2  
 COMPARATIVE ANALYSIS OF MICROSCOPY AND MACROSCOPIC RESULTS

Cases	Multisticks		Microscopy			Culture		
	Nitrite	Leukocyte esterase	Bacteriuria	Pyuria	Negative	+ve $\geq 10^5$ cfu/ml	+ve $\leq 10^4$ cfu/ml	-ve No growth
20	+	+	0	20	0	20	0	0
108	-	+	4	89	15	31	50	27
50	+	-	41	6	3	12	23	15
151	-	-	5	7	139	12	7	132
<b>Total</b>			50	122	157	75	80	174
	329		329			329		

In this study we compared the sensitivity, specificity and predictive values of the LE test and the test of urinary NIT and in combination as screening tests for bacteriuria in 329 clinical urine specimens. The LE - NIT combination had a sensitivity of 84.00%, a specificity of 90.00% and a negative predictive value of a negative test of 91.66% for specimens with  $\geq 10^5$  CFU/ml. When both tests are negative, one can predict with a high degree of confidence (predictive value of a negative tests, 95.00%) that the urine specimen will contain  $< 100000$  CFU/ml. These findings are in agreement with those reported in previous studies (10, 12).

In our study, The LE and NIT combination had a significantly higher predictive value of a negative test than either test alone at all two levels of bacteriuria. The presence of both pyuria and bacteriuria from a fresh urine sample are highly indicative for UTI. In similar study carried out by Pfaller MA et al. (1985), Semeniuk et al. (1999), and Santos JC et al. (2007) showed that the

combination of a host response-specific test (LE) with a bacteria-specific test (NIT) result was more sensitive screen for bacteriuria than either test alone. This was true at level of bacteriuria ranging from  $\leq 10^4$  CFU/ml to  $\geq 10^5$  CFU/ml. Perry JL et al. (1982) found LE activity to be a better indicator of significant bacteriuria. In the present study, the sensitivity of the NIT test was low for both patient groups. The NIT test proved to be a specific but relatively insensitive test. The low sensitivity and high specificity presented by the NIT test make it important in cases in which culture is negative. Some microorganisms that cause UTI, such as enterococci and *S. saprophyticus*, are unable to reduce nitrate to NIT. False negative results may occur due to frequent urinations, which lower the exposure of the microorganisms to nitrate; this can also occur with a diet poor in vegetables (11). The results obtained in this study demonstrate that Dipstick tests for LE has poor sensitivity and specificity with respect to UTI. Therefore, the use of results for LE from dipstick testing has a high likelihood

of being misleading because pyuria can occur due to leucorrhea, fever, pregnancy and administration of adrenocortical steroids, in female patients without infection (11).

According to data presented in table 2, Best results were obtained by combining both microscopy (Bacteriuria and Pyuria) and Macroscopic (NIT and LE) test. Positive result of microscopic and macroscopic examination indicates cent percent significant bacteriuria. Similar results have shown in study carried out by Sawalha RMH (2007), Yuent et al., (2001) and Taneja N et al., (2009). Microscopic examination of the urine for the presence of WBCs and bacteria is usually performed after centrifugation. More than five WBCs per high-power field suggest a possible infection. Elevation in number of WBCs in urine is a result of an inflammatory response of urogenital mucosa to colonizing bacteria.

We conclude that urinalysis methods are good predictors of urine-culture diagnosis and can be used as predictors of UTI. Individually, parameters such as intense bacteria, LE and NIT had good predictive power. An association among the different urinalysis techniques improved accuracy over single analysis.

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