Indian Journal of Pathology and Oncology 2024;11(1):3–7

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Indian Journal of Pathology and Oncology

Journal homepage: www.ijpo.co.in

Original Research Article

# Evaluation of Leukocyte esterase and Nitrite dipstick tests with routine urine microscopic analysis in detecting urinary tract infections

**Agam Hans**[1]*, **Abhishek Yadav**[1], **Parneet Kaur**[1], **Archana Kumari**[1]

[1]*Dept. of Pathology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Ambala, Haryana, India*

ARTICLE INFO

*Article history:*
Received 22-12-2023
Accepted 17-01-2024
Available online 17-04-2024

*Keywords:*
Pyuria
Culture
Uropathogenic escherichia coli
Pyelonephritis
Cystitis

ABSTRACT

**Background**: Urinary tract infection is a global health problem affecting all age groups. *E coli* is the most common cause of UTI followed by *klebsiella*, staphylococcus haemolyticus and enterococci etc. The gold standard for detecting an UTI is the presence of pathogen in urine along with clinical symptoms and pyuria. Nitrite (NIT) and leukocyte esterase (LE) tests are two important dip stick tests used for screening UTI.
**Materials and Methods:** A total of 202 patients who presented with clinical symptoms of UTI from January 2023 to December 2023 were evaluated for urine routine and culture examination. LE and NIT dipstick tests were evaluated and change of colour was considered positive. Microscopic examination of urine was performed manually and urine culture with count of > $10^5$ CFU/ml was considered positive. Statistical data was analysed using IBM SPSS v 29.0.2.0 (20) and Microsoft Excel. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for both tests.
**Results**: LE had a sensitivity of 47.22% whereas NIT was much less sensitive (15.27%). Specificity of NIT (99.23%) was higher in comparison to LE (81.53%). NIT had overall better PPV and NPV (91.66%, and 67.89%) as compared to LE (58.62% and 73.61%). The accuracy of LE and NIT were 69.3% and 69.8% respectively. A positive correlation was also seen with increasing WBC count and positive urine culture.
**Conclusion**: Urine culture along with clinical and routine analysis is necessary for definitive diagnosis of UTI but importance of dipstick chemical examination should not be underestimated. LE and NIT have an additional benefit of quick results in comparison to culture which takes at least 24 hours.



## 1. Introduction

Urinary tract infections (UTIs) are considered as a severe public health problem with an estimated incidence of more than 150 million per year globally with the health care expenditure on UTIs being in billions.[1,2] Urinary tract infections occur frequently in people across all age groups with varying incidences in neonates and elderly. The overall self-reported annual incidence of UTIs in women were 12.1% and 3% among men.[3] Multiple classifications have been used across literature for UTIs but for all practical purposes, UTIs are clinically classified as uncomplicated or complicated.[4,5] Uncomplicated UTI affects individuals where no relevant functional or anatomical abnormality is noted in the urinary tract. It is further divided into lower (cystitis) or upper (pyelonephritis) UTIs. Complicated UTIs are defined as UTIs with a compromised host defence or a compromised urinary tract.[6] This includes urinary obstruction by stones, urinary retention by neurological or anatomical causes, immunosuppression, renal failure, renal transplantation, pregnancy, and presence of any foreign body in the tract including calculi, catheter, or any other drainage devices.[5,6] A bacterial etiology is usually demonstrated for most of the UTIs, and the most common bacterial species in various patient groups have

* *Corresponding author*.
*E-mail address*: agamhans94@gmail.com (A. Hans).

https://doi.org/10.18231/j.ijpo.2024.002

been studied thoroughly. *Escherichia coli* is the most common pathogen (≈80%) isolated in acute community-acquired uncomplicated urinary tract infections, followed by staphylococcus saprophyticus (≈15%). Klebsiella, enterococci, enterobacter and proteus species although infrequently, can also cause uncomplicated cystitis and pyelonephritis.[7] The gold standard for the diagnosis of a urinary tract infection is the detection of the pathogen in the presence of clinical symptoms. Common symptomology of UTI includes increased urinary frequency, urgency, dysuria and suprapubic discomfort. Although in recent times, molecular diagnostic approaches using Antigen detection or DNA hybridization and amplification techniques are being applied to the diagnosis of many infectious disorders, UTIs are still diagnosed by urine culture results. As UTI is caused by a variety of bacteria, a diagnostic approach with specific targeting of bacterial antigens or genes is much less sensitive and expensive. Urine dip stick chemical tests along with microscopic analysis can be good screening tests for diagnosing UTI. Nitrite and leukocyte esterase (LE) tests are two important dip stick tests used for screening UTI. As urine culture is expensive, laborious and takes at least 24 hours for the result interpretation, evaluating the diagnostic efficacy of LE and Nitrite along with statistical comparison of microscopic analysis with these tests could be useful.

## 2. Materials and Methods

This study was a laboratory based observational study conducted in Central Laboratory, MMIMSR, Mullana, Ambala. The study period was of 11 months starting from 15th January 2023 to 15th December 2023. Two hundred and two cases who were subjected to urine culture and routine urine analysis with clinical suspicion of urinary tract infection based on inclusion and exclusion criteria were included in the study. All samples of patients of any gender above 18 years of age subjected to urine culture and routine analysis with clinical suspicion of UTI including urinary urgency, increased frequency, or dysuria were included in the study. Patients with any history of urinary tract stones, urinary obstruction, neurogenic bladder or any developmental anomaly or patients currently with urethral catheterization were excluded from the present study. Pregnant females, immunosuppressed individuals, patients suffering from chronic kidney disease, any autoimmune disorder or malignancy were also excluded from the study.

### 2.1. Methodology

Patients with suspected UTI provided a fresh voided (midstream) urine sample for routine urine analysis and culture censitivity analysis. Chemical analysis of urine was done using Sysmex UC-3500 using meditape UC-9A and microscopic analysis was done manually within 2 hours of receiving the sample. Any change in color of the dipstick was considered a positive test result. For culture and sensitivity analysis urine were collected in sterile containers and immediately processed. To isolate the pathogens, urinary samples were speckled on the cysteine lactose electrolyte deficient (CLED) media and then incubated at 37°C for at least 24 hours. A sterile calibrated wire loop was used to inoculate a 0.01 ml urine sample and then this isolate was used for a colony count. Kass criteria was used for determining the significant colony counts and a single species count of $>10^5$ organisms per ml was considered to be significant. Biochemical characterization of the colonies was performed for confirmation. MacConkey agar was used to subculture the colonies in order to get pure growth of the microorganisms. In the instance of culture of multiple microorganisms, only the predominant microorganism was considered and assessed.

### 2.2. Data analysis

Data was analysed using IBM SPSS v 29.0.2.0 (20) software and Microsoft Excel. Sensitivities, specificities, positive predictive values, negative predictive values and accuracy of both the nitrite and LE tests were calculated with urine culture being the gold standard. The following formulas were used for statistical analysis.

$$\text{Sensitivity} = \frac{True\ positives\ (TP)}{True\ positives(TP) + False\ negatives(FN)}$$

$$\text{Specificity} = \frac{True\ negatives(TN)}{True\ negatives(TN)+False\ positives(FN)}$$

$$\text{Positive Predictive Value} = \frac{True\ positives(TP)}{True\ positives(TP)+False\ positives(FP)}$$

$$\text{Negative Predictive Value} = \frac{True\ negatives(TN)}{True\ negatives(TN)+False\ negatives(FN)}$$

$$\text{Accuracy} = \frac{True\ positives+True\ negatives}{TP+TN+FP+FN}$$

## 3. Results

The study population consisted of 202 patients who presented with urinary tract infection symptoms and were analysed for routine urine examination and urine culture. Out of 202 patients, 104 were male and 98 were female with a sex ratio of 1.06. The mean age of the study population was 48.1 years with Standard Deviation (SD) of ± 19.2 years. The minimum and maximum age of the patients were 18 and 90 years respectively. Out of 202 patients who had UTI symptoms, 72 had a positive urine culture test. Out of 72 culture positive patients, 25 (34.7%) were male and 47 (65.3%) were female. Among the 72 patients most commonly isolated organism was *E. coli* contributing to approximately 45% of the culture positive cases out of which 10 were male and 23 females. Second most common isolated organism was *Klebsiella pneumoniae* with 18 (25%) cases followed by

*Enterococcus* species with 14 cases (19.4%). *Pseudomonas aeruginosa* was isolated in 2 cases, whereas Proteus mirablis, Methicillin resistant Staphylococcus aureus, Staphylococcus haemolyticus, Candida and Serratia were positive in one case each. Sex wise graphical distribution of all culture positive cases is illustrated in Figure 1. Comparing leukocyte esterase with nitrite test, LE was more sensitive with a sensitivity of 47.22%, whereas nitrite was more specific with a specificity of 99.23%. Specificity, PPV and NPV of Leukocyte esterase test was 81.53%, 58.62% and 73.61% respectively with an accuracy of 69.3%. Sensitivity, PPV and NPV of nitrite test was 15.27%, 91.66%, and 67.89% respectively with an accuracy of 69.8%. Combined characteristics of LE along with Nitrite is shown in Figure 2. A positive correlation was also seen with increasing WBC count and positive urine culture as shown in Figure 3. Our result revealed that the probability of acquiring an UTI when pus cells in urine were less than 5/hpf, was 16.4%, the probability rose to 46.51%, 55.93%, 68% and 75% when urine pus cells were >5/hpf, >10/hpf, >20/hpf and >40/hpf respectively. Also, there was a positive correlation of culture positivity when the LE score was ≥ 2+ with 75% positivity rate, whereas positivity rate declined when LE score was ≤ 1+ with only 29.31% cases showing growth of organism in urine culture. LE scores distribution with culture positivity is described in Figure 4.

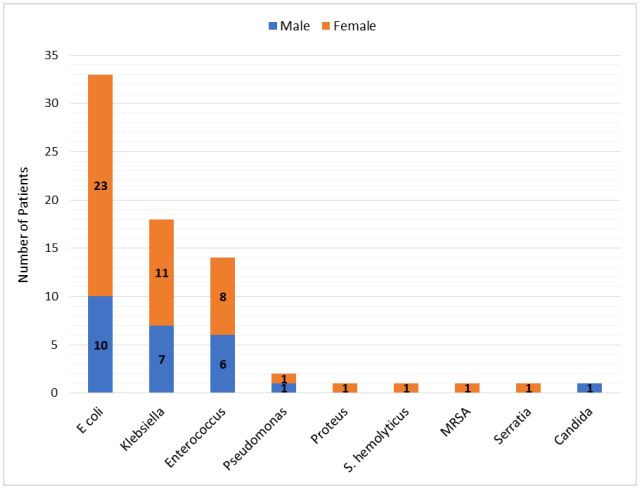


**Figure 1:** Microorganisms isolated from urine cultures

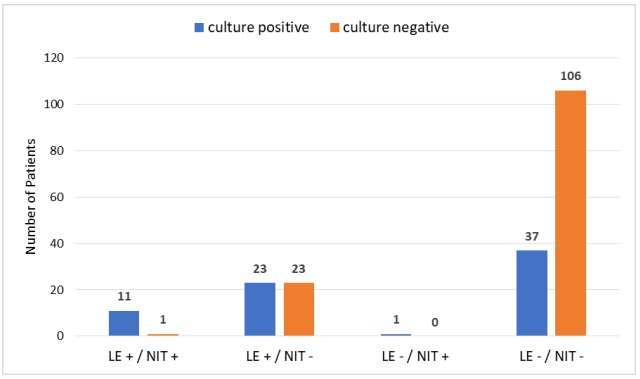


**Figure 2:** Combined characteristics of LE and NIT in patients

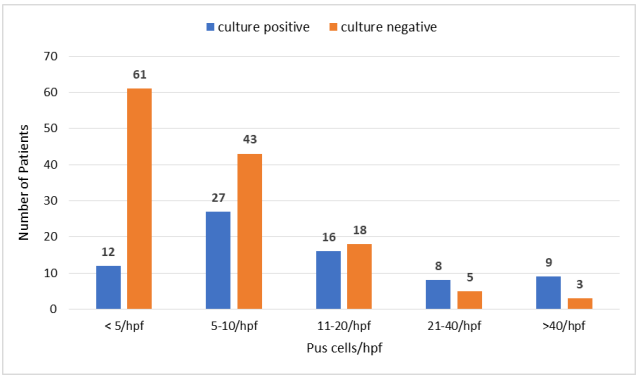


**Figure 3:** Correlation of pyuria with culture positivity

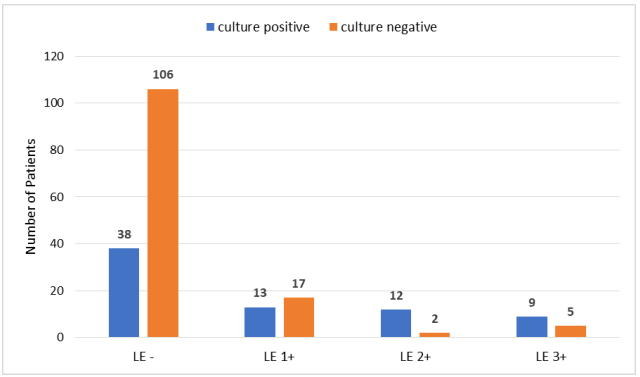


**Figure 4:** Correlation of LE grading with culture positivity

## 4. Discussion

Urinary tract infection is the infection of urothelium anywhere along the urinary tract, starting from kidneys till urethra. Defining UTI is complex as it involves multiple clinical and laboratory parameters. In clinical settings as well research, a combination of symptoms and positive laboratory parameters are often necessary to come at a conclusion.[12] European Medicine Agency (EMA) and United States Food and Drug Administration (FDA) have mainly used four parameters for characterising UTIs. The major four factors consist of the symptoms, host factors, pyuria and bacteriuria.[13,14] The gold standard for diagnosing UTI is urine culture with $>10^5$CFU/ml of a single pathogen or bacteria. Although urine culture holds the prime importance in diagnosing UTIs, Urine analysis is invaluable and contributes significantly in diagnosing urinary tract infections. This quick, easy and consistent method using dipsticks can give results with in minutes

**Table 1:** Comparison of diagnostic efficacy of LE and NIT in various studies

| | Our Study (n= 202) | Zaman et al.[8] (n = 420) | Gieteling et al.[9] (n=104) | Sultana et al.[10] (n=400) | Demilie et al.[11] (n=37) | Bellazreg F et al[2] (n=431) |
|---|---|---|---|---|---|---|
| **LE** | | | | | | |
| Sensitivity (%) | 47.22 | 74 | 69 | 72 | 71 | 87 |
| Specificity (%) | 81.53 | 76 | 92 | 86 | 90 | 64 |
| PPV (%) | 58.62 | 39 | 79 | NA | 62 | 57 |
| NPV (%) | 73.61 | 93 | 87 | NA | 93 | 89 |
| **NIT** | | | | | | |
| Sensitivity (%) | 16.66 | 33 | 28 | 48 | 57 | 48 |
| Specificity (%) | 99.23 | 94 | 99 | 96 | 96 | 95 |
| PPV (%) | 92.30 | 52 | 79 | NA | 80 | 85 |
| NPV (%) | 68.25 | 87 | 87 | NA | 90 | 74 |

as compared to culture which takes at least 24 hours. Leukocyte esterase (LE) and Nitrite (NIT) test are two main tests useful in laboratory diagnosis of UTIs. Nitrites are not normally present in urine but can be present when bacteria with reducing properties convert nitrates to nitrites. Many gram positive as well gram negative bacteria have the ability to reduce nitrates to nitrites when present in significant quantities in urine.[15] Leukocyte esterase is a marker of pyuria and is produced by neutrophils in urine. Ureaplasma and chlamydia have been isolated in many cases with leukocyte esterase positivity but urine negative culture.[15] Various sensitivity analysis studies across the literature have depicted varied sensitivity and specificity of NIT and LE, but almost all studies reveal Leukocyte Esterase to be more sensitive and Nitrite to be more specific in diagnosing UTIs. Same was the case in our study. Our results revealed that LE had a sensitivity of 47.22% whereas NIT was much less sensitive with a sensitivity of 15.27%, proving LE to be more sensitive than NIT. Specificity of both LE and NIT were high with a significant edge of NIT having a specificity of 99.23% in comparison to 81.53%. Nitrite test had overall better PPV and NPV (91.66%, and 67.89%) as compared to Leukocyte esterase test (58.62% and 73.61%). The accuracy of LE and NIT were 69.3% and 69.8% respectively, suggesting NIT to be more accurate. A comparison of few studies is described in Table 1. Studies conducted by Zaman et al. in Belgium, Gieteling et al. in Netherlands, Sultana et al. in Australia, Demelie et al. in Ethopia and Bellazreg et al. in Tunisia concluded that leukocyte esterase test was more sensitive marker whereas nitrite test is more specific in diagnosing UTI.[2,8–11] This is also in accordance with the present study with comparable sensitivity and specificity.

## 5. Conclusion

Although Leukocyte esterase, Nitrite dipstick tests and pyuria are not 100% sensitive or specific, yet they are must for diagnosing urinary tract infections. In this study we saw that the accuracy of NIT and LE test were almost similar (69.8% and 69.3% respectively) with a small edge of NIT over LE. Quick, easy assessment and repeatability of dipstick test is an added benefit. Urine Culture, although the gold standard for diagnosing urinary tract infections, is laborious and takes 24-48 hours for interpretation of results. In that case, a Nitrite positive dipstick test, which has a high PPV, an empirical antibiotic course against *E. coli* can be considered. But for definitive diagnosis of urinary tract infections, a triad of Clinical symptoms, Routine chemical and microscopic analysis along with Urine culture is utmost necessary.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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## Author biography

**Agam Hans,** Junior Resident https://orcid.org/0009-0004-7117-5788

**Abhishek Yadav,** Junior Resident https://orcid.org/0009-0008-6835-1639

**Parneet Kaur,** Assistant Professor https://orcid.org/0009-0006-2061-7149

**Archana Kumari,** Junior Resident https://orcid.org/0009-0000-0810-0223

**Cite this article:** Hans A, Yadav A, Kaur P, Kumari A. Evaluation of Leukocyte esterase and Nitrite dipstick tests with routine urine microscopic analysis in detecting urinary tract infections. *Indian J Pathol Oncol* 2024;11(1):3-7.