



## COMPARISON OF LABORATORY METHODS FOR THE DIAGNOSIS OF URINARY TRACT INFECTION

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### ABSTRACT

**Introduction:** Urinary Tract Infection (UTI) is most frequent bacterial infection. Conventional urine culture is widely used for its diagnosis. This method is time consuming, expensive and patients are often treated before results are available. Dipsticks Nitrite Test (NT), Leucocyte Esterase (LET) and microscopic examination are commonly used primarily to predict the outcome of urinary tract infection.

**Objective:** To compare reagent strip testing with microscopy and culture on different medias to identify significant bacteriuria.

**Methods:** 500 samples were tested by semi-quantitative culture on cysteine lactose electrolyte deficient agar (CLED) with Andrade indicator, Mac conkey agar, Nutrient agar, microscopic examination of urine for significant pyuria, dipstick LET and NT. Culture was used as gold standard to evaluate the performance of direct microscopy and dipstick tests

**Results:** Out of 500 urine sample 162(32.4%) were culture positive, out of these 162 samples 100(61.7%) samples showed significant bacteriuria ( $>10^5$  CFU/ml). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the dip-stick LET were 71.6%, 75.7%, 58.5%, and 84.7% respectively; those of the dip-stick NT were 59.2%, 84.0%, 64%, and 81% respectively; and those for microscopic significant pyuria detection were 66%, 81.3%, 62.9% and 83.3% respectively. Highest sensitivity (87.6%) and NPV (93.1%) was obtained on combining microscopy and dip-stick LET and NT.

*Escherichia coli* was the most common organism isolated (n75=46.3%).

**Conclusion:** Study suggests that positive cultures cannot be accurately predicted by single method, but Combined use of all three methods have > 90% prediction (NPV) for negative culture. Use of CLED agar with Andrade indicator proven to be useful as primary medium & helped to reduce the plate burden & work load

### INTRODUCTION:

Urine cultures constitute a significant proportion of the specimens processed in most clinical microbiology laboratories. The majority of urine samples received for routine culture in a hospital setting do not contain culturable pathogenic bacteria, although the prevalence of urinary tract infection varies for different patient populations.<sup>(1,2)</sup>

UTI is one of the most frequent bacterial infection in all age groups. During the preschool years, UTI is more common in girls than in boys. The prevalence of bacteriuria increases in the female population with at least 10-20% of females experiencing a symptomatic UTI episode some time during their life.<sup>(3)</sup>

The most widespread reference method for diagnosis is conventional urine culture but it is time-consuming, expensive and patients are often treated before results are available. A rapid and sensitive method for screening out negative urine specimens would therefore benefit the laboratory and patient care by reducing costs and improve response time to clinicians.

There are many urine-screening tests including microscopic examination of urine sediment and dipstick analysis of nitrite and leukocyte esterase.

### MATERIALS AND METHODS:

**Study Design:** Prospective study

**Study Area:** Sheth Vadilal Sarabhai General hospital, Ahmedabad.

**Study Population:** Urine Samples coming for routine culture Identification & Sensitivity testing to the hospital bacteriology laboratory

**Study Period:** July 2012 to August 2012.

**Sampling technique:** Simple random Sampling.

**Sample Size:** 500 urine samples.

### STANDARD LABORATORY METHODS AND TECHNIQUES WERE USED AS FOLLOWS:

**Sample Collection:** The patients were advised to collect clean catch, mid-stream preferably early morning specimen of urine in sterile containers.

**Culture :** Specimens were streaked with a 0.01mL calibrated loop on Nutrient agar, Mac conkey agar & Cysteine Lactose Electrolyte Deficient agar (CLED) with Andrade indicator (Microexpress.) incubated at 35-37°C aerobically overnight .Bacterial isolates were confirmed by the observation of colony characteristics and by using battery of biochemical tests.

**Microscopy:** Urine specimens were centrifuged in 10ml amount at 2000 rpm for 5 minutes. White blood cells counts (cut-off at 10 WBC/HPF) per high-powered field (HPF) were compared with urine culture results. <sup>(4)</sup>

**Dipstick Testing:** Dip-stick leucocyte esterase test (LET) and nitrite test (NT) were evaluated using the Ultra stik-10 test strips (Agappe Diagnostic LTD) . Kit insert instructions were 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. Dipstick test was done on uncentrifuged urine sample.

**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, dip-stick LET and NT) using standard formulae.

The following values were calculated for the performance of the test in screening for bacteriuria : sensitivity =  $TP/(TP+FN)$ ; specificity =  $TN/(TN+FP)$ ; positive predictive value =  $TP/(TP+FP)$ ; and negative predictive value =

$TN/(TN+FN)$ , where TP is true positive, TN is true negative, FP is false-positive, and FN is false-negative.(5)

**RESULTS:**

Total 500 urine samples were collected out of them 290 were males & 210 were females. 162 (32.4%) were cultures positive of them, 154 (30.8%) samples showed growth of single organism, 8(1.6%) showed mixed growth and 338 (67.6%) samples yielded no growth. (Fig.1).

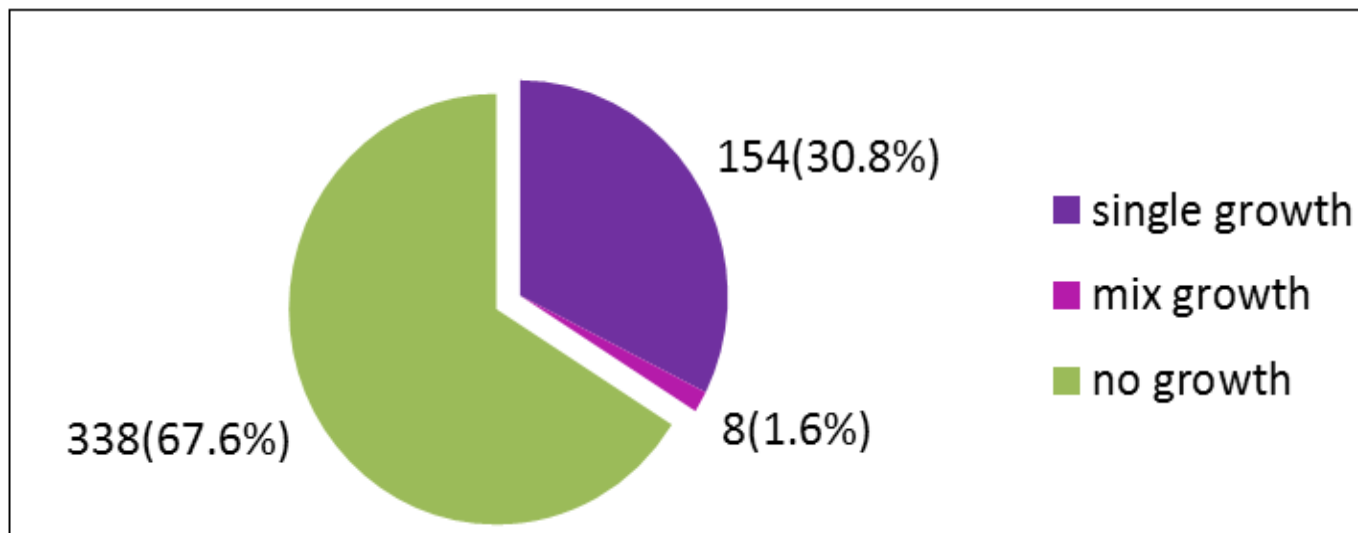
162 (32.4%) were cultures positive , out of these 162 samples 100(61.7%) samples showed significant bacteriuria ( $>10^5$  CFU/ml). 162 culture were positive out of them 92 females (56.7%) & 70(43.2%)males.

*Escherichia coli* was the most common organism isolated (n=75, 46.2%) followed by *klebsiella pneumonia* (n=42, 25.9%), then *Pseudomonas aeruginosa* (n=21,12.9%), *Acinetobacter baumannii* (n=8,4.9%), *Enterococcus*(n=7,4.3%), *proteus mirabilis*(n=4,2.4%), *staphylococcus coagulase negative*(n=4,2.4%), *staphylococcus aureus*(n=1,0.6%).

The results of urine microscopic examination, dipstick of nitrite test and leukocyte esterase test compared with their relationship to the results of urine culture are explained in table (1).

The sensitivity of leukocyte esterase test was (71.6%) higher than the nitrite test (59.2%) while its specificity was (75.7%) lower as compared to nitrite test (84%). The Positive and negative predictive value for nitrite test were (64%) and (81%) respectively and for leukocyte esterase test was (58.5%) and (84.7%) respectively and the combination of either of three tests positive had the highest sensitivity (87.6%) and NPV( 93%) (Table 2).

Figure 1: RESULTS OF URINE CULTURE



ORGANISMS GROWTH RESULTS ON CLED WITH ANDRADE INDICATOR

Escherichia coli	Orange-red to red colonies with rose to pink halos
Proteus mirabilis	Blue-green, transparent colonies
Klebsiella	Grey-green or orange to blue, mucoid colonies
Staphylococcus aureus	Smooth, opaque, golden-yellow colonies with rose halos
Enterococcus	Small opaque yellow to orange colonies, small rose to pink halos

Table 1: Results of Dipstick, Urine culture and Urine microscopy.

Urine culture	Nitrite test			Leukocyte esterase Test			Urine microscopy		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	96	66	162	116	46	162	107	55	162
Negative	54	284	338	82	256	338	63	275	338
Total	150	350	500	198	302	500	170	330	500

Table2. Sensitivity, specificity, predictive values of dipstick and urine microscopy

Screening test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Nitrite	59.2	84.0	64	81.1
Leukocyte esterase	71.6	75.7	58.5	84.7
Urine microscopy	66	81.3	62.9	83.3
Any one of three tests positive	87.6	79.8	67.6	93

**DISCUSSION:**

In this study the UTI was found in 162 of 500 outpatients, more females had UTI than males (56.7%,43.2%).

In the present study, from 500 culture positive cases a total of 162 strains were isolated. Of which, 69 (46.2%) were *Esch.coli* followed by 42(25.9%) *Klebsiella* spp, Various studies in Bangladesh noted that *Esch.coli* is the predominant organism<sup>(6,7)</sup>

CLED with Andrade indicator media inhibit proteus species from swarming, and distinguish lactose & non lactose fermenter, also supported growth of Gram positive bacteria, bacteria with low colony count better than routine media.

On CLED with Andrade indicator medium the presence of Enterococci is frequently masked by larger colonies of Gram negative species, for this reason isolation of the organism may be less in CLED with Andrade indicator.<sup>(8)</sup>

The nitrite test was found to have high specificity (84%); however, it also showed a low sensitivity (59.2%). This was similar to findings reported by Eidelman Y.<sup>(9)</sup> who found low sensitivity for nitrite test (39%). The Nitrite test give lower sensitivity as compared to leukocyte esterase can be explained by the fact that a minimum of 4 hours is required for pathogenic bacteria to reduce nitrate to nitrite .so the nitrite test is likely to be negative in random urine sample ,than the first morning voided specimens<sup>(10)</sup>.

In contrast, KacmazB. *et al*<sup>(11)</sup> found that sensitivity for nitrite test was 60.0% and Koeijers *et al*<sup>(12)</sup> found the positive predictive value of a positive nitrite test result was 96%. This difference may be because of different sample populations for these studies or because of the different brands of strips used for urinalysis. It may also be due to improper techniques for collection or transportation to the laboratory, allowing the colonizing bacteria to multiply, which result in false positive nitrite test which may result in under treatment and as

consequences could cause real damage or sepsis to the urinary tract system.

In the present study, pyuria (>10WBCs/HPF) was detected in 170 specimens however; 107 specimens were culture positive (Table 1). This suggests that pyuria alone cannot be used for detecting bacterial pathogens in patients with significant bacteriuria. MacDermott<sup>(13)</sup> concluded that there was no correlation between the degree of pyuria and a significant urine culture.

On the other hand Shaw *et al* reported that urine WBC count was sensitive in detecting UTI<sup>(14)</sup>, but had more false positive results than the urine dipstick (leukocyte esterase or nitrite). A report from Jordan showed that urine dipstick results (leukocyte esterase and nitrite) and pyuria significantly correlated with the results of urine

cultures but demonstrated more false positive, which ranged from 13.4 to 26.6%<sup>(15)</sup>. False-positive tests that result in misdiagnosis of urinary tract infections may lead to the wrong diagnosis, increase costs, and expose patients to the risks of unnecessary antibiotics.

In our study, High negative predictive value (in both dipstick and urine microscopy) is extremely useful as it to help to decide which urine sample should be cultured and which to be discarded.<sup>(16)</sup> There will be few cases (20 out of 162 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.

Table 3: Comparison of our study results with others studies.

Study	Nitrite test				Leukocytes esterase test			
	Sensitivity %	specificity %	Positive Predictive Value%	negative Predictive Value%	Sensitivity %	specificity %	Positive Predictive Value%	negative Predictive Value%
Our study	59.2	84.0	64	81.1	71.6	75.7	58.5	84.7
Weiunberg and Gan (17)	56	98.1	54.7	98.1	85.4	92.7	33.7	99.3
Sadika et al(18)	38.2	88.4	87.2	40.9	85.4	58	80	65.8
Sheriff et al(19)	54.6	96.8	37.5	98.4	100	78	13.9	100
Ayazi and Daneshi(20)	56	81	88	71	69	60	72.6	80
Loher et al(21)	37.3	100	100	90.2	79.4	72.7	33.6	95.3
Cannon et al (22)	72.7	99.6	96.8	94.6	71.4	33.6	96.9	91

**CONCLUSION:**

A combination of microscopic examination and dipstick tests improve the sensitivity (87.6%) of detecting urinary tract infections. Negative samples could be screened out with a very high degree of confidence (NPV 93%)

A positive screening test will require culture and sensitivity examination so that proper antibiotic can be prescribed.

Although study is expensive but for research purposes & minimum chances of missing positive culture a combination of different media should be used to detect both Gram negative and Gram-positive bacteria.

CLED with Andrade indicator media may be considered as primary screening medium to use in laboratory & help to reduce the plate burden & work load.

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