Validity of Nitrite and Leukocyte Esterase Tests for Laboratory Detection of Urinary Tract Infection in Specimens Submitted to the Philippine Heart Center: A prospective study from October 2005 to September 2006

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Background --- Forced expiratory volume in six seconds (FEV6) is an acceptable alternative to forced vital capacity (FVC) for diagnosing airway obstruction in adults. The use of FEV6 simplifies testing procedures, reduces test variability, and may improve accuracy in diagnosing airway obstruction. The study was conducted to determine the relationship of FEV1/FEV6 with FEV1/FVC in the spirometric detection of severity of airway obstruction among Asians at Philippine Heart Center.

Methods --- This is a one year cross sectional study comparing the FEV1/FEV6 versus FEV1/FVC in the spirometric diagnosis of airway obstruction among Asians at the Philippine Heart Center. Patients who underwent spirometric studies at Philippine Heart Center Pulmonary Laboratory from May 2005 to April 30, 2006 were evaluated. Baseline demographic data, smoking history and spirometric results were evaluated. The highest post-bronchodilator FEV1, FEV6 and FEV1/FEV6% from tests of acceptable quality were used for analysis. Each subject was categorized as having "airway obstruction " by comparing both FEV1/FVC and FEV1/FEV6 with the respective lower limits of normal defined by Hankinson and coworkers. We used FEV1/FVC as the "gold standard" for diagnosing airway obstruction. The severity of airway obstruction was graded into one of four categories; possible normal variant (FEV1>100% predicted), mild (FEV1 70-100% predicted), moderate (FEV1 50-70% predicted), and severe (FEV1 <50% predicted).The sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of FEV1/FEV6% in predicting airway obstruction as defined by FEV1/FVC were calculated. The agreement between test result classification based on FVC and FEV6 was calculated using the Kappa test.

Results --- Of 597 spirometric tests analyzed, 352 were males and 245 were females. 78% of males were smokers. FEV1/FEV6 has 97.6% sensitivity and has 83.6% specificity in detecting mild airway obstruction, with a positive and negative predictive values of 93.1% and 93.3% respectively. Indeed, with a kappa value of 0.837, a very good overall performance was obtained for FEV1/FEV6% in detecting mild airway obstruction. In addition , a kappa value of 0.694 was a substantial agreement for FEV1/FEV6 in detecting moderate airway obstruction.

Conclusion --- FEV6 is an acceptable surrogate for FVC in detecting airway obstruction in Asian adults. Using FEV6 instead of FVC has the advantage that the end of a spirometric examination is more explicitly defined and is easier to achieve. ***Phil Heart Center J 2008; 14(1):56-60.***

Key Words: Spirometry ■ FEV1 ■ FVC ■ Obstructive Lung Disease ■ Validity Study

Routine urinalysis using dipstick is ordered to screen for Urinary Tract Infection (UTI) in general. The two tests included in the dipstick urinalysis that indirectly assess UTI are the urinary nitrite test (NT) and the leukocyte esterase test (LE). While urine culture remains the gold standard for the laboratory diagnosis of UTI, the use of NT and LE test to determine the necessity of such costly procedure can be a cost effective measure.

Urinary nitrite is a substance produced from the breakdown of dietary nitrates by most urinary pathogens. More than 105 to 106 bacteria per ml of bladder

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urine will generate a positive NT.[1] Common organisms include Escherichia coli, Klebsiella, Enterobacter, Proteus, Staphylococcus, and Pseudomonas species. Whenever the nitrite test is positive, a culture is done to confirm and identify the organism as well. The test is based on Griess' test and is specific for nitrite.

Leukocyte esterase is an enzyme produced by granulocytes, mostly neutrophils. It is a more reliable indicator of pyuria because it detects both the lyzed and intact granulocytes. The LE test is negative if there are less then 100 neutrophils per 10 high power field.[4]

Many studies have evaluated the accuracy of dipstick urinalysis as a screening for UTI in different populations and different centers. A study was done by D'Souza and D'Souza in 2004 wherein they compared dipstick urinalysis with culture and sensitivity in 100 urine samples. They have concluded that bedside dipstick urinalysis maybe a useful and cost-effective way of reducing numbers of urine samples sent for further testing.[6] Leman P in 2002 studied urinalysis results of 60 female patients with triage diagnosis of UTI. Forty percent have proven UTI after culture. Microscopy alone done on these patients showed 100% sensitivity, specificity of 38.9% and Positive Predictive Value (PPV) of 45.1%. But if both NT and LE tests were positive, the PPV is increased to 100%. [8]

These encouraging results were taken further by Patel, et. al. One thousand and seventy six urine samples were dipstick tested at point of care and the quantitative results for markers of UTI - LE, nitrites, blood and protein were compared with the results of routine microscopy and culture. They were found to have greater than 98% NPV and a sensitivity and specificity of 98.3% and 19.2% respectively.[9]

However, some studies done do not agree with the reported benefits of NT and LE. Van Nostrand et al in 2001 studied 225 urine cultures and the result showed that there is lack of sensitivity for NT, LE and presence of bacteriuria as indicators of UTI.[10] A study done by Eidelman Y et al in 2002 showed dipstick urinalysis based on LE, NT, RBC and CHON is not sufficiently sensitive for diagnosis of UTI.[11] Buchsbaum GM, et al evaluated the utility of urine reagent strip in general in screening women with incontinence for UTI. A total of 265 pairs of reagent strips and urine cultures were evaluated. They have concluded that for women presenting with urinary incontinence, the sensitivity of a urine reagent test for diagnosing UTI was low.[12]

These major differences in reports of accuracy and predictive values in these several studies left room for a study in one's own laboratory setting. A retrospective analysis of statistics at the Philippine Heart Center showed a total of 15,255 urinalysis and 1,108-urine culture done for year 2004. Considering the bulk of urinalysis and urine culture that were requested, and the cost of these procedures, this study is significant in our center, as it will serve as a baseline reference in our laboratory work up for UTI. Thus, this study was carried out to determine the validity of NT, LE and a combination of both tests using urine culture as the gold standard in the laboratory detection of UTI in a general population tested at the Philippine Heart Center from October 2005 to September 2006.

Methods

This is a prospective cross-sectional study done at Philippine Heart Center Biological and Clinical Laboratory Division from October 2005 to September 2006. Included were all first morning urine specimens submitted for simultaneous routine urinalysis and urine culture and sensitivity at our laboratory during this time frame. Excluded were the following: urine specimens submitted for routine urinalysis with subsequent separate specimen for culture and sensitivity; urine specimen submitted for either routine urinalysis or urine culture and sensitivity only; randomly collected specimens and urine collected from draining catheters.

All first morning urine specimens for urinalysis and culture were recorded by the researcher including the patient's name, age, sex, and the time and date of entry. Using a 1 uL calibrated loop, a loopful of the urine specimen was streaked on MacConkey Agar and Blood Agar Plate using aseptic technique. The plates were incubated at 35 degrees Centigrade for 18 to 24 hours. The isolated colonies were counted and multiplied by 1000 to obtain the number of Colony Forming Units (CFU)/ml of urine. Representative colonies were tested using Gram stain and biochemical methods to identify the species. Urine culture with isolation of 3 or more different species with a colony count in the range of 104 to 105 was reported as suggestive of genital contaminants. Urine specimens were incubated for 48 hours before having been reported as negative growth.

Immediately after obtaining an aliquot for urine culture, the rest of the specimen was subjected to routine urinalysis, which included physical examination, chemical dipstick test (Roche Combur UX10 test, Roche Miditron M) in which the NT and LE test are included, and microscopic examination. The unspun urine sample was thoroughly mixed. The test strip (Roche Combur UX10) was briefly dipped into the urine sample ensuring that all test areas were moistened. The long edge and the back of the test strip was briefly dabbed to a sterile paper towel and immediately

inserted to the machine (Roche Miditron M). All results were recorded accordingly. The NT, LE and combined NT - LE test results were compared with the culture results. The specificity, sensitivity, PPV and NPV of LE alone, NT alone and combined LE - NT were computed and compared.

Results

Of the 324 urine specimens included in the study, 100 (30.86%) have significant growth in urine culture.

Table1. Frequency Distribution of Microorganisms Isolated in the 100 Urine Cultures

Microorganisms Isolated	Culture with Growth (%)
Escherichia coli	37
Candida non-albicans	12
Klebsiella pneumoniae	10
Candida albicans	7
Enterococcus faecalis	6
Staphylococcus coagulase negative	4
Proteus mirabilis	4
Pseudomonas species	3
Klebsiella oxytoca	2
Stenotrophomonas maltophilia	2
Streptococcus agalactiae	2
Morganella morgani	1
Pseudomonas aeruginosa	1
Staphylococcus epidermidis	1
Microscopy and culture suggestive of genital contamination	8
Total	100

The most frequent isolate in the urine culture was Escherichia coli (37%). Most of the patients with growth in their urine culture were adult females. The growth of mixed isolates indicating genital contamination was also common in females in all age group.

Table 2. Leukocyte Esterase test results with urine culture results

Urine Culture	Growth (+)	Growth (-)	Total
LE (+)	77	118	195
LE (-)	23	106	129
Total	100	224	324

In the result, 118 of LE (+) urine samples have no growth in culture (Table 2). This may be due to the presence of leukocyturia in-patients with interstitial nephritis. In these cases, there was significant leukocyturia with no accompanying bacteriuria. It may also be due to interferences in the reagent strip such as the contamination of urine with strong oxidizing agents, which could have caused a false positive LE test. Twenty-three were LE (-) but with growth in urine culture. This may be due to a high concentration of protein, glucose, oxalic acid, or ascorbic acid, which caused a false negative LE test. Eight of the 23 have proteinuria ranging from trace to +3: one has glucose of +3, and two have both proteinuria and glucosuria. The other 12 patients may have had a history of antibiotic intake prior to urine collection.

Table 3. Measure of association of the LE test results with the urine culture

Measure of Association	%	(95% confidence interval)
Sensitivity	77	67.3 , 84.6
Specificity	47.3	40.7 , 54.1
Predictive Value Positive	39.5	32.6 , 46.7
Predictive Value Negative	82.2	74.2 , 88.1

The LE test had a low sensitivity, specificity and predictive values. It had a poor agreement with culture result (Kappa coefficient - 0.1926). It had no significant difference with the NT (on the p value >0.05), but there was a significant difference from the combined LE-NT tests (p value <0.05). These results made the LE test not valid when used alone as a screening tool for UTI.

Table 4. Nitrite test results with urine culture results

Urine Culture	Growth (+)	Growth (-)	Total
Nitrite (+)	22	6	28
Nitrite (-)	79	217	296
Total	101	223	324

The positive NT results with negative culture growth can be due to postcollection bacterial proliferation, which has produced measurable amounts of nitrite. The high number of NT negative results with a positive culture growth was attributed to the growth of non-nitrate reducing bacteria and yeasts in the culture. Eighteen percent were identified as Candida, 4% Enterococcus, 1% Stenotrophomonas maltophilia, and 10% mixed flora. These were non-nitrite-reducing organisms. The rest of the urine specimens, which were NT positive but were culture negative, may have come

from patients with low nitrate diet. This is a limitation of this study in as much as not all patients who undergo urinalysis are instructed by their physicians to observe nitrate-rich diet three days before specimen collection.

Table 5. Measure of association of the Nitrite test results

Measure of Association	%	95% Confidence Interval
Sensitivity	21.8	14.4 , 31.3
Specificity	97.3	94.0 , 98.9
Predictive Value Positive	78.6	58.5 , 91.0
Predictive Value Negative	73.3	67.8 , 78.2

The NT had a low sensitivity and predictive values but had a high specificity .It had a poor agreement with the culture results (Kappa coefficient 0.2379). It has no significant difference from either the LE test (p value >0.05) or the combined NT-LE test (p value <0.05). The low sensitivity and low predictive values, and its poor agreement with culture result made NT not valid when used alone as screening for UTI. Its high specificity, however, makes the test of value when one is ruling out UTI.

Table 6. Combined LE and Nitrite test results with urine culture results

Urine Culture	Growth (+)	Growth (-)	Total
LE and Nitrite (+)	19	6	25
LE and Nitrite (-)	22	104	126
Total	41	110	151

When taken into combination, the LE-NT results had a better agreement with the culture result (Kappa coefficient 0.4659) compared with either test done alone. The positive results of both LE and NT on six culture negative urine could be again attributed to post collection bacterial proliferation, and three of these specimens have +1 to +3 urinary protein and glucose which could have caused a false positive LE. Six of the 22 urine specimens with (-) LE-NT but with growth in culture grew non-nitrite reducing organisms (Candida -3, Enterococcus -1 and contaminants -2). The rest may again be attributed to low nitrate diet of the patient (NT-) and interference in the reagent strip (LE+).

Table 7. Measure of association of combined NT-LE test results with urine culture results

Measure of Association	%	(95% confidence interval)
Sensitivity	46.3	31.0 , 62.4
Specificity	94.5	88.0 , 97.8
Predictive Value Positive	76.0	54.5 , 89.8
Predictive Value Negative	82.5	74.5 , 88.5

The combined NT-LE tests had low sensitivity and low predictive values but had high specificity. Its high specificity makes the combined LE-NT results of value when ruling out UTI. It has a better agreement with culture (Kappa coefficient 0.4659) compared with either LE or NT alone.

Table 8. Comparison of the measures of association of LE, NT, and combined LE-NT

Tests	Sensitivity	Specificity	PPV	NPV	Kappa Coefficient	+/- SD	p-value
LE	77%	47.3%	39.5%	82.2%	0.1926	0.0466	0.0000
NT	21.8%	97.3%	78.6%	73.3%	0.2379	0.0420	0.000
Combined LE-NT	46.3%	94.5%	76%	82.5%	0.4659	0.0775	0.000

Taking into consideration all the measures of association of the tests, all were statistically significant with the combined LE-NT having the closest agreement with culture results, and with the NT and combined LE-NT having high specificity.

While our results disproved our first hypothesis that there is no difference in the accuracy of NT, LE and combined NT-LE tests, the differences on the tests' measure of association tend to overlap. Our second hypothesis stating that combined NT-LE test result is not more accurate compared with either test done alone does not hold true in all the measures of association.

Conclusion

The LE test has very low measures of association and poor agreement with culture results to be used alone as a screening test for UTI. The NT has a slightly better agreement with culture result compared with the LE test. The combined LE-NT tests have better agreement with culture result compared with either LE or NT alone. Both NT alone and combined LE-NT has high specificities hence either NT alone and combined LE-NT is useful when one is ruling out UTI. Depending on a patient's clinical presentation, a urine specimen with a negative NT or a combined negative LE-NT can be reasonably excluded from costly urine culture.

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