

URINALYSIS AND STANDARDIZATION

Aziz Marjan Khattak¹, Bushra Ashiq²

¹Department of Pathology, Gomal Medical College, D.I. Khan and

²Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi

The identification of bacteria more than a century ago, soon lead to the finding of microorganisms in the urine.¹ The presence of significant number of bacteria ($>10^5$ organisms /ml) in urine reflects urinary tract infection (UTI).² UTI is among the most common infections encountered by physicians.³ It can be asymptomatic or symptomatic.⁴ Asymptomatic infection may lead to symptomatic infection in a week's time⁵ or complications of urinary tract and other systems without developing to symptomatic UTI.⁶

Examination of urine is the most common screening laboratory procedure utilized for the early detection of renal or urinary tract disease.⁷ Urine for bacterial culture is among the most common specimen submitted to the clinical microbiology laboratories and 10 to 20% are subsequently found to be positive for bacteriuria.⁸

The complete examination of urine includes: 1) Gross examination 2) Chemical examination 3) Microscopic examination and 4) Culture.⁹ The whole process of urine examination just from collection to storage and transport to laboratory and method applied for examination will affect the quality of reporting. In addition to the skill of operator, the reagents used also contribute to standard reporting on specimen provided.¹⁰

Complete urine examination is a vast topic. This review article will emphasize on specimen collection, transport, storage, microscopy and processing for culture.

COLLECTION OF URINE SPECIMEN

Except for the urethral mucosa which supports the growth of microflora, the normal urinary tract is devoid of bacteria.¹¹ As male and female urethra and the female peri-urethral area harbor microorganisms, urine becomes easily contaminated with bacteria from vaginal canal or perineum. To avoid contamination, there are three methods for collection of specimen: a) Midstream collection technique, b) Catheter collection and c) Supra-pubic aspiration.¹²

Midstream collection technique: Urethral colonies will contribute only small number of bacteria if collection of specimen is delayed until the ure-

thra has been flushed by passage of the first portion of voiding. Such clean catch, midstream, specimens are quite satisfactory for culture¹² and can be collected from anyone, male or female, who retains voluntary control of micturition.¹³

For urine obtained by bag technique two samples with homogenous growth of at least $>10^5$ CFU/ml is considered significant.¹

Where possible, patients should be evaluated in the early morning first voiding. If this is not possible a period of at least 4 hours since the last voiding is required before the collection of initial urine for culture.¹⁴ Women should be examined for exclusion of vaginitis, before collection of specimen.^{3,14}

Catheter collection: Catheterization for the purpose of obtaining urine specimen should be avoided if possible because of the high risk of introducing nosocomial infections. Catheterization should be restricted to those patients who are unable to produce midstream urine sample. The culture results obtained from midstream clean catch urine did not differ in sensitivity, specificity and positive or negative predictive value from a parallel in-out catheter specimen.¹⁵ Stamm et al¹⁴ (1982) found results obtained by catheterization samples similar to suprapubic aspiration.

Supra-pubic aspiration: In urine obtained by supra-pubic aspiration, any growth is considered significant.¹

TRANSPORT AND STORAGE

Urine specimen delayed in transport to the laboratory more than one hour or with visible sign of contamination should be rejected.¹⁶ The samples ideally should be transported immediately and processed in laboratory because samples that sit at room temperature for several hours can have falsely elevated colony counts. If the sample cannot be transported to the laboratory quickly it should be refrigerated; samples at 4°C are relatively stable for approximately 24 hours, recommendations given by Millar and Cox 1997.¹⁷

Boric acid as preservative was evaluated and proved to be the best chemical for urine specimen before culture.¹⁸

When immediate delivery to laboratory is not possible refrigerate the urine at 4- 6°C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine. Specimens containing boric acid need not to be refrigerated.¹⁹

MACROSCOPIC EXAMINATION

Sediment microscopy can be deleted if macroscopic and dipstick analysis are normal.¹⁶ Only 2.4%¹⁶ or 3%²⁰ would be found to have abnormal sediment microscopic findings after negative macroscopic findings and dipstick analysis. The five most common causes of turbidity in the urines are leukocytes, erythrocytes, epithelial cells, bacteria and amorphous material.²⁰

Dipstick urine screening has been augmented with nitrite and leukocyte esterase test strips available as nine test dipstick. Nitrite test is based on the ability of enteric gram-negative bacteria to reduce dietary-derived nitrate to nitrite. Leukocytes can be detected by the action of esterase present in granulocytes and histiocytes, enabling the detection of even lysed leukocytes that may not be recognized on sediment microscopy.²¹ Leukocyte esterase strip test is able to detect 10 WBC/ μ l by chamber counting, denoting sensitivity of 87.9% and 95.3% respectively.²²

Much time and expense could be saved by omitting the microscopic examination on those urine specimens which are yellow and clear and have a negative chemical reaction to the reagent strips.²⁰

The reagent strips having the panels to detect protein, blood, and nitrite and leukocyte esterase were used in a study²³ for urine of asymptomatic bacteriuria during pregnancy. The sensitivity was 33% when all the four tests were used in combination and specificity was 99%. It was concluded that reagent strips are not sufficiently sensitive for use in the screening for asymptomatic bacteriuria and therefore, many patients would be missed.

In another study by Rehmani²⁴ (2004) urine dipstick evaluation was performed for symptomatic patients. The sensitivity of nitrite test was 81% and that of leukocyte esterase 77% for positive cultures. However, the sensitivity for combined nitrite and leukocyte esterase test was 94%. Nitrite test was more specific (87%) than leukocyte esterase test (54%) or both tests taken together (50%). The predictive value of nitrite and leukocyte esterase together for a negative urine culture was 95%. Dipstick alone cannot accurately predict urinary tract infection in emergency department.

CHEMICAL EXAMINATION

Patients usually bring their first morning specimen on each follow up visit. This specimen is used

primarily for the detection of protein and glucose. Urinalysis with microscopic analysis may also be done in attempt to detect bacteriuria. Although morning specimen has been used for detecting UTI, there is skepticism about its value in routine screening.²⁵

Controversy exists over the biochemical tests. The use of strips to test for the presence of nitrites and the ability to reduce triphenyl-tetrazolium chloride are helpful if positive, but they often yield false negative results. Use of a leukocyte esterase (LE) test can also yield false positive and false negative results.³ The LE test detects esterase released from degraded white blood cells. It is therefore, an indirect measure of WBCs whose presence is induced by urinary bacteria.²⁶

MICROSCOPIC EXAMINATION

Correlation studies between sediment microscopy leukocyte count/HPF compared with chamber counting WBC/ μ l, had variable results due to lack of uniformity of sediments microscopy methods.^{16,26} Allwall²⁷ found the number of WBC/HPF to be approximately 11% of the number of WBC/ μ l, or approximately 9 WBC/ μ l for each WBC/HPF. Microscopic examination of urine that shows >10 WBCs/mm³ from clean catch midstream urine indicates infection. If bacteria are seen in un-spun urine specimen, this indicates significant bacteriuria i.e. >10⁵ CFU/ml.³

Sediment microscopy was performed on ten fold concentrated sediment suspension obtained by centrifugation of 10 ml urine at 2000 rpm (radius of 9 cm) for 5 minutes.²⁵ The sediment after decantation was re-suspended to a volume of 1 ml. A minimum of 10 HPF were scanned for formed elements, the average of ten fields was reported (0-4 WBC/HPF and 0-3 RBC/HPF taken as normal range).¹⁶ For sediment microscopy, specimen was centrifuged in 15 ml amounts at 2000 rpm for 5 minutes. Significant pyuria was inferred by the presence of more than 10 leukocytes/ HPF. For urine gram staining, a 0.01 ml loop was used to apply mixed un-centrifuged urine to a slide. A positive smear was defined as more than 2 organisms per oil immersion field.²⁵

Pyuria was defined as five or more white blood cells per HPF (un-spun specimen). The un-spun specimen yields fewer false positive and is almost as sensitive as the spun specimen.²⁸

Urine cytological examination is an efficient tool that has a good diagnostic yield in detecting malignant urothelial lesions.²⁹

Gram staining and urine culture were carried out on urine samples from catheterized patients. The sensitivity, specificity, positive predictive values and negative predictive values of the test were 73.5%,

77.7%, 76.4% and 75.0% respectively. It is concluded that due to high false negative (26.5%) and false positive (22.3%) rate, Gram staining is not a reliable screening test for the diagnosis of urinary tract infections.³⁰

The presence of $> 10^5$ organisms/ml frequently means infection where as contamination with less than 10^4 organisms/ml usually do not.³¹

CULTURE

Urinary infections are characterized by periods when bacteria, almost always coliform organisms, multiply in the urine within the urinary tract. When this happens the urine usually contains 100,000 or more bacteria per ml (Significant bacteriuria), where as lower numbers of organisms are most often contaminants which have entered the urine from urethra or external genitalia while the specimen was being voided.⁶

The traditional definition for asymptomatic urinary tract infection is measurement of 100,000 of a single uropathogen per milliliter on more than one separate clean catches, was used by few authors while most considered one specimen of 100,000 uropathogen per milliliter as positive culture.²⁵

Urine culture is the gold standard method for diagnosis of urinary tract infection.²⁶ Culture of urine specimen could be performed by 1) Uricult dip-slide 2) Swab method of plating out 3) The standard loop method of plating out and 4) Pour plate culture.³¹

The presence of UTI is defined as the existence of urinary symptoms such as frequency, urgency of urination and dysuria with or without bacteria ($> 1 \times 10^2$) or pyuria (8 leukocytes per milliliter).^{5,32}

A concentration of 10^3 colony forming units / ml can cause an acute urinary tract infection in healthy women.^{3,14}

Quantitative urine culture is most accurately performed by the classic dilution pour plate method which is too expensive of time, personnel and material for use in laboratory.³³ Surface inoculation culture simple quantitative method was developed based on standard, volume calibrated bacteriologic loops.³³ Two standard loops with the following characteristics were used by Hoeprich (1960)³³ to prepare quantitative streak plate cultures: (1) to deliver a 0.01 ml sample – 4 mm inside diameter fused loop with 75 mm, shank fashioned of 3.5% rhodium and 96.5% wire of Brown and Sharp gauge-19 (0.036 inch in diameter); (2) to deliver 0.001 ml-1.45 mm inside diameter fused loops with 50 mm shank fashioned of 3.5% rhodium and 96.5% platinum, of Brown and Sharp gauge-26 (0.016 inch in diameter) was used.

Platinum and Nicrome closed loops are also recommended.

Stamm et al (1982)¹⁴ found pathogens in acutely dysuric women with Coliforms 52.40% and less commonly, Staph saprophyticus, S. aureus, Enterococci. Neu (1992)³ found E. coli as the most common and other organisms in the order of frequency as Staph. Saprophyticus, Klebsiella, Proteus mirabilis, Enterococci. While Bachman et al (1993)²⁵ did not consider Staph coagulase negative and Staph aureus as uropathogens while pathogens in order of frequency were Escherichia coli, Klebsiella, Staph saprophyticus, B streptococcus. Tincello & Richmond (1998)²³ found in asymptomatic pregnant women uropathogens as Coliforms the most common and Group B Streptococcus, Staph aureus, Other Streptococci, Enterococcus, Staph albus, Proteus as less common. Hooton et al (2000)⁵ found the following uropathogens in order for frequency as Gram negative bacilli, Staph. saprophyticus, Staph. aureus, Enterococci and Group B Streptococci. While coagulase-negative staphylococci, alpha haemolytic streptococci, lactobacilli, diphtheroids and mixed gram-positive flora as non-pathogens. Khan & Shah (2000)⁴ found uropathogens in UTI in order of frequency as E. coli, Klebsiella pneumoniae, Proteus spp and Pseudomonas aeruginosa. Mohanna & Raja'a (2005)³⁴ reported uropathogens in children in order of frequency as E. coli, Staph. saprophyticus, Proteus spp and Enterococcus spp.

The uropathogens isolated in asymptomatic bacteriuria are the same as in symptomatic bacteriuria.¹⁷

CONCLUSION

Treatment based only on microscopic urinalysis would lead to many patients not receiving appropriate treatment (high false negative rates) and many being treated unnecessarily (high false positive rates).²⁶ Standard methods of processing should be adopted from the time of specimen collection, storage and transport to complete examination. The following recommendations are suggested briefly:

- The specimen should be early morning one if possible, otherwise at least of a 4 hour stay in bladder before collection. Midstream clean catch is convenient method; however catheter and supra-pubic collection methods can be applied if indicated. The method of collection should be described in case of collection by the two methods.
- Processing of urine should be started immediately. In case of delay urine should be refrigerated at 4 to 8°C ideally up to 2 hours but not more than 12 hours. In case refrigeration facility is not available, boric acid (1% w/v) can be used as preservative.

- Dipstick analysis of urine is controversial however it can be applied in areas with out sophisticated facilities.
- Lysed WBCs can be detected by the LE test.
- The results of spun and un-spun specimens remain the same on microscopy provided standard fixed volume of urine is applied.
- The classical number of pathogens established is $>1 \times 10^5$ CFU/ml of urine in asymptomatic UTI but less number $>1 \times 10^2$ CFU/ml in symptomatic patients is accepted.
- Escherichia coli is the commonest pathogen. For the next common pathogens, there is great variation in different communities.

REFERENCES

- Hansson S, Martinell J, Stokland E, Jodal. The natural history of bacteriuria in childhood. *Infect Dis Clin N Am U* 1997; 11: 499-512.
- Kass EH, Finland M. asymptomatic infections of the Urinary tract. *Trans Assoc Am Physicians* 1956; 69: 56-6.
- Neu HC. Urinary tract infections. *Am J Med* 1992; 92 (suppl 4A): 63-69.
- Khan MT and Shah SH. (2000) Experience with gram negative bacilli isolated from 400 cases of UTI in Abbottabad. *J Ayub Med Coll*; 12(4):21-23.
- Hooton TM, Scholes D, Stapleton AE, Roberts PL, Winter MSC, Gupta K, Samadpour M, Stamm WE. A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl J Med* 2000; 343: 992-7.
- Kass E H. Chemotherapeutic and antibiotic drugs in the management of infections of the Urinary tract. *Am J Med* 1955; 18: 764-81.
- Berryschumann G, Greenberg NF. Usefulness of macroscopic urinalysis as a screening procedure. *Am J Clin Pathol* 1979; 71: 452-56
- Aathithan S, Plant JC, Chaudry AN, and French GL. Diagnosis of Bacteriuria by Detection of Volatile Organic Compounds in Urine Using an Automated Headspace Analyzer with Multiple conducting Polymer Sensors. *J Clin Micro* 2000; 39: 2590-93.
- Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Washington WC Jr. Introduction to Microbiology In: *Color Atlas and Text Book of Diagnostic Microbiology 5th Edition*. Lipponcott Williams and Wilkins, Philadelphia: 1997; 121-162.
- Cheesbrough M. Quality assurance and sources of error in district laboratory practices In: *District Laboratories Practice in Tropical Countries Part 1*; University Press Cambridge UK 1999: pp 34-7.
- Kaye E. Antibacterial activity of human urine. *J Clin Invest* 42:2374-2390.
- Monzon OT, Ory E M, Dobson H L, Carter E, Yom E M. A Coparasion of Bacterial Counts of the Urine Obtained by Needle aspiration of the Bladder , Catheterization, MidstreamVoided Method, *New Englad J Med* 1958; 259: 764.
- Riley , HD Jr. Evaluation of a Method for Detecting and Following Urinary Tract Information in Fmales Without Catheterization. *J Lab & Clin Med* 1958; 52: 840.
- Stamm WE, Counts GW, Running KR, Fhin S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. *N Eng J Med* 1982; 307: 463-68.
- Walter HG, Knopp RK. Urinary sampling in ambulatory women: midstream clean catch urine versus catheterization. *Ann Emerg Med* 1989; 18: 166-72.
- Loo SYT, Scottolini AG, Luangphinth S, Adam AL, Jacobs LD, Mariani AJ. Urine screening strategy employing dipstick analysis and selective culture: An evaluation. *AJCP* 1984: 81; 634-642.
- Millar LK and Cox SM. Urinary tract infection complicating pregnancy. *Infec Dis Clin North Am* 1997; 11: 13-26
- Goodman LJ, Kaplan RL, Landou W, et al. A urine preservative system to maintain the bacterial counts. A laboratory and clinical evaluation. *Clin Paediatr* 1985; 24: 383-6.
- Cheesbrough M. Microbiological tests. In: *District Laboratories Practice in Tropical Countries Part 2*. Gopsaons Paper Limited NOIDA, India 2002: pp 14,106.
- Schumann GB, Greenberg FN. Usefulness of Macroscopic Urinalysis as a Screening Procedure. *Am J Clin Pathol* 1979; 71:452-6
- Aven J, Schumann GB, Vars L. Coparasion of chemstrip Leukocyte test with standardized Piponicolaou-stained urine sediments evaluation. *Lab Med* 1983;14:163-66.
- Kusumi RK, Grover PU, Kunin CM. Rapid detection of pyuria by Leukocyte esterase activity. *JAMA* 1981; 245: 1653-55.
- Tincello DG, Richmond DH. Evaluation of reagent strips in detecting asymptomatic bacteriuria in early pregnancy: Prospective case series. *Br Med J* 1998; 316: 435-7.
- Rehmani R. Accuracy of Urine Dipstick to predict Urinary Tract infections in an Emergency Department. *J Ayub Med Coll Abbottabad*: 2004; 16: 4-7.
- Bachman JW, Heise RH, Naessens JM, Timmerman MG. A study of various tests to detect asymptomatic urinary tract infections in an obstetric population. *JAMA* 1993; 270: 1971-74.
- Smith MBH. Screening for urinary infection in asymptomatic infants and children. *Canadian Guide to Clinical Preventive Health Care*; 1994: 220-230.

-
- 27 Allwall N Pyuria deposit in high power microscopic field WBC/HPF vs WBC /cmm³ in counting chamber Acta Med Scand 1973;194:537-40
- 28 Lenke R R, Dorsten J P V. The efficiency of the Nitrite test and microscopic urinalysis in predicting urine culture results. Am J Obstet Gynecol 1981; 140: 427.
- 29 Mansoor I. Analysis of urine cytology at a community hospital. J Ayub Med Coll 2003; 15: 20-3.
- 30 Mumtaz S, Ahmad M. Value of urinary Gram-staining in the diagnosis of urinary tract infection in catheterized patients. Pakistan J Med Res 2000; 39:168-9.
- 31 Weissenbachs ER, Reason barges K. Uncomplicated Urinary tract infection in pregnant and non-pregnant women. Cuss opine obstet Gyneco 1993; 5: 513
- 32 Savage DCL, Wilson MI, McHrdy M, Dewar DAE, Fee WM. Covert bacteriuria of Childhood: A clinical and epidemiological study. Arch of Disease in Childhood 1973; 48: 8-20.
- 33 Hoeprich PD. Culture of the urine. J Lab Clin Med 1960; 56: 899-907.
- 34 Mohanna MA, Raja'a YA. Frequency and treatment of UTI in children subjected to urine culture in Sana'a Yemen. J Ayub Med Coll 2005; 17: 20-22..
- Address for Correspondence:**
- Dr. Aziz Marjan Khattak
M Phil Microbiology
Assistant Prof. Pathology
Gomal Medical College
D.I.Khan, Pakistan
Phone: 0966-9280338