

LABORATORY DIAGNOSIS OF URINARY TRACT INFECTIONS

Chapter

13

A diagnosis of a urinary tract infection (UTI) is based on the patient's history, physical examination and laboratory investigations. Overuse of antibiotics for the empiric management of patients with UTIs has resulted in many uropathogens developing resistance to common antibiotics. Management of UTIs needs a clear plan that considers the patient's age and other factors such as pregnancy, complications and symptom recurrence. Since the sensitivities of uropathogens have become much less predictable, urine culture to identify the causative uropathogen and performing susceptibility testing has become mandatory in the management of all UTIs, especially in the case of 'complicated UTIs'.

Laboratory investigations include:

- Rapid tests: urine dipstick
- Urine specimens for microscopy, culture and sensitivity (MC&S)

INDICATIONS TO PERFORM URINE MC&S

- Especially important in recurrent infections, treatment failures and paediatric patients due to growing antimicrobial resistance that necessitate antimicrobial susceptibility testing to guide antibiotic therapy.

URINE MC&S IS INDICATED FOR ALL PATIENTS WITH A 'COMPLICATED UTI'. THESE INCLUDE:

- All neonates/children with suspected UTI, including any young child with unexplained fever
- All male patients with suspected UTI
- All elderly patients, especially if dysuria, pyrexia, or incontinence is present
- All pregnant patients: Urine MC&S should be performed at the first antenatal visit to detect asymptomatic bacteriuria which is associated with pyelonephritis and premature labour. The presence of bacteriuria in urine should be confirmed with a second urine culture. Periodic screening for recurrent bacteriuria should be performed following treatment.
- All immunocompromised patients with a suspected UTI
- All patients with comorbidities such as diabetes
- Patients with suspected acute pyelonephritis
- Catheterised patients, if symptomatic or features of systemic infection are present
- Patients who have failed empiric antibiotic therapy
- Prior to performing transurethral resection of the prostate or any urological procedure where mucosal bleeding is anticipated

URINE MC&S IS INDICATED IN YOUNG WOMEN UNDER THE FOLLOWING CIRCUMSTANCES:

- Suspicion of complicated infection, including pyelonephritis
- Atypical symptoms
- Absence of leucocyte esterase (LE) and/or nitrites on dipstick screening in symptomatic females
- Failure to respond to initial empiric therapy, raising the possibility of a resistant organism
- Recurrent symptoms less than one month after empiric treatment of a previous UTI for which no culture was performed

Most experts agree that urine culture is not necessary in a symptomatic sexually active young woman with pyuria (detected by dipstick) that has an apparent uncomplicated cystitis.

URINE SPECIMEN COLLECTION AND TRANSPORT

- Early morning specimens provide the best yield as bacteria in the urinary tract would have been able to multiply overnight.
- The method of urine collection must be specified on the laboratory requisition form.
- The best urine specimen for the assessment of a UTI is one that accurately represents the bladder bacterial count with minimal contamination by colonising bacteria.

TYPE OF SPECIMEN	NOTES
Midstream urine (MSU)	<p>This is the most recommended method for urine collection as it is simple, inexpensive and results correlate reasonably well with suprapubic aspiration or straight catheterisation.</p> <p>To minimise contamination with commensal bacteria in the distal urethra, patient education regarding how to collect a MSU is important.</p> <ul style="list-style-type: none"> • Perform local disinfection of the meatus and adjacent mucosa with a non-foaming antiseptic solution and dry this region with a sterile cotton wool swab. • Minimise contact of the urinary stream with the mucosa by spreading the labia in females or by retracting the foreskin in uncircumcised males. • Discard the first part of the voided urine as this flushes away urethral contaminants. • Collect the second, midstream specimen in an appropriate sterile container.
Suprapubic aspirates (SPA)	<ul style="list-style-type: none"> • Suprapubic aspirates obtained from babies and young children are considered the 'gold standard' in difficult cases as it is associated with the lowest contamination rate. • Any isolate should be considered clinically relevant. • A SPA is an invasive procedure. Before the procedure is attempted, ultrasound guidance should be used to demonstrate the presence of urine in the bladder.

Cystoscopy or nephrostomy urine specimens	<ul style="list-style-type: none"> • A urine specimen collected from the ureter (at cystoscopy) or from the kidney (via a nephrostomy) is treated in the laboratory as a fluid from a sterile site and all bacterial and fungal growth viewed as clinically relevant.
Straight (“in-and-out”) catheterisation	<ul style="list-style-type: none"> • Sometimes used for children and adults unable to produce a self-collected specimen. • After disinfection, a catheter is inserted into the bladder, the first few millilitres of urine discarded and the remaining specimen is collected in a sterile container. • Due to the small risk of bacteria being introduced into the bladder and inducing infection, this method is no longer recommended unless an alternative collection method is not available.
Indwelling catheters	<ul style="list-style-type: none"> • Urine specimens should only be taken when signs and symptoms suggest a possible infection. • If required, a specimen should be taken from the sampling port of a newly inserted catheter with a sterile needle and syringe after disinfection of the port, or if a port is not present, by puncturing the catheter tubing with a sterile needle and syringe. • Sampling should be done immediately prior to initiating antimicrobial therapy. <p>NOT RECOMMENDED</p> <ul style="list-style-type: none"> • Culture from indwelling catheters frequently contain colonising flora which may not represent infection or be representative of urine in the bladder. • Culture of specimens from the catheter collection bag as bacteria multiply in catheter bags. • Cultures of Foley catheter tips are of no clinical value.
Suprapubic catheter	<ul style="list-style-type: none"> • Processed and interpreted as a catheter specimen of urine (CSU) rather than a true bladder aspiration.
Adhesive urine collection bag	<ul style="list-style-type: none"> • A non-invasive alternative to catheterisation for children in whom a midstream specimen may be difficult to collect. • The perineum should be cleaned before a sterile adhesive bag is applied. • The baby is held in an upright position until urine is passed into the bag. • Due to the high incidence of contamination, a positive result is difficult to interpret and should be confirmed by a straight catheterisation or suprapubic aspiration urine specimen where appropriate. • Most studies confirm that a negative culture from a urine bag specimen rules out infection indicating a high negative predictive value for this method.
Diapers and urine collection pads	<ul style="list-style-type: none"> • Diapers and urine collection pads may be used but the contamination rate is similar to that of urine bag specimens and results should be interpreted with caution.

Cotton wool balls, gauze and sanitary towels	<ul style="list-style-type: none"> • These should not be used to collect urine in infants, children and elderly patients.
Urine specimens for the diagnosis of prostatitis	<ul style="list-style-type: none"> • Urine may be collected after massage of the prostate via the rectum because this releases any sequestered bacteria or inflammatory cells. • The traditional Meares-Stamey four-glass specimen (collecting the first 10 mL void, a midstream specimen, expressed prostate secretions (EPS) and 10 mL post prostate massage urine) is regarded as positive if there is a ten-fold higher bacterial count in the EPS than the midstream urine. • A two-specimen variant, involving only the midstream and the EPS specimens, can also be used. • It should be remembered that prostatic massage in a patient with acute bacterial prostatitis may precipitate sepsis and should not be performed.

URINE SPECIMEN TRANSPORT

- Urine should not be kept at room temperature for more than 30 minutes as this will permit the growth of bacteria.
- If there is to be a delay in transport to the laboratory, urine specimens should be refrigerated at 4°C as refrigeration stops bacterial growth.

URINE ANALYSIS

'Urinalysis' refers to the physical, chemical and microscopic examination of urine and should be performed within two hours of collection. Although it is not recommended as a routine screening tool, except in pregnant women, doctors should know how to interpret the results correctly.

PHYSICAL	
Colour	<p>Various substances, e.g. food, medication and infection can cause abnormal urine colours.</p> <p>Cloudy urine often is a result of precipitated phosphate crystals in alkaline urine or pyuria due to infection.</p>
Odour	<p>Although the normal odour of urine can be strong in concentrated specimens it does not imply infection.</p>
pH	<p>May range from 4.5–8 but normally is slightly acidic (pH 5.5–6.5) because of metabolic activity.</p> <p>Useful in the diagnosis and management of UTIs and calculi, as alkaline urine in a patient with a UTI suggests the presence of a urea-splitting organism, which may be associated with magnesium-ammonium phosphate crystals and increased risk of staghorn calculi formation.</p>

DIPSTICK URINALYSIS

GENERAL INTERPRETATION

- **A positive dipstick:** can support the diagnosis of a UTI. It has a low positive predictive value and should not be performed in patients without UTI symptoms.
- **A negative dipstick:** can refute the diagnosis if clinical suspicion is low, although a negative result does not exclude bacteriuria.
- **False-positive and false-negative results:** not unusual and although abnormal leucocyte esterase or nitrites results have a high sensitivity, the specificity remains low.

DIPSTICK	NOTES	TESTS AND INTERPRETATION
Haematuria	<p>Definition: three or more red blood cells per high-powered field (HPF) in two of three urine specimens.</p> <p>Classification: based on glomerular, renal and urologic aetiologies with infections presenting as a urologic haematuria (haematuria without proteinuria or dysmorphic red blood cells or casts).</p>	<p>Urine dipstick: detect red blood cells by reacting with the peroxidase enzyme in erythrocytes. Myoglobin and haemoglobin will also catalyse this reaction and a positive result might be due to haematuria, myoglobinuria or haemoglobinuria.</p> <p>Microscopic examination: used to confirm haematuria.</p>
Proteinuria	<p>Definition: persistent protein excretion above 150 mg/day (10–20 mg/dL), which is a marker of kidney disease. Urinary proteins secreted by the kidney include albumin, serum globulins and other smaller proteins.</p>	<p>Urine dipstick: usually an incidental finding and frequently benign and transient in nature.</p> <p>A positive dipstick test for proteinuria is a poor indicator of infection on its own, with a high rate of false positives and negatives.</p> <p>Other causes for a positive test: alkaline urine, gross haematuria or the presence of mucus or semen in the urine. It may also indicate several other renal pathologies, including glomerulonephritis and pre-eclampsia.</p>

Nitrites	Urine normally does not contain nitrites and if present, is highly specific for the presence of bacteria.	<p>A positive dipstick test for nitrites indicates that the causative organism is present in significant numbers. This test is specific but not highly sensitive for UTIs.</p> <p>False-positive results: After just one week of air exposure up to one third of test strips can give false-positive nitrites results.</p> <p>A negative dipstick test does not rule out a UTI.</p> <p>False-negative results: Infections with non-nitrate reducing organisms may cause false-negative results, as could low-nitrate diets.</p>
Leukocyte esterase	Leukocyte esterase is an enzyme released by neutrophils and macrophages and may indicate pyuria associated with UTI.	For accurate interpretation, five minutes should be allowed for the dipstick reagent strip to change colour.

MICROSCOPIC URINE ANALYSIS

Microscopic examination is important as it allows for the identification of casts, leucocytes, erythrocytes, crystals, squamous epithelial cells and bacteria and aids in the diagnosis of a variety of conditions.

Measurement by haemocytometry is preferred, although the counting of cells in the sediment of centrifuged urine is more commonly used.

Assessment of pyuria through cell count is subject to variability due to possible contamination of the urine with vaginal secretions and variation in the volume of supernatant used for assessment.

TYPE	DESCRIPTION	INTERPRETATION
WBC	<p>Normal</p> <p>Men: < 2 WBCs per high-power field Women: < 5 WBCs per high-power field</p> <p>Abnormal</p> <p>Pyuria: >10 000 WBC's per mL in uncentrifuged urine OR 2–5 WBCs per HPF in centrifuged sediment</p>	Pyuria reflects an inflammatory condition in the urinary tract and is not always due to an infection.
RBC	Dysmorphic red blood cells suggest glomerular disease.	<p>Haematuria is commonly seen in acute cystitis but is not diagnostic of the infection.</p> <p>Rarely seen in other dysuria syndromes but is often seen in non-infective renal tract disease.</p>

Casts	Casts in the urinary sediment reflects pathology in specific regions of the urinary tract, such as the distal convoluted tubule or collecting duct. Cellular elements determine the type of cast: hyaline, erythrocyte, leukocyte, epithelial, granular, waxy, fatty, or broad.	Erythrocyte and leukocyte casts: seen in glomerulo- and pyelonephritis as well as other inflammatory conditions. Hyaline casts: associated with pyelonephritis and chronic kidney disease.
Bacteriuria	Asymptomatic bacteriuria: 5 bacteria/HPF or 100 000 CFU/mL Symptomatic bacteriuria: a colony count as low as 100 CFU/mL may suggest a UTI.	Clean catch specimens are furthermore frequently contaminated by urogenital flora, but in the presence of any bacteria in a non-contaminated male urine specimen is suggestive of infection, and necessitates further culturing.
Crystals	Various types including calcium oxalate, uric acid, cystine and triple phosphate crystals.	Crystals may be seen in the urinary sediment of healthy patients. Triple phosphate crystals are often associated with alkaline urine and UTIs (usually associated with <i>Proteus</i> species).
Squamous epithelial cells		Presence suggests contamination.

LABORATORY INTERPRETATION

PYURIA	BACTERIURIA	
Yes	Yes	Usually seen in UTIs because of the strong association between pyuria and infections.
No	Yes	Suggestive of contamination, especially in asymptomatic patients. Does not exclude infection in patients with neutropaenia or diabetes as they may have an inadequate white cell response to infection.
Yes	No	Sterile pyuria causes: <ul style="list-style-type: none"> • Patient has already taken antimicrobials • Contamination of the urine sample by the antiseptic solution • Contamination of the urine sample with vaginal leucocytes from vaginal secretions • Chronic interstitial nephritis (such as analgesic abuse nephropathy) • Nephrolithiasis (renal stones) • Uroepithelial tumour • Infection with atypical organisms such as <i>Chlamydia trachomatis</i>, <i>Ureaplasma urealyticum</i> and <i>Mycobacterium tuberculosis</i>

URINE CULTURE

The purpose of urine culture is to confirm the presence of bacteriuria, identify the causative pathogen and provide antibiotic susceptibility. Urine culture is recommended for most symptomatic patients with suspected UTIs, except for healthy, non-pregnant young women with typical symptoms of an uncomplicated UTI.

The most common cause of urinary tract infections is *E. coli*. Other bacterial species include *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., enterococci and staphylococci, including *S. saprophyticus*.

A sterile calibrated loop is dipped into the urine. The agar plate is then inoculated and incubated. The bacterial count is calculated from the number of colony forming units (CFUs) on the plate after overnight incubation at 35–37°C. In immunosuppressed patients, including those in intensive care or neonatal units, culture for *Candida* spp. should be performed because urinary colonisation may precede, or may indicate a candidaemia.

In asymptomatic patients, the threshold for bacterial growth that correlates with a true bacteriuria as opposed to specimen contamination is set at 100 000 CFU/mL. However, in symptomatic women with pyuria, counts as low as 100 CFU/mL have been associated with the presence of bacteriuria. Thus the standard definition of a positive urine culture ($\geq 100\ 000$ CFU/mL together with pyuria) does not apply to all patients and it has been suggested that a CFU count ≥ 100 /mL be considered significant in a midstream urine specimen in women with acute symptoms and pyuria. Lower colony counts still representing UTIs is also seen in men, patients on antimicrobial treatment and infections due to organisms other than *E. coli* and *Proteus* species.

Catheter-associated UTI in patients with indwelling urethral, indwelling suprapubic, or intermittent catheterisation is defined by the presence of symptoms or signs compatible with a UTI with no other identified source of infection, along with 1000 CFU/mL of one bacterial species in a single catheter urine specimen or in a midstream voided urine specimen from a patient whose urethral, suprapubic or condom catheter has been removed within the previous 48 hours.

If symptoms persist and the standard bacterial culture is negative, a pelvic examination should be performed with culture and PCR for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

If mixed enteric bacteria in high numbers are recovered from a second, well-collected straight-catheterised sample from the same patient, a rectal-urinary fistula should be considered.

Recovery of a yeast, usually *Candida* spp., even if the CFU/mL is high, is not infrequent in patients who do not actually have a yeast UTI. It may be due to:

- Colonisation of an indwelling catheter
- Concurrent vaginal thrush in a female patient
- Systemic infection: additional tests must be conducted for confirmation (e.g. blood cultures and β -D-glucan levels).

Recovery of *Mycobacterium tuberculosis* is best accomplished with first-void morning specimens of > 20 mL and requires a specific request to the laboratory so that appropriate processing protocols and media are employed.

SENSITIVITY TESTING

The laboratory routinely reports a susceptibility profile on a urinary pathogen in significant numbers. The choice of agents to test will depend upon local antibiotic policies and resistance patterns. In general, the primary agents tested target coliforms and enterococci.

CONTAMINATION

Distinguishing between genuine bacteriuria and specimen contamination is often difficult as urine may become contaminated by normal flora as it passes through the distal urethra and in women over the perineum. The appropriate collection of urine specimens is therefore critical for the evaluation of culture results.

SPECIMEN CONTAMINATION SHOULD BE CONSIDERED WHEN:

- Low colony counts are reported. There is increasing controversy regarding the significance of low-level bacteriuria. Recent studies suggest that lower levels of bacteriuria (100 to 10 000 CFU/mL) may be considered indicative of UTI.
- There is growth of two or more isolates at $\geq 10^5$ CFU/mL or the growth of one or more non-pathogens.
- There are three or more species of bacteria in a urine specimen.