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The laboratory diagnosis of urinary tract infection

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Abstract
Urinary tract infection is common, and it is not surprising that urine specimens make up a large proportion of those samples submitted to the routine diagnostic laboratory. Many of these specimens will show no evidence of infection and several methods can be used to screen out negative samples. Those that grow bacteria need to be carefully assessed to quantify the degree of bacteriuria and hence clinical relevance. To influence treatment, a final report should be produced within 24 hours of specimen receipt, with turnaround times continuously monitored. Much work needs to be done to determine the cost effectiveness involved in processing urine specimens and the evidence base for the final report provided.
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The aim of the microbiology laboratory in the management of urinary tract infection (UTI) is to reduce morbidity and mortality through accurate and timely diagnosis with appropriate antimicrobial sensitivity testing. Although optimal specimen collection, processing, and interpretation should provide the clinician with a precise answer, no single evaluation method is foolproof and applicable to all patient groups. In practice, laboratories will not be able to approach each specimen individually, and standard operating procedures are generated to cover the processing of most samples, with the aim of detecting the abnormal presence of bacteria and fungi within the urinary tract.[1] The interpretation of results requires an understanding of the limitations of local laboratory protocols and of the clinical context in which the specimen was taken.

Urine specimens make up a large proportion of the samples submitted to a routine diagnostic laboratory. A large laboratory may examine 200–300 urine samples each day. This heavy workload reflects the frequency of UTI both in general practice and in hospital settings. In children, infection is more common in young girls, except in the neonatal age group, where boys predominate. It is estimated that 20% of

women develop a UTI during their lifetime; the incidence increases at puberty and remains high throughout adult life, only after the age of 50 years is a similar incidence seen in males. UTI accounts for approximately 23% of all hospital acquired infections.[2] Although the incidence of infection is high, most specimens received will show no evidence of infection and several methods have been developed to screen out negative samples to minimise expense and improve turnaround times. These will also be reviewed.

Most infections at all ages are the result of enteric bacteria, especially *Escherichia coli*, which colonise the perineum and then ascend the urethra to multiply and infect the bladder, kidney, and adjacent structures.[3] The most common site of infection is the bladder. Haematogenous infection of the urinary tract occurs most notably with *Mycobacterium tuberculosis* and *Salmonella* spp, and direct introduction of organisms during instrumentation of the urinary tract is also well recognised. Structural and functional abnormalities result in a wider range of possible infecting organisms.

Urinary tract infection may occur with or without symptoms; the latter is known as covert or asymptomatic bacteriuria. Because urine must pass through the distal urethra and in women over the perineum, it may become contaminated by the normal flora of these regions. Isolation of more than one bacterial strain suggests such contamination, but even when a single strain is isolated, quantitative culture is required to determine whether it indicates true bacteriuria. Kass, in his original studies validating the midstream urine specimen (MSU), showed that 95% of hospitalised patients with acute pyelonephritis had more than 10^5 colony forming units (cfu)/ml of urine, whereas only 6% of asymptomatic patients had this degree of bacteriuria.[4 5] Subsequent studies have shown that lower bacterial counts can be important; this applies both to men and symptomatic women in whom 30–50% have fewer than 10^5 organisms/ml.[6 7] These cut off values can be applied to all rapidly growing bacteria but not fungi or fastidious organisms. Patients with frequency dysuria syndrome in whom urine cultures show no appreciable growth should be investigated

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for other agents that cause non-specific urethritis, such as *Chlamydia trachomatis*.[8] It should be noted that above the distal urethra the urinary tract is normally sterile, and any bacteria isolated from urine samples taken directly from the bladder, ureter, or kidney must be viewed as clinically relevant. The detection of polymorphonuclear cells (pyuria) and red blood cells (haematuria) in urine is useful for the diagnosis of infection or other renal tract pathologies.

Each laboratory should aim to have available a final report with microscopy, culture, and sensitivity on a substantial number of specimens (for example, > 90%) within 24 hours after the receipt of the specimen, and laboratories should monitor their turnaround times as part of quality assurance. The ability to screen out negative specimens as quickly as possible should also be considered; ideally this is done at source. The physician can make a clinical judgement as to whether a negative screen (for example, by reagent strip testing or bedside microscopy) is sufficiently accurate to rule out infection in that individual patient, given the limitations of the method used.

This broadsheet deals specifically with the diagnosis of bacterial UTI causing cystitis or pyelonephritis. It does not include the laboratory investigation of prostatitis, infection with mycobacteria, or the examination of urine for parasites.

Collection and transport of specimens
Rigorous care during the collection of urine is vital to prevent contamination by commensal flora, especially in female patients and children. Most samples are MSUs, and patients should be given clear instructions on discarding the first part of the stream before collection in an appropriate sterile container. Female patients should be instructed to part the labia while passing urine to avoid contamination. The initial few millilitres of urine wash away distal urethral organisms and hence the MSU is representative of bladder urine. It requires good control of micturition and an adequate volume of urine in the bladder. It may prove difficult to get such a sample in the elderly or those with hip joint problems. Early morning samples may harbour greater bacterial counts but are less amenable to outpatient clinical practice. However, they are recommended for the diagnosis of renal tuberculosis.

Catheter specimens of urine (CSUs) are often obtained from patients with long term indwelling catheters. Bacteria are frequently recovered but only a few are important and samples should only be taken when signs and symptoms such as fever, loin pain, or suprapubic pain suggest infection. Urine should be aspirated directly from the catheter using a sterile needle and syringe and then placed in a sterile container. Bacteria multiply in catheter bags so specimens from this site are unsuitable. Temporary catheterisation was often used to diagnose urinary infection in women but because of the risk of bacteria being introduced into the bladder this method is no longer acceptable. Intermittent catheterisation may be

used by patients with neurogenic bladder and these specimens should be treated as CSUs.

Increasingly, samples are being received from patients with urological problems, including patients with ileal conduits and those who have undergone bladder reconstruction, and these can present difficulties with interpretation. Specimens should only be taken if there are clinical signs of infection (for example, malaise, pyrexia, or vomiting) and they should be obtained via careful catheterisation of the stoma or reconstructed bladder using an aseptic technique. The interpretation of the cultures should then be as for any catheter specimen.

Suprapubic aspirates (SPAs) were often obtained from babies and young children and are still considered the "gold standard" and are used in difficult cases. Any isolate should be considered clinically relevant. Obtaining an SPA involves an invasive procedure; however a sterile adhesive bag or pad (that is, a sanitary towel with the appropriate absorption characteristics, lining a nappy) is much simpler but can still achieve a definitive answer in 50–75% of cases.[9] After cleansing the perineum, the baby is maintained in an upright position until urine is passed into the bag, pad, or alternatively via a clean catch into a container.

Urine samples collected from the ureter (at cystoscopy) or from the kidney (via a nephrostomy) should be treated in the laboratory as a fluid from a sterile site and all bacterial and fungal growth viewed as clinically relevant. For the diagnosis of prostatitis, urine specimens may be collected after massage of the prostate via the rectum because this is said to release any sequestered bacteria or inflammatory cells from this site.

As can be seen from the above, not all specimen types are the same and correct interpretation of urine cultures requires accurate data being clearly present on the request form. Urine will permit growth of bacteria and if there is to be a delay in transport (≥ 2 hours) or in setting up cultures in the laboratory then it should be stored refrigerated at 4°C; this will also preserve the white cell count.[10 11] Boric acid is often used to retain the bacterial count, but its antibacterial activity can reduce the number of organisms present, especially if an inadequate volume of urine is dispensed into the container.[12] Hence, rapid transportation/processing with refrigeration (if necessary) is the preferred method. A dip slide or dip inoculum is useful in overcoming delays in culturing urine specimens, but because no microscopy can be performed on the specimen, it is most useful for the follow up of patients. It is more expensive than routine methods.

Initial processing of specimens
A clear specimen of urine is unlikely to grow bacteria in great numbers but a cloudy specimen can result from bacteria, crystals, or leucocytes. Because gross visual examination cannot always be relied upon, several rapid screening methods have been developed; these are best performed at the bedside and accurately detect pyuria or the presence of bacteria.
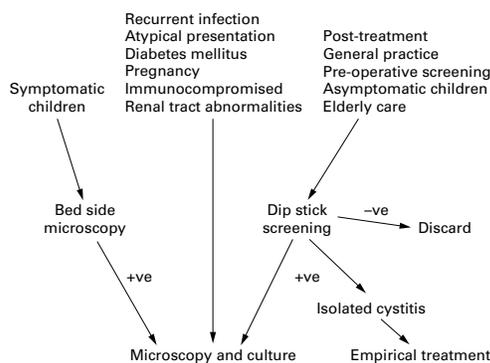
Figure 1 Guidelines on the use of dipsticks in clinical practice.

However, culture remains the preferred method of detecting and quantifying bacterial growth.

REAGENT STRIP TESTING

Several tests are available, those most commonly used to assist in the diagnosis of urinary tract infection are protein, blood, leucocyte esterase, and nitrate reductase.[13] Their principal role is in excluding infection; combining the last two tests has a negative predictive value of 98% but much lower positive predictive value (38%). Strips may be read visually or by machine. A suggested protocol for their use is given in fig 1.

Protein

A positive urine test for protein is a poor indicator of infection on its own, with a high rate of false positives and negatives; however, it may indicate several other renal pathologies, including glomerulonephritis and pre-eclampsia.

Haemaglobin

Haematuria may also be detected in UTI but can also result from a variety of conditions, including calculi and neoplasia. The strips detect peroxidase activity of haemoglobin and myoglobin, but because ascorbic acid can inhibit peroxidase reactions, false negatives may ensue. Haemolysis may result in negative microscopy.

Leucocyte esterase

A chloroacetate stain reacts with the enzyme leucocyte esterase found in primary neutrophil granules. The detection of pyuria by this test is reasonably sensitive (72–97%) and may be more accurate than microscopy because enzyme activity is still retained when white cells have disintegrated. False negatives or reduced reactions occur in the presence of ascorbic acid, boric acid, doxycycline, cefalexin, gentamicin, nitrofurantoin, glycosuria, urobilinogen, or high concentrations of protein; false positives occur with clavulanic acid, imipenem, or contaminated specimens.[14 15] More recently, strip testing of urinary lactoferrin, a protein found in neutrophil nuclei and granules, has been suggested as an alternative to leucocyte esterase testing.[16]

Nitrate reductase (Greiss test)

This enzyme reduces nitrate to nitrite and is present in coliforms but not other bacteria such

as *Staphylococcus saprophyticus* and enterococci. The test is best performed on early morning urine and, although specificity is high, sensitivity ranges from 35% to 85%. Combined with the leucocyte esterase test, the sensitivity rises to 70–100% with only a small decrease in specificity.[14]

OTHER METHODS USED

Glucose oxidase

Glucose is usually present in urine at low concentrations, and bacteria will utilise this energy source; hence, a positive test is indicated by the absence of glucose. The test is quite sensitive but false positives can occur along with false negatives, in patients with diabetes mellitus, or in those patients infected with bacteria that do not metabolise glucose.

Catalase activity

Catalase is found in most bacteria that cause UTI (but not streptococci) and in associated somatic cells (leucocytes and erythrocytes). It is measured with a tube based assay (API Uriscreen; BioMérieux, Lyon, France) and has been promoted for screening asymptomatic populations. Field trials have given variable results.[17]

Bioluminescence

UTIscreen (Coral Biomedical, San Diego, California, USA) is a semiautomated system that measures bacterial ATP. A releasing agent is added along with the firefly enzyme luciferin-luciferase and the sample is incubated at room temperature for 15 minutes, during which time the integrated light output is measured. The specificity of this method at detecting > 10^5 cfu/ml is 70%, sensitivity 96%, negative predictive value 98%, and positive predictive value 55%. Hence, its principal role is in rapidly screening out negative specimens. False negatives may be seen with candida and enterococcal infection and false positives with gross haematuria.[18–20]

Malthus

The Malthus system (Malthus Ltd, Stoke on Trent, UK) measures conductance between two electrodes immersed in media. Growth is indicated by a change in electrical impedance and has been used for the detection of bacteraemia. Its use in detecting bacteriuria is limited and early evaluations showed that no single medium allows the detection of all bacteria. The system is not suitable if a preservative such as boric acid is used because this alters conductance. If the cut off time is set at 2.5 hours (somewhat longer than most rapid screening methods), 80% of true positives are detected. However, there is a high false positive rate and it has not gained wide acceptance.[21]

Electrochemical detection method

This technique, which determines molecular hydrogen production using a platinum electrode, was first applied to the detection of coliforms in water. In one study, 94% of positive urines (> 10^5 cfu/ml) were detected at four hours, but it should be noted that certain

Table 1   Common causes of "sterile" pyuria

Female genital tract infection/non-specific urethritis in male
    patients
Prostatitis
Neoplasia of the renal tract
Renal calculi
Catheterisation
Renal tuberculosis
Fever in children, independent of cause
Prior antimicrobial chemotherapy

microorganisms such as staphylococci, strepto-cocci, *Acinetobacter* spp, and *Candida albicans* require longer detection periods.[22]

Enzyme immunoassay

An indirect immunoassay has been developed to detect antibodies specific to antigens on bacteria most commonly associated with UTI. Preliminary evaluation found the test to be labour intensive, costly, and to have relatively low sensitivity and specificity (62% and 65%, respectively).[23]

Microcalorimetry

Minor changes in temperature as a result of bacterial metabolism can be used to indicate high numbers of bacteria in a urine specimen. No large scale studies of this technique have been published.[24]

Photometry

Detects bacterial growth based on changes in light transmission and is more rapid than conventional culture techniques. However, if the interval for detection is prolonged to detect certain pathogens such as *Pseudomonas aeruginosa*, then there is an increase in false positive results. Several automated systems are available.[25]

Colorimetric filtration

Urine is passed through a filter that traps cells, Safronin O dye stains these cells which can then be detected visually or using a semiautomated colorimetric system. False positive results caused by cellular elements often occur and sensitivity rates fall dramatically when trying to detect $< 10^5$ cfu/ml.

Turbidimetric screening

Measuring turbidity using a double beam turbidometer can screen out negative samples. At a 94% sensitivity, 55% of samples are screen negative.[26]

MICROSCOPY

Detection of pyuria

A urinary excretion rate of more than 4×10^5 leucocytes/hour is found in 96% of patients with symptomatic bacteriuria, but in only 10% of patients with covert bacteriuria. This is more readily determined by finding > 10 leucocytes/ml in uncentrifuged urine; in symptomatic infection counts are often much higher than this. However, it should be noted that pyuria only indicates inflammation and does not always mean infection. Table 1 lists the causes of "sterile" pyuria. Pyuria and/or bacteria on microscopy are highly suggestive of UTI and are useful criteria to select specimens for direct sensitivity testing.[27] However, the absence of pyuria does not exclude infection because patients with neutropenia may have an inadequate white cell response to infection. Alkaline urine, such as that encountered with *Proteus* spp infection, results in white cells disintegrating before microscopy being performed. Pyuria is considered by some to be a poor predictor of infection.[28]

Detection of haematuria

Haematuria is commonly seen in acute cystitis but is not diagnostic of that condition. It is rarely seen in other dysuria syndromes but is often seen in non-infective renal disease. Table 2 indicates the importance of other elements seen on urine microscopy.[29]

Detection of bacteriuria

Microscopy of uncentrifuged, unstained urine will detect more than 10^4 bacteria/ml of urine. Sensitivity increases if the urine is centrifuged and/or Gram stained; this also permits preliminary identification of the pathogenic bacteria. These methods are labour intensive and impractical for routine specimens.[18]

Methods

MICROSCOPY

For routine use, a semiquantitative method using a flat bottomed microtitre tray and an inverted microscope is recommended. Pipette a fixed volume (80 μl, now the recommended volume) from a well mixed sample of urine and dispense into a microtitre tray.[30] After leaving to settle for 10 minutes, examine each well—for example, with a ×32 objective and ×10 eyepiece (FOV number 18)—count the number of white and/or red blood cells, and multiply this by a conversion factor to determine the number of cells/ml or cells/litre. This method is useful for detecting casts and can be integrated into a multipoint inoculator system. Cell numbers may be more accurately determined by using a counting chamber, but this is too labourious for routine diagnostic work. Reporting accurate numbers of white cells is probably not necessary and bands of reporting, such as 0, < 10, >10, < 50, > 50, < 200 and > 200, may be more practical. The reporting of red cells as present or absent is usually sufficient for most clinical practice.

Table 2   Importance of elements seen on direct microscopy of urine

Elements	Importance
Cells	
White blood cells	Urinary tract infection or inflammation (see causes of sterile pyuria (table 1))
Red blood cells	Urinary tract infection or inflammation Calculi or neoplasia
Eosinophils	Acute interstitial nephritis
Epithelial cells	Contamination of specimen
Casts	
Hyaline	Normal finding in concentrated urine
Granular	Renal parenchymal disease (non-specific)
Red blood cell	Glomerulonephritis, vasculitis
White blood cell	Interstitial nephritis, pyelonephritis
Epithelial cell	Acute tubular necrosis, interstitial nephritis, glomerulonephritis
Crystals	Several different types of crystals may develop in the urine including uric acid, calcium phosphate, calcium oxalate, cystine and sulphur. Their evaluation is outside the remit of the diagnostic microbiology laboratory

AUTOMATED MICROSCOPY

The DiaSys R/S 2000 is an automated system for the microscopic examination of urine. It simply draws a sample from a specimen container to an optical slide assembly on the microscope stage. In comparative studies it has a high degree of correlation with standard microscopic examination. Laboratory costs can be reduced by adopting this method.[31]

An image processing computer (Yellow IRIS; International Remote Imaging Systems, Chatsworth, California, USA) is able to recognise different particle sizes and can be used to analyse stop motion pictures from a video camera on a flow microscope. Several types of cellular elements present in uncentrifuged urine are recognised; however, the application of this technology is limited by cost.[32]

BACTERIAL CULTURE

The most common cause of urinary tract infection is *E coli*. In hospital practice, other bacterial species commonly seen include enterobacter, klebsiella, proteus, pseudomonas, enterococci, and staphylococci.[33] *Staphylococcus saprophyticus* is a common cause of infection in young sexually active women. The laboratory must also quantify culture results to determine the clinical relevance of an isolate. Table 3 shows a comparison of the organisms isolated in the community with those found in hospital.

CULTURE METHODS

Before culturing the urine should be mixed by inverting the container.

Choice of media

The media chosen must be able to support the growth of urinary pathogens and possible contaminants, inhibit *Proteus* spp from swarming, and distinguish lactose and non-lactose fermenters. Cysteine lactose electrolyte deficient medium (CLED) fulfils these criteria. Culture plates should be incubated overnight at 35–37°C in air. More recently, a new chromogenic agar has been described for the detection of urinary tract pathogens that may provide better differentiation of bacteria than conventional media.[34] Anaerobes rarely cause UTI but culture should be considered in a selected group of patients, such as those with persistent pyuria or foul urine and symptoms of UTI.[35] In immunosuppressed patients (including those on intensive care or neonatal units), culture for *Candida* spp should be performed because the urine may be positive before, or may indicate, the development of fungaemia. Screening for methicillin resistant *Staphylococcus aureus* should include urine samples from catheterised

Table 3 Organisms causing urinary tract infections in the community and in hospital[33]

Microorganism	Community (%)	Hospital (%)
Escherichia coli	69.4	50.8
Proteus mirabilis	4.3	5.1
Klebsiella/Enteobacter spp	4.7	7.3
Enterococcus spp	5.5	11.9
Staphylococcus spp	4.0	8.4
Pseudomonas aeruginosa	0	11.1
Others	12.1	5.4

patients. An appropriate selective medium, such as mannitol salt agar with oxacillin (2 mg/litre), should be used.

Media and culture conditions to aid the isolation of more fastidious organisms such as lactobacilli, corynebacteria, *Gardnerella vaginalis*, *Mycoplasma* spp, and *Haemophilus* spp may be worth pursuing for symptomatic patients with pyuria and negative routine culture. The use of multipoint inoculation to perform routine anaerobic culture has been suggested by some authors as being clinically valuable.[35] A microtitre method may be used in the identification of enterobacteriaceae, and this technique may also be applied to sensitivity testing using an automated reader.[36]

Standard loop method

A sterile calibrated loop (1, 2, or 10 µl) is dipped into the urine. The agar plate is then inoculated and spread. Up to four samples may be inoculated on to a 9 cm plate using one of the smaller loops. The bacterial count is calculated from the number of cfu on the plate after overnight incubation and the quantity of urine originally inoculated. If a 1 µl loop has been used one colony on the plate represents 10^3 organisms/ml in the original specimen. If the detection of lower counts is to be achieved a larger volume of urine must be used.

Filter paper method

A strip of commercially prepared sterile filter paper is dipped to the prescribed mark and then, after the removal of excess fluid, touched against an agar plate. Growth is recorded after overnight incubation: 25 colonies of bacilli and 30 of cocci represent 10^5 cfu/ml.[37]

Multipoint inoculation

Several specimens are analysed in parallel by this method, which takes a fixed volume of urine that is then inoculated on to several plates. Twenty specimens can be inoculated on to each 9 cm plate. Usually this method will only detect $\geq 10^4$ organisms/ml. Each plate may contain a variety of antibiotics or biochemical reagents so that direct sensitivity profiles can be determined and the provisional identification of most isolates is possible.[38] The method may be automated using a Mastascan (Mast Group Ltd, Bootle, UK).

Other methods

These include pour plates (serial dilutions of urine incorporated into 50°C agar) and roll tubes (urine poured into an agar coated plastic tube); neither of these techniques is suitable for routine laboratory use.

IDENTIFICATION OF BACTERIA

Clear protocols for the identification of bacteria and fungi should be in place. In general, it is adequate to report coliforms as such without full identification. *Proteus* spp are urease positive and resistant to nitrofurantoin. *Pseudomonas aeruginosa* is an oxidase positive lactose non-fermenter, resistant to most first line antibiotics. If clinical details suggest that a

non-lactose fermenting coliform may be a *Salmonella* spp and the urease and oxidase tests are negative then slide agglutinations with "O" and "H" antisera should be performed from cultures on blood (or other non-selective) agar plates. Any positive results should be followed up with biochemical confirmation. If typhoid fever is suspected, 5–10 ml of uncentrifuged urine should be inoculated into double strength selenite, incubated overnight at 37°C in air, then subcultured on to deoxycholate citrate agar, which in turn is incubated overnight at 37°C in air. Any suspect isolate should be dealt with in containment level 3 accommodation.

One advantage of using blood agar alongside CLED is that Gram positive bacteria are more easily characterised. If uncertainty exists, a catalase test will distinguish streptococci (negative) from staphylococci (positive). *Staphylococcus aureus* is DNase, slide, and tube coagulase positive. *Staphylococcus saprophyticus* can be identified by its resistance to novobiocin and this makes a useful distinction from other coagulase negative staphylococci, which are usually only important in specific situations such as instrumented or catheterised patients. If the appearance of the colony is typical of *Enterococcus faecalis* report the organism as such; if uncertain, perform a bile aesculin test. β-Haemolytic streptococci can be readily identified by Lancefield group testing.

Other isolates are identified using standard laboratory techniques, all multiply antibiotic resistant organisms need to be fully identified.

Fungi need only be identified if there is evidence to suggest that the isolate is clinically relevant. Because cut off values vary from author to author, we recommend that repeat sampling is performed to determine that there is persistent funguria (catheters should be changed). *Candida albicans* is germ tube positive and usually sensitive to fluconazole, itraconazole, and amphotericin. Sensitivity testing and the identification of other candida and fungal species are only necessary in selected patients, such as those who are severely immunocompromised.

The detection of antimicrobial substances is not routinely recommended but should be incorporated in the multipoint set to exclude false negative culture results. The detection of antibody coated bacteria in urine is not recommended in the routine diagnostic laboratory but may be useful to distinguish between upper and lower urinary tract infection in selected patients.

SENSITIVITY TESTING
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, and second line sensitivities need only to be performed if less common bacteria or resistant isolates are encountered. The suggested first line agents include amoxicillin, trimethoprim, cefalexin (or other oral cephalosporins), nitrofurantoin, co-amoxiclav, and ciprofloxacin. Urine is used as the primary inoculum when there is

evidence of infection (pyuria and/or bacteriuria) so as to permit rapid reporting. This method may be more representative than picking individual colonies for subculture, particularly given the heterogenous nature of urinary tract infection.[39] The degree of pyuria that triggers the performance of direct sensitivity testing should be decided locally depending on the patient group examined. It is suggested that all urines that show bacteria on microscopy and those with pyuria > 100 white cells/mm^3 should be tested. Recent recommendations for disc content and zone size interpretation have been published by the British Society for Antimicrobial Chemotherapy.[40]

Each sensitivity report is tailored to guide clinicians to the most appropriate agents and it is often necessary to suppress antibiotics if the isolate is not deemed to be clinically relevant. Suppressing antibiotic sensitivities on the results of positive specimens may be a particularly useful way of educating users that treatment of a positive catheter urine is not normally warranted. In addition, the presence or absence of pyuria may be used to decide which sensitivities are reported. However because the definition of UTI is based on bacterial counts and not on the presence or absence of pyuria, performing sensitivities should be related to the number of bacteria present and the relevant clinical situation. Specific agents may be unsuitable in particular situations—for example, the reporting of intravenous antibiotics to general practitioners—and certain antibiotics are relatively contraindicated in pregnancy. If sensitivity testing is not performed (for example, on mixed cultures) then culture plates should be kept for five days so that further testing may be performed if necessary.

Interpretation and reporting of culture results

The interpretation of culture results can be considered as more of an art than a science. A urine culture result depends on so many variables, such as appropriate collection, transport, and the limits of the methods of detection. The reliability of single positive urine culture in diagnosing UTI is only 80%, rising to 90% if a repeat culture shows identical results.[5] Traditionally, > 10^5 bacteria/ml of urine showing a single isolate is taken to indicate bacteriuria and distinguishes infection from contamination in asymptomatic patients. This degree of bacteriuria is usually used in surveillance and epidemiological studies to allow standardisation of data. Mixed culture with a predominant organism should also be considered as clinically relevant, although the possibility of contamination exists. Counts as low as 10^2/ml in symptomatic women are relevant when enterobacteriaceae are grown, but this is not necessarily the case with other microorganisms.[6] A count of 10^3/ml is viewed as the lower limit of clinical relevance in symptomatic men.[7] Therefore, pure culture of even a low count of bacteria should always be considered as potentially important and sensitivity

Table 4 Common causes of falsely low bacterial counts in urine specimens

Colonisation without infection
Dilutional through excessive rehydration
Acidification or alkalinisation of urine
Urinary frequency
Concurrent use of a systemic antimicrobial agent and/or other growth inhibitor
Use of a topical cleansing agent with antimicrobial activity during specimen collection
Haematogenous infection of the urinary tract
Obstruction of the renal tract distal to the site of infection
Infection with a fastidious or slow growing organism

testing performed if there are appropriate clinical details. Table 4 lists the causes of low bacterial counts in urine specimens. These recommended cut off values are taken from carefully conducted studies and in routine diagnostic work specimen collection, storage, and transportation may be suboptimal. Each laboratory should define which groups of patients or wards or departments warrant additional work or consideration of low bacterial counts so that appropriate sensitivity testing and reporting can occur. In general, repeat culture should be requested before treatment is started in any patient in whom the diagnosis of UTI is doubtful, or if contamination is suspected. When doubt exists about a culture result, the comment "Please repeat if clinically indicated" may be added to the standard comment. Table 5 indicates a possible schedule for reporting specimens. Bacterial counts or the presence or absence of pyuria cannot be used to localise the level or severity of infection; however, other laboratory based tests may be used—for example, quantification of antibody coated bacteria in urine or serum C reactive protein values.[41 42]

CATHETER SPECIMENS
Asymptomatic bacteriuria commonly develops and approximately 95% of patients will have notable bacteriuria (> 10^5 cfu/ml) after catheterisation for two weeks or more. Overt infections with fever, acute pyelonephritis, and bacteraemia occur in both short and long term catheterised patients. Covert infection occurs in the long term catheterised patient with symptoms such as catheter obstruction, calculi formation, periurinary infection, and chronic interstitial nephritis or pyelonephritis. Specimens should only be sent when these conditions are suspected clinically. These patients may have polymicrobial bacteriuria and should not be dismissed, especially if repeat cultures from correctly taken specimens are positive.

However, for most CSUs sensitivity testing on several isolates is unnecessary provided culture plates are available for five days in case symptomatic infection develops. Exposure to antibiotics in hospital favours alteration of regional flora and the acquisition of resistant strains which, as a consequence of cross infection, may result in outbreaks. To avoid unnecessary treatment with antibiotics, suppressing results of antibiotic sensitivity is recommended with an appropriate comment such as "catheter associated bacteriuria does not require treatment unless there are clinical signs of infection" and if appropriate add "sensitivities available on request".

SPECIMENS FROM CHILDREN
These may be from a pad specimen or a urine bag and therefore contamination can readily occur. Negative cultures or a slight growth may be diagnostically useful. However, positive cultures should be confirmed by a repeat specimen, more than 10^3 cfu/ml is taken as relevant, especially if there is a single isolate.[28] Further investigation using a DMSA (^{99}Tc-dimercaptosuccinic acid) scan or ultrasound is warranted in children under 5 years to exclude urinary tract abnormalities that might be amenable to surgery.[43] Routine screening of urine in infants or children is not recommended, although if renal abnormalities are suspected, including those infants who have been exposed to cocaine in utero, then screening should be performed.[44]

URINARY TRACT INFECTION IN PREGNANCY
Asymptomatic bacteriuria occurs in 2–10% of pregnant women. In the absence of specific treatment it tends to persist and in one third of those affected progresses to cause acute pyelonephritis.[45] Infection may be complicated by low birth weight and prematurity, pre-eclampsia, maternal anaemia, amnionitis, and intrauterine death. The treatment of asymptomatic bacteriuria is thought to reduce these risks.[45] The optimal method for screening is urine culture, which should be repeated to exclude contamination. Reagent strip testing only is less effective in identifying those patients who will develop pyelonephritis, but it does offer considerable cost savings. In the future, other rapid methods described above may be useful screening tests to complement urine culture.

Symptomatic urinary tract infection during pregnancy usually presents in the second

Table 5 Guide to reporting positive urine cultures

Count	No. of isolates	Specimen type	Sensitivity required	Comments
>10^5 cfu/ml	1 or 2	MSU	Yes	Report with sensitivities
		CSU	Yes	Report organism, suppress sensitivities unless appropriate clinical details, CSU comment
	>2	MSU and CSU	No	Report as contaminated specimen—repeat if clinically indicated
10^4–10^5 cfu/ml	1–2 (not skin flora)	MSU and CSU	No	Report as "no significant growth", unless the patient is immunocompromised, on relevant antibiotics, or has raised WCC (>50)
	1–2 (skin flora)		No	No significant growth
	>2		No	No significant growth
<10^4 cfu/ml	Any	MSU and CSU	No	If ⩾ 100 WCC and no antibiotics stated or detected consider culture for fastidious organisms, etc
	1–2		Yes	Immunocompromised or MSU and clear evidence of symptoms (lower limits of 10^2 cfu/ml for women with the urethral syndrome and 10^3 cfu/ml for men)

cfu, colony forming units; CSU, catheter specimens of urine; MSU, midstream specimens of urine; WCC, white blood cell count.

trimester. Unlike asymptomatic bacteriuria it has a low relapse rate after treatment and progression to pyelonephritis is uncommon. Because pregnancy itself may result in frequency and nocturia it may be difficult to distinguish symptomatic from asymptomatic bacteriuria.

Several antibiotics used to treat urinary tract infection should be avoided during pregnancy. These include aminoglycosides, quinolones, tetracyclines, and trimethoprim (first trimester). Laboratory reporting of sensitivity data should reflect this.

OTHER PATIENT GROUPS

Asymptomatic bacteriuria in the elderly is not associated with an increased mortality rate or with morbidity, such as hypertension or renal dysfunction. UTI may present atypically—for example, as falls, immobilisation, or confusion—and although urine culture should be considered as part of any geriatric assessment, routine screening is not recommended. Incidental detection of bacteriuria in men warrants further investigation, in particular for prostate or bladder outflow problems. Infection is more common in diabetic women, but not in diabetic men or school age diabetics, and routine screening in the absence of symptoms is not recommended. Urinary tract infection is seen more frequently after renal transplantation: it occurs in up to 79% of patients and is often asymptomatic. It can result in graft dysfunction, so routine screening, as part of a regular review, is recommended. All isolates need to be carefully evaluated and repeat cultures requested as appropriate. Screening for UTI is also recommended in patients who require urological procedures, including extracorporeal shock wave lithotripsy. Many of these patients may have uteretic stents in place that also predispose to infection.

Human immunodeficiency virus infection, particularly when CD4 counts are below 200 cells/mm^3, appears to be associated with an increased risk of bacteriuria. Other immunosuppressed patients (for example, transplant patients, patients on chemotherapy, or high dose corticosteroids) also have an increased risk of UTI.

CANDIDURIA

The kidney is involved in 90% of patients with disseminated candida infection and candiduria is an early indicator of systemic candidosis. However, *Candida* spp may colonise the perineum and urethral meatus resulting in contamination of urine during collection. Treatment is started on the basis of other risk factors for disseminated infection. Clinically relevant candiduria in other situations is more difficult to define and, in the absence of a recognised clear cut off in colony count that distinguishes between contamination and infection, repeated isolation of candida is a useful guide in deciding on further evaluation. Risk factors for funguria include urinary tract abnormalities, diabetes mellitus, antibiotic treatment, and immunosupression. Chronically catheterised patients may develop asymptomatic candiduria and, in this situation, the

catheter should be changed whenever possible. Some authors recommend alkalinisation of the urine but consideration should also be given to systemic fluconazole or amphotericin bladder washouts or irrigation. Sometimes, cystoscopy will provide visual proof or histological evidence of candida infection, thereby avoiding the need for repeat sampling. Urethral candidiasis occurs as an extension of candida vaginitis in women and in men as a result of sexual contact.[46]

Quality issues

Laboratories should seek Clinical Pathology Accreditation (CPA (UK) Ltd) or other appropriate accreditation for all clinical microbiology work. Inherent in this is the performance of internal audit, internal quality assessment, participation in the National External Quality Assurance Scheme (NEQAS), and involvement in internal quality assessment of near patient testing facilities. Involvement in the "Q" probes and Q Track system of the College of American Pathologists allows comparative audit with other laboratories. Q probes specific to the diagnosis of UTI include studies on transport, handling of urine samples, and urine culture contamination.[10 47] In addition, The Clinical Benchmarking Company (UK) has looked at specific UTI projects with a view to improving cost effectiveness.[48] Evidence based practice for the reporting of urine culture results is still unfortunately lacking.

Conclusion

Because the sensitivities of uropathogens are relatively predictable, uncomplicated cystitis is often treated with empirical short course antibiotics without urine culture. If, however, antimicrobial prescribing is to focus on the treatment only of true bacterial infections, as has been recommended in the recent National Health Service Executive guidance,[49] then urine culture should be performed. The first step in controlling the workload of the diagnostic laboratory is to have clear and appropriate guidelines for sending urine cultures from symptomatic and asymptomatic patients, both in general practice and in hospitals, and to be certain that bedside screening tests are applied as appropriate. Specimens should be collected and transported in a correct manner, so that contamination and bacterial overgrowth are minimised. The use of automation will allow large numbers of specimens to be processed with reduced technical input, but results are not as well standardised as they are for quantitative culture, which permits the detection of low numbers of bacteria, mixed samples, and sensitivity results. Thus, urine culture will remain the "gold standard" until these issues can be dealt with.

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