



NATIONAL STANDARD METHOD

INVESTIGATION OF URINE

BSOP 41

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



Association of Medical Microbiologists
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AMENDMENT PROCEDURE

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

| Amendment Number/ Date | Issue no. Discarded | Insert Issue no. | Page | Section(s) involved in BSOP 41.7 | Amendment |
|------------------------|---------------------|------------------|-------|--|---|
| 8/ 20/10/09 | 6.1 | 7 | 1 | Front page | CMN logo added |
| | | | 15-18 | Technical information/limitations 1.2 Specimen transport and storage 2.2.1 Midstream Urine (MSU) 2.3 Adequate quantity and appropriate number of specimens 3.2 Special considerations to minimise deterioration | The term “CE marked leak proof container” replaces “sterile leak proof container”; endnote ^a added to clarify the change and referenced to IVD Directive 98/79/EC ² |
| | | | All | All | Department name changed from ESL to DEST Document edited. |
| | | | 32 | Appendix 3 | Section added and multiplicative formula included |
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| Types of specimens: | Bag urine | Ureteric urine |
| | Catheter urine | Mid stream urine |
| | Clean catch urine | Nephrostomy urine |
| | Prostate massage/secretions | Urostomy urine |
| | Pad urine | Cystoscopy urine |
| | Ileal conduit urine | Suprapubic aspirate |
| | | |

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the processing and bacteriological investigation of urine samples. These include mid stream and clean catch specimens and those collected via bag, ileal conduit, ureter, catheter, urostomy, nephrostomy, cystoscopy, supra pubic aspirate, prostate massage/secretions, and pad urine.

INTRODUCTION

Urinary Tract Infection

Urinary tract infection (UTI) results from the presence and multiplication of microorganisms in one or more structures of the urinary tract with associated tissue invasion. This can give rise to a wide variety of clinical syndromes. These include acute and chronic pyelonephritis (kidney and renal pelvis), cystitis (bladder), urethritis (urethra), epididymitis (epididymis) and prostatitis (prostate gland). Infection may spread to surrounding tissues (eg perinephric abscess) or to the bloodstream.

Protection against infection is normally given by the constant flow of urine and regular bladder emptying. Urine is a poor culture medium for many bacteria due to its acidity, high urea concentration and variable osmolality and, in men, possibly partly as a result of antibacterial activity of prostatic secretions³.

Bacteriuria – Implies that bacteria are present and may be cultured from urine. The patient may or may not be symptomatic.

Symptomatic patients – They may be bacteriuric or abacteriuric. Symptoms in children and the elderly, when present, may be non-specific and difficult to interpret.

Frequency – The average bladder capacity is about 500 mL. Significant reduction in capacity accompanies acute inflammation which can lead to an increase in the frequency of micturition.

Dysuria – Painful and difficult micturition.

Urgency – A strong desire to empty the bladder, which can lead to incontinence.

Nocturia – Waking in the night one or more times to void the bladder⁴.

Nocturnal enuresis – The involuntary voiding of urine during sleep, ie bed-wetting.

Incontinence – The involuntary leakage of urine. The commonest form of this is stress incontinence where leakage accompanies an increase in intra-abdominal pressure due to sneezing, coughing or laughing. Overflow or dribbling incontinence accompanies an overfilled bladder.

Prostatism – Symptoms include: hesitancy or delay in initiating micturition, intermittency or interruption, and reduced force of the urine stream resulting from prostate gland pathology.

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Renal colic – This is characterised by very severe cramping pain resulting from distension of the ureter and pelvis above an obstruction such as a renal stone. Often accompanied by frequency and urgency.

Asymptomatic bacteriuria – Common in several patient groups, particularly the elderly, pregnant women and diabetic patients.

Clinical manifestations of UTI

Acute urethral syndrome – Occurs in women with acute lower urinary tract symptoms with either a low bacterial count⁵⁻⁸ or without demonstrable bacteriuria or vulvovaginal infection.

Uncomplicated UTI – Occurs in otherwise healthy individuals. There are no underlying structural or neurological lesions of the urinary tract and no other systemic diseases predisposing the host to bacterial infection. Recurrences are usually reinfections with organisms ascending via the urethra.

Acute uncomplicated cystitis – This condition usually occurs in young women. It has an abrupt onset and produces severe symptoms which are usually accompanied by pyuria and bacteriuria. Uncomplicated cystitis can occur in some men⁹.

Complicated UTI – Occurs in patients in whom there may be residual inflammatory changes following recurrent infection or instrumentation, obstruction, stones, or anatomical or physiological abnormalities or pathological lesions. These interfere with drainage of urine in part of the tract which encourages prolonged colonisation¹⁰. Relapses with the same organism may occur¹⁰.

The following are examples of complicated UTI:

Acute pyelonephritis (pyelitis) – An inflammatory process of the kidneys and adjacent structures¹⁰. Symptoms include loin, low back or abdominal pain and fever. Symptoms of cystitis may also be present. Severity ranges from mild disease to full blown Gram-negative sepsis¹¹ with a few patients developing complications such as intrarenal and perinephric abscess. Such cases often require aggressive diagnostic and therapeutic measures.

Chronic pyelonephritis (chronic interstitial nephritis, or reflux nephropathy) – Controversy exists over the definition and cause of this syndrome. It is the second most common cause of end-stage renal failure. It is thought to be a result of renal damage caused by UTI in infants and children with vesicoureteric reflux, or by obstructive uropathy in adults. However, it is still unclear whether recurrent infection causes progressive kidney damage.

Perinephric abscess¹² – A complication of UTI, although uncommon, that affects patients with one or more anatomical or physiological abnormalities. The abscess may be confined to the perinephric space or extend into adjacent structures. Pyuria, with or without positive culture, is seen on examination of urine. Causative organisms are usually Gram-negative bacilli but can also be staphylococci or *Candida* species. Mixed infections have also been reported.

Prostatitis^{5,12} – An inflammatory condition of the prostate gland that occurs in a variety of different forms, some involving infection. Routes of infection of the prostate include ascending urethral infection, reflux of infected urine into the prostatic ducts that empty into the posterior urethra, invasion of rectal bacteria by direct extension, or by lymphatic or haematogenous spread.

- **Acute bacterial prostatitis** – An abrupt, febrile illness with marked constitutional and genitourinary symptoms¹³
- **Chronic bacterial prostatitis** – Relapsing and recurrent UTIs, caused by the organisms persisting in the prostatic secretions despite antimicrobial therapy^{12,13}. The method of Meares and Stamey¹⁴ compares white blood cell (WBC) and bacterial counts of urethral, midstream and post prostatic massage urine specimens and expressed prostatic secretions (EPS). Prostatic massage should not be undertaken in patients with acute prostatitis because of the

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risk of precipitating bacteraemia. All specimens are taken at the same time and processed immediately¹⁴. Chronic bacterial prostatitis is less common than non-bacterial prostatitis. Bacterial prostatitis is associated with UTI. Organisms responsible are similar to those that cause UTI

Pyonephrosis¹² – The bacterial infection of an obstructed ureter which fills with pus. This may follow surgical intervention. Diagnosis is made from blood culture or pus drained from the kidney.

Renal abscesses – Localised in the renal cortex and may occur as a result of *Staphylococcus aureus* bacteraemia. Pyuria may also be present, but urine culture is usually negative. Renal abscesses are increasingly being seen as complications of acute pyelonephritis caused by Gram-negative bacilli. The rare condition of emphysematous pyelonephritis, which results in multifocal intrarenal abscesses and gas formation within the renal parenchyma, is usually seen in diabetic patients or as a complication of renal stones. *Escherichia coli* is the commonest cause and the condition carries a 70% mortality rate¹².

Urethritis⁵ – Common in both male and female patients and is often associated with UTI or occasionally with bacterial prostatitis.

In men urethritis is commonly caused by sexually transmitted diseases and is associated with urethral discharge. The main organisms responsible are: *Neisseria gonorrhoeae* (gonococcal urethritis), *Chlamydia trachomatis* and *Ureaplasma urealyticum* (non-gonococcal urethritis or NGU)⁵.

In female patients the condition may appear as acute urethral syndrome or urethrocystitis caused by Enterobacteriaceae, *Staphylococcus saprophyticus* and less commonly by *C. trachomatis* and *N. gonorrhoeae*.

Incidence of UTI

The incidence of UTIs is influenced by age, sex or by predisposing factors that may impair the wide variety of normal host defence mechanisms³.

Children – UTI is a common bacterial infection that causes illness in children, in whom it may be difficult to diagnose as the presenting symptoms are often non-specific¹⁵. In children the condition is often associated with renal tract abnormalities and is most common in males in the first 3 months of life as a result of congenital abnormalities. In older children, females are more commonly affected. Infection in preschool boys is often associated with renal tract abnormality¹². Failure to diagnose and treat UTI quickly and effectively may result in renal scarring and ultimately loss of function. The phenomenon of vesicoureteric reflux, while predisposing children to UTI, may also be caused by UTI^{16,17}.

Adults – The incidence of UTI is highest in young women. Around 10 – 20% of women will experience a symptomatic UTI at some time. Most infections in adult men are complicated and related to abnormalities of the urinary tract¹⁰ although a low incidence occurs spontaneously in otherwise healthy young men¹⁸.

The elderly – UTI incidence increases with age for both sexes. It is estimated that 10% of males and 20% of females over the age of 65 have asymptomatic bacteriuria¹². According to some, no treatment is indicated for asymptomatic patients except before invasive genitourinary procedures¹⁹.

Pregnancy – Studies in the UK have shown that asymptomatic bacteriuria (persistent colonisation of the urinary tract without urinary symptoms) occurs in 2 – 5% of pregnant women. Unless detected and treated early there is an increased risk of preterm birth and pyelonephritis affecting maternal and fetal outcome²⁰. In about 30% of patients acute pyelonephritis occurs, especially at the time of delivery^{21,22}. It has been reported that 20 – 40% of pregnant women with untreated bacteriuria will develop pyelonephritis²¹. In pregnancy, routine and sensitive urinary screening programmes are essential for the detection of bacteriuria in pregnancy. The screening can be done by midstream urine culture early in pregnancy.

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Diabetes – Women with diabetes have a higher incidence of asymptomatic bacteriuria than those without. There is no difference in the prevalence of bacteriuria between men with diabetes and men without diabetes²³. There is debate as to whether factors such as glycosuria, age or instrumentation are contributory to the high prevalence of UTI, but bladder dysfunction as a result of diabetic neuropathy may be the major predisposing factor²⁴. The relative incidences of symptomatic infection in patients with or without diabetes remain unclear but, when they do occur, UTIs tend to be more severe in patients with diabetes¹⁰.

Neuromuscular disorders – Patients with impaired bladder innervation as a result of congenital or acquired disorders (spinal bifida, spinal cord injury) are at increased risk of UTI. This may be due to impaired function of the bladder leading to incomplete emptying or an increased requirement for instrumentation of the urinary tract to assist voiding.

Renal transplantation – Most infections occur soon after transplantation, usually as a result of catheterisation, the presence of a ureteric drainage tube, or a previous UTI whilst on dialysis²⁵. Less commonly, infection may be introduced via the donor kidney.

Immunosuppression – Overall the incidence of UTI is not higher in patients who are immunocompromised compared with those who are not. The exceptions to this include patients who are diabetic or have undergone renal transplants²⁶. There have also been studies that suggest that men who are suffering from acquired immunodeficiency syndrome (AIDS) may also be at increased risk from bacteriuria, and symptomatic UTI with severe episodes resulting in bloodstream infection and death have been reported²⁷. However, because of long-term antibiotic use for other infections, UTI in such patients is often due to more unusual or resistant organisms. Steroid treatment may induce reactivation of tuberculosis of the urinary tract.

Community acquired UTI – Most commonly occurs when bacteria that colonise the anterior urethra or vaginal introitus ascend into the bladder. Less commonly, haematogenous spread of organisms and relapsing infection from unresolved foci in the prostate, kidney or calculi may seed other parts of the urinary tract. Rarely, bacteria spread from the bowel via a fistula, as in Crohn's disease or malignancy.

Healthcare Associated UTI – Usually a direct result of instrumentation, most commonly catheterisation. Catheter associated bacteriuria is usually asymptomatic and is not synonymous with clinically significant infection. Bacteriuria occurs in 10 – 20% of patients who are catheterised, but urinary tract infection in only 2 – 6%²⁸. Bacteraemia, most commonly Gram-negative, occurs more commonly in patients with UTI and develops in 1 – 4% catheterised patients with urinary tract infection causing significant morbidity (increasing hospital stay and costs). It has a mortality of 13 – 30%²⁹. Organisms originating from the patient's perineal flora or the hands of health care staff may be introduced to the bladder during catheterisation, or via the periurethral route along the external catheter surface, or the intraluminal route as a consequence of faulty catheter care¹³. In patients' catheterised long term (>30 days), prevalence of bacteriuria is virtually 100%: infecting strains change frequently and polymicrobial bacteriuria may be present³⁰. Treatment of asymptomatic bacteriuria has not been shown to be of any benefit in reducing complications in patients who are catheterised³⁰ and is likely to encourage the emergence of resistant strains.

Organisms implicated in UTI

Acute, uncomplicated UTIs

Acute, uncomplicated UTIs are usually caused by a single bacterial species.

E. coli – The most common organism involved in UTI. An international survey of midstream urine (MSU) samples taken at 252 centres in 17 countries reports that *E. coli* accounts for 77% of isolates³¹.

Only a few serotypes frequently cause UTI. This might reflect their prevalence in the faecal flora, or reflect differences in virulence factors. Certain virulence factors specifically favour the development of

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pyelonephritis whereas others favour cystitis or asymptomatic bacteriuria³². Generally, the more virulence factors a strain expresses, the more severe an infection it is able to cause³².

Recognised virulence factors include:

- Increased adherence to vaginal and uroepithelial cells predisposes to acute infection but not asymptomatic bacteriuria³³
- Resistance to bactericidal activity³²
- Greater quantity of K antigen³⁴
- Haemolysin production³⁴

Proteus mirabilis – Common in young boys and males and is associated with renal tract abnormalities, particularly calculi. In hospital patients it may cause chronic infections³⁵.

S. saprophyticus – Studies have shown that this organism was found to be responsible for 4% of UTIs³⁶. *S. saprophyticus* adheres to uroepithelial cells significantly better than *S. aureus* or other coagulase-negative staphylococci¹².

Other coagulase-negative staphylococci – Often considered as urinary contaminants as they are part of the normal perineal flora. However, they may cause complicated infections in patients of both sexes with structural or functional abnormalities of the urinary tract, prostatic calculi or predisposing underlying disease³⁷.

Streptococci – Rarely cause uncomplicated UTI, although Lancefield Group B streptococci may cause infection in some women. Enterococci may occasionally cause uncomplicated UTI³⁵.

Complicated UTIs³⁵

Complicated UTIs which occur in the abnormal or catheterised urinary tract are caused by a variety of organisms, many of them with increased antimicrobial resistance as a result of the prolonged use of antibiotics.

E. coli remains the most common isolate. Other frequent isolates include *Klebsiella*, *Enterobacter* and *Proteus* species, *Enterococcus* species (usually associated with instrumentation and catheterisation), and *Pseudomonas aeruginosa* (associated with structural abnormality or permanent urethral catheterisation). *S. aureus* rarely causes infection and is associated with renal abnormality or as a secondary infection to bacteraemia, surgery or catheterisation. It is frequently seen as a contaminant due to perineal carriage³⁵.

Bladder colonisation with *Candida* species is associated with indwelling catheters, but may also be present as contamination from the genital tract. *Candida albicans* is the most frequently isolated species³⁸.

Mycobacterium tuberculosis and other *Mycobacterium* species may infect the urinary tract (see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)).

Less common organisms causing infection include *Haemophilus influenzae*, *C. trachomatis*³⁹, *Mycoplasma hominis*, *U. urealyticum*^{40,41} and *Corynebacterium urealyticum* (CDC Group D2)⁴².

There is still debate over the role of fastidious organisms such as anaerobes, *Lactobacillus* species, *Gardnerella vaginalis* and fastidious streptococci⁴³⁻⁴⁶.

Many viruses may be cultured from urine but their role remains uncertain apart from adenoviruses (particularly type 11) and BK virus which have been implicated in haemorrhagic cystitis⁴⁷.

Urine samples are not suitable for the isolation of leptospires due to the presence of other contaminating bacteria and the poor viability of leptospires in urine⁴⁸.

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Specimen collection

Suprapubic aspirate (SPA) is seen as the "gold standard" but is usually reserved for clarification of equivocal results from voided urine in infants and small children. Before SPA is attempted it is preferable to use ultrasound guidance to determine the presence of urine in the bladder¹⁵.

Midstream urine (MSU) and clean-catch urine are the most commonly collected specimens and are recommended for routine use. Thorough periurethral cleaning is recommended before collection of the specimen, although the need for this has been questioned in both men⁴⁹ and women⁵⁰⁻⁵².

Catheter urine (CSU) ("In and out" or intermittent self catheterisation) samples are occasionally collected to ensure that they are contamination free.

Bag urine is commonly collected from infants and young children although it should be discouraged. Artificially elevated leucocyte counts may be seen as a result of vaginal reflux of urine, recent circumcision or confusion with round epithelial cells found in urine from neonates³⁸. Negative cultures provide useful diagnostic information, but significant growth should be confirmed with SPA⁵³. Pad urines may be a suitable alternative⁵⁴.

Other specimens obtained during or as a result of surgery include those from ileal conduit, cystoscopy, nephrostomy and urostomy, prosthetic massage/secretions. Specimens may also be taken after bladder washout.

Transport of specimens

Rapid transport and culture, or measures to preserve the sample aid reliable laboratory diagnosis. Delays and storage at room temperature allow organisms to multiply which generates results that do not reflect the true clinical situation. Where delays in processing are unavoidable, refrigeration at 4°C is recommended or the use of a boric acid preservative^{55,56-58} may be beneficial.

Boric acid preservative at a concentration of 1 – 2% holds the bacterial population steady for 48 – 96 hours, and other cellular components remain intact^{55,56}. Toxicity to certain organisms has been reported⁵⁷. The toxic effect is delayed⁵⁸ and often reflects underfilling of the container⁵⁹.

Laboratory investigation of UTI

Laboratory investigation of UTI normally involves microscopy (or an alternative method of measuring cellular components) and quantitative culture (or an alternative non-culture method such as a semi-automated urine analyser).

Except in a few patient groups, interpretations of culture results are made with regard to clinical presentation, the presence or absence of pyuria (which are associated with infection) and squamous epithelial cells (SECs) (which indicate contamination).

A reference guide for the diagnosis of UTI is available for use by clinicians⁶⁰. Clinical evaluation of the patient helps the interpretation of laboratory results and assists in the diagnosis of UTI.

Adequate internal control measures are critical, especially when chemical tests are deployed away from the laboratory near to the patient and where culture is not performed on the basis of negative results.

Microscopy

Microscopy is used to identify the presence of white blood cells (WBCs), red blood cells (RBCs), casts, SECs, bacteria and other cellular components in the urine. Semi-quantitative methods using a microtitre tray with an inverted microscope⁶¹ or a disposable counting chamber are recommended for routine use. This NSM contains a table of multiplicative factors based on the varying volumes of urine dispensed, the diameter of well and the field of vision diameter⁶¹ (refer to Appendix 1).

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Microscopy need not be performed on all urine samples where screening for asymptomatic bacteriuria is required (eg antenatal clinic screening) and may be omitted for such indications if in compliance with local protocols. Automated screening systems such as those based on biochemical tests offer flexible, cost effective alternatives to microscopy⁶². Microscopy (or an alternative) is recommended for all symptomatic patient groups, to assist in the interpretation of culture results and the diagnosis of UTI.

Microscopy of uncentrifuged, unstained urine has been used as a method of screening for bacteriuria without the need for culture⁶³, but is unreliable to detect counts $<10^7$ colony forming units per litre (cfu/L) ie $<10^4$ colony forming units per millilitre (cfu/mL). The sensitivity increases if the specimen is centrifuged and/or stained⁶⁴.

Significant pyuria – In a carefully taken specimen significant pyuria correlates well with bacteriuria and symptoms in most patients to suggest a diagnosis of UTI. Significant pyuria is defined as the occurrence of 10^7 or more WBC/L (10^4 WBC/mL)⁶⁵ although higher numbers of WBC are often found in healthy asymptomatic women. A level of $>10^8$ WBC/L ($>10^5$ WBC/mL) has been suggested as being more appropriate in discriminating infection³⁷. Pyuria is present in 96% of symptomatic patients with bacteriuria of $>10^8$ cfu/L (10^5 cfu/mL), but only in $<1\%$ of asymptomatic, abacteriuric patients. Counts are determined more accurately in uncentrifuged urine⁶⁶.

Significant pyuria and/or bacteriuria may be used as criteria for selection of specimens for direct sensitivity testing.

Pyuria without apparent bacteriuria (ie no growth on routine culture media) may be the result of many factors including: lysis of the WBCs in alkaline urine, which occurs in infections with *Proteus* species; a result of prior treatment with antimicrobial agents; catheterisation; calculi (stones); or bladder neoplasms. Other conditions which may lead to sterile pyuria include genital tract infection; sexually transmitted diseases (eg *C. trachomatis*)⁸ or an infection with a fastidious organism. Renal tuberculosis may also be implicated in sterile pyuria but is uncommon, although should be considered if clinically indicated (eg in high risk populations⁶⁷).

Haematuria – Haematuria is observed in 40 – 60% of patients with acute cystitis but is rarely seen in association with other dysuric syndromes³⁸. Finding 1 – 2 RBCs/high power field is not considered to be abnormal. Haematuria may be caused by non-infective pathological renal conditions or by renal mycobacterial infection, with or without associated pyuria³⁸. Apparent haematuria may be the result of menstruation. Differentiation of dysmorphic RBCs to determine those of glomerular origin⁶⁸ is sometimes requested by specialist units, although its reliability is disputed⁶⁹. RBC lysis may occur in hypertonic and hypotonic urine, rendering them undetectable by microscopy. Laboratories should consult with local urologists regarding the reporting of RBCs in urine.

Casts⁷⁰ – Casts are cylindrical protein mouldings formed in the renal tubules and often giving clues to renal pathology. Recognition of casts is important in helping to establish the existence of renal disease, but is less useful in the differentiation of renal disorders.

Large numbers of hyaline casts are associated with renal disease, but may also be found in patients with fever or following strenuous exercise. Cellular and densely granular casts indicate pyelonephritis or glomerulonephritis. RBC casts usually indicate glomerular bleeding and are excreted in large numbers in the acute phase of post-streptococcal nephritis or rapidly progressive nephritis. Less commonly, epithelial cell and fatty casts accompany acute tubular necrosis and nephrotic syndrome.

Crystals⁷¹ – These may be asymptomatic or associated with the formation of urinary tract calculi. Some crystals such as cystine are rarely seen and may indicate an underlying metabolic disease.

Squamous epithelial cells – SECs are a useful indicator of the degree of contamination from the perineal region.

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Alternatives to microscopy

Urine analyser and automated (and manual) chemical screening methods may be useful in laboratories as a more rapid alternative to microscopy for the majority of urines⁶². See section below: Non-culture methods.

Culture and non-culture methods

The three main methods for the detection of UTIs involve culture, non-culture semi-automated systems (eg particle counting, electrical impedance, colorimetric filtration, photometry, bioluminescence, radiometry) and chemical (eg leucocyte esterase, nitrite, protein, blood detection):

Culture methods

There are several culture methods for the quantification of bacteria in urine. The easiest and most commonly used are the calibrated loop technique⁷², the sterile filter paper strip⁷³ and multipoint technology⁷⁴. Of these, multipoint methodology using CLED or chromogenic media, are considered to be the most versatile and efficient for large numbers of specimens.

Multipoint inoculation of CLED agar alone may contribute to the under-reporting of mixed cultures that are more readily identified using chromogenic agar or a range of identification and susceptibility media. The culture of urine by multipoint methods may be automated, or performed manually using either microtitre trays containing agar or by using 9 mM agar plates. Microtitre trays may be read manually or with an automated system where the resulting data are transferred to the laboratory information management system for reporting. Microtitre trays examined manually require background light and some form of magnification to facilitate the recognition of mixed cultures and small colonies.

Chromogenic media contain various substrates which permit presumptive identification of several common species through a change in either colony pigmentation or colour of agar. They perform satisfactorily compared to CLED and have the advantage that mixed cultures are easier to detect. However, chromogenic media from different manufacturers can vary in specificity⁷², and are relatively expensive^{75,72}.

The use of agar plates (rather than microtitre trays) may lead to false negative reporting if antimicrobial substances present in some urines diffuse to neighbouring inocula. When this occurs, repeat culture of the affected inocula is required.

Other methods include use of dipslides, pour plates and roll tubes. These methods are not recommended for routine use in this NSM but may be useful in specific circumstances and in accordance with local protocols.

Non-culture methods

Many non-culture methods for screening for bacteriuria and pyuria have been described and reviewed⁷⁶, but there are no recent publications. Most urine analyser systems⁷⁷⁻⁷⁹ and chemical methods^{80,81} are not sufficiently sensitive to detect low levels of bacteriuria that may be clinically significant.

Urine analysers may be used to screen for 'negatives' to allow earlier reporting. Regardless of screening result, culture is still recommended for all specimens from children, pregnant women, patients who are immunocompromised, and requests for repeat culture.

Methods that detect pyuria as well as bacteriuria may be useful for the exclusion of non-infected patients⁸².

Non-culture semi-automated methods – Urine analyser systems are expensive and vary in their performance⁷⁷⁻⁷⁹. They are intended to identify red and white blood cells, bacteria, yeasts, epithelial

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cells, mucus, sperm, crystals and casts (or some of these depending on the technology). Currently available technologies include the following:

- Flow cytometry: This works by measuring electrical impedance (for volume), light scatter (for size) and use of fluorescent dyes (for nuclear and cytoplasmic staining). The particles are characterised using these measurements and the results are displayed as scattergrams. Sensitivity and specificity results can vary depending on the parameters and cut-offs employed⁸³⁻⁸⁶. Cut-off criteria are chosen for an analyser to balance the levels of sensitivity and specificity required according to a local assessment of clinical need
- Particle recognition system: The urine specimen passes through the analyser and a camera captures up to 500 frames per specimen. Each image is classified by size, shape, contrast and texture features. This technology has been shown to be more reliable for identifying cellular components but less suitable for detection of bacteriuria⁸⁷
- Detection of bacterial metabolites: This system analyses volatile bacterial metabolites. These collect in the 'headspace' of the system where they are sampled by conducting polymer sensors. This technology is more often associated with identifying microbes from wound and skin infections but it is being investigated for application with urine specimens

Non-culture chemical screening tests – may be used for screening negative urines according to selected criteria^{88,89}. Most chemical tests are available commercially as dipsticks and are quick and easy to use. Reading colour changes in dipstick strips using colorimetric measurement is preferred, as results are more reliable and reproducible and free from observer error⁹⁰ particularly if an automated reading system is used. Boric acid and some antimicrobial agents such as nitrofurantoin and gentamicin adversely affect the leucocyte esterase test^{38,91}.

Chemical tests for the presence of blood may be more sensitive than microscopy as a result of the detection of haemoglobin released by haemolysis.

Interpretation of culture

Studies conducted in the 1950s remain the basis for interpreting urine culture results⁶⁶ showing that bacterial counts of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) are indicative of an infection and counts below this usually indicate contamination^{66,67}. The most common organism implicated in UTI in this group is *E.coli*³¹.

In specific patient groups (see below), counts between 10^8 cfu/L (10^5 cfu/mL) and 10^5 cfu/L (10^2 cfu/mL) may be significant^{67,92,93}. A pure isolate with counts between 10^7 and 10^8 cfu/L (10^4 - 10^5 cfu/mL) should be evaluated based on clinical information or confirmed by repeat culture. Overall the confirmation of a UTI requires the demonstration of significant bacteriuria by quantitative culture (defined according to patient group or specimen type). Routine culture methods may not be sensitive enough to detect low bacteria levels (eg $\geq 10^7$ cfu/L / $\geq 10^4$ cfu/mL) and increased sensitivity will be achieved by increasing the inoculum size (see section 4.4.2).

Increased inoculum sizes are also required for persistently symptomatic patients without bacteriuria if the patient has recurrent "sterile pyuria", or for specimens where lower counts are to be expected, such as SPAs or other surgically obtained urine

Women – in acutely symptomatic women UTI may be associated with counts of a single isolate as low as 10^5 cfu/L (10^2 cfu/mL) in voided urine^{67,92,93}. Interpretation of culture results must be made with care however, and take into account factors such as age and storage of specimen, level of contamination indicated by SECs, and the sensitivity of the method.

Growths of $<10^8$ cfu/L ($<10^5$ cfu/mL) in asymptomatic, non-pregnant women are rarely persistent and usually represent contamination³⁸.

The presence of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) in asymptomatic, pregnant women indicates infection but should be confirmed in a repeat sample⁹⁴.

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Men – Counts as low as 10^6 cfu/L (10^3 cfu/mL) of a pure or predominant organism have been shown to be significant in voided urine from men⁹⁵. Where there is evidence of contamination, a carefully collected repeat specimen should be examined.

Diagnosis of prostatitis may be achieved by comparing the levels of pyuria in sequential specimens taken in association with prostatic massage¹⁴ (see Section 2.2.1). If the level of pyuria after prostatic massage is 10 times that of the initial urine, then bacterial prostatitis is likely. More than 15 WBCs per high power field in expressed prostatic secretions is considered abnormal, even if the WBCs in the urethral and bladder urine are within the normal range.

Children¹⁵ – Confirmation of UTI in children is dependent on the quality of the specimen, which is often difficult to obtain cleanly. The probability of UTI is increased by the isolation of the same organism from two specimens.

Colony counts of $\geq 10^6$ cfu/L ($\geq 10^3$ cfu/mL) of a single species may be diagnostic of UTI in voided urine. Generally, a pure growth of between 10^7 - 10^8 cfu/L (10^4 - 10^5 cfu/mL) is indicative of UTI in a carefully taken specimen.

Negative cultures or growth of $< 10^7$ cfu/L ($< 10^4$ cfu/mL) from bag urine may be diagnostically useful. Counts of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) should be confirmed by culture of a more reliable specimen, either a single urethral catheter specimen or, preferably, an SPA⁵³.

Bacteriuria usually exceeds $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) in SPAs from children with acute UTI, although any growth is potentially significant.

Catheterisation

Patients with indwelling catheters – These samples may not accurately reflect the true bladder pathogen and often contains several bacterial species. Culture results should be interpreted with caution. The criteria have not been established for differentiating asymptomatic colonisation of the urinary tract from symptomatic infection²⁹. Urine cultures may not reflect bladder bacteriuria because sampled organisms may have arisen from biofilms on the inner surface of the catheter⁹⁶. Therefore the quality of the specimen collected and clinical circumstances in the individual patient are critical in the interpretation of bacterial counts. In carefully collected specimens, taken under controlled study conditions in short term catheterised patients, counts of $< 10^8$ cfu/L ($< 10^5$ cfu/mL) have been shown to be significant⁹⁷. In specimens of unknown quality and those from long term-catheterised patients, interpretation of significance on the basis of bacterial counts alone may be impossible. Significance of isolates and reporting of sensitivities may be indicated in certain groups – such as urology or post operative patients, especially if future operative intervention is planned on the urinary tract.

Bacterial counts from catheterised patients may be affected by the administration of medication or fluids that increase urine flow, rapid transit of urine from the catheterised bladder, or colonisation with relatively slow growing organisms such as *Candida* species²⁸.

Catheterisation is occasionally used to collect a contamination free sample ("in and out") when any bacterial growth is significant. Specimens from patients with intermittent self-catheterisation should be treated as a mid stream urine.

Other urine investigations

Screening for antimicrobial substances – This may be useful to detect false negative cultures where the inoculum contains an antimicrobial agent which diffuses into the agar and inhibits bacterial growth. Where microtitre trays are used for multipoint culture the highest concentration is localised to the small area of medium in the microtitre tray. Where agar plates are used for multipoint culture (rather than microtitre trays) both the primary and neighbouring inocula may be affected as a result of the diffusion through the medium.

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A seeded plate is inoculated after other plates and the absence of growth after incubation indicates the presence of an antimicrobial substance. The procedure is simple if multipoint replicating devices are used⁹⁸ (see section 4.2.2) and may reduce further testing of the specimen (eg for fastidious organisms).

Screening for *Salmonella Typhi* and *Salmonella Paratyphi* – *S. Typhi* and *S. Paratyphi* are present in urine in the early stages of typhoid and paratyphoid fever. Screening urines may be received from suspected cases and/or their contacts for selective enrichment and culture.

Diagnosis of *Schistosoma haematobium* – May be undertaken on urine taken at a specific time coinciding with maximum egg excretion, or on the terminal portion of voided urine. Haematuria is the most common presentation of *S. haematobium* infection. Chronic infection can lead to bladder cancer⁹⁹ (see [BSOP 31 – Investigation of specimens other than blood for parasites](#)).

Screening for *Chlamydia trachomatis* – May be undertaken on urine specimens from patients with sterile pyuria.

TECHNICAL INFORMATION/LIMITATIONS

In National Standard Methods, the term “CE marked leak proof container” is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)¹⁰⁰ state that such devices must “reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Although SI units have been adopted in other NSMs, they have been left as optional for urines. Most current literature still refers to the old nomenclature when defining “significant bacteriuria”. The following is a list of imperial units and their SI equivalents.

| | | |
|--------------------|---------------|-------------------|
| $\geq 10^5$ cfu/mL | equivalent to | $\geq 10^8$ cfu/L |
| $< 10^5$ cfu/mL | equivalent to | $< 10^8$ cfu/L |
| 10^4 cfu/mL | equivalent to | 10^7 cfu/L |
| $< 10^4$ cfu/mL | equivalent to | $< 10^7$ cfu/L |
| 10^3 cfu/mL | equivalent to | 10^6 cfu/L |
| $< 10^3$ cfu/mL | equivalent to | $< 10^6$ cfu/L |
| 10^2 cfu/mL | equivalent to | 10^5 cfu/L |

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1 SAFETY CONSIDERATIONS¹⁰¹⁻¹¹³

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

CE marked leak proof container^a in a sealed plastic bag.

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard Group 3 organism, for example *Mycobacterium* species, *Salmonella* Typhi or *Salmonella* Paratyphi A, B and C is suspected on clinical grounds, in which case work should be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet, isolator or be otherwise suitably contained.

Refer to current guidelines on the safe handling of all organisms documented in this NSM.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION^{38,115}

Boric acid preservative should be used where delays are likely to occur. It should be noted that boric acid may be inhibitory to some organisms⁵⁶⁻⁵⁸ and may inhibit tests for leucocyte esterase^{38,88}.

2.2.1 MIDSTREAM URINE (MSU)

MSU is the recommended routine collection method.

Periurethral cleaning is recommended (water is considered sufficient)⁵¹.

The first part of voided urine is discarded and, without interrupting the flow, approximately 10 mL is collected into a CE marked leak proof container^a. The remaining urine is discarded. If boric acid preservative is used the container is filled up to the mark in a similar manner and the contents mixed well.

Clean-catch urine

A reasonable alternative to MSU.

Periurethral cleaning is recommended. The whole specimen is collected and then an aliquot sent for examination in a CE marked leak proof container^a.

Suprapubic aspirate (SPA)¹⁵

Urine is obtained aseptically, directly from the bladder by aspiration with a needle and syringe. The use of this invasive procedure is usually reserved for clarification of equivocal results from voided urine (eg in infants and small children). Ultrasound guidance should be used to show presence of urine in the bladder before carrying out SPA.

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Catheter urine (CSU)

The sample may be obtained either from a transient ("in and out") catheterisation or from an indwelling catheter. In the latter case, the specimen is obtained aseptically from a sample port in the catheter tubing or by aseptic aspiration of the tubing. The specimen should not be obtained from the collection bag.

Bag urine

Used commonly for infants and young children. The sterile bags are taped over the freshly cleaned and dried genitalia, and the collected urine is transferred to a CE marked leak proof container^a. There are frequent problems of contamination with this method of collection.

Pad urine⁵⁴

An alternative to collecting bag urine from infants and young children. After washing the nappy area thoroughly, a pad is placed inside the nappy. As soon as the pad is wet with urine (but no faecal soiling), push the tip of a syringe into the pad and draw urine into the syringe. Transfer specimen to a CE marked leak proof container^a. If difficulty is experienced in withdrawing urine, the wet fibres may be inserted into the syringe barrel and the urine squeezed directly into the container with the syringe plunger.

Ileal conduit – urostomy urine

Urine is obtained via a catheter passed aseptically into the stomal opening after removal of the external appliance. Results from this type of specimen may be difficult to interpret.

Cystoscopy urine

Urine is obtained directly from the bladder using a cystoscope.

Ureteric urine

Paired urine samples are obtained from each ureter during cystoscopy via ureteric catheters inserted from the bladder.

Urine samples may also be sent following nephrostomy, other surgical procedures, or bladder washout.

Meares and Stamey localisation culture method for diagnosis of prostatitis¹⁴

The following specimens are collected:

The initial 5 – 8 mL voided urine (urethral urine)

MSU (bladder urine)

Expressed prostatic secretions following prostatic massage

The first 2 – 3 mL voided urine following prostatic massage

Urines for *S. Typhi* and *S. Paratyphi* culture

Any urine samples from suspected cases or contacts of cases.

Early morning urines

Three entire, first voided, early morning urine specimens are required for culture for *M. tuberculosis* (see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)).

Alternatively, if there are no appropriate containers available for the whole EMU, a midstream sample of the EMU is acceptable.

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Urine for *S. haematobium* detection

Total urine sample passed into CE marked leak proof container^a without boric acid preservative is required. Alternatively, a 24 h collection of terminal urine may be examined (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

A minimum volume of 1 mL for specimens in plain CE marked leak proof container^a for bacterial pathogens.

Fill to the line marked on containers with boric acid preservative according to manufacturer's instructions.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed within 4 h if possible^{116,117}, unless boric acid preservative is used.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

If processing is delayed for up to 48 h, refrigeration is essential⁵⁵ or the specimen may be collected into a CE marked leak proof container^a with boric acid preservative^{56-58,118}. This increases the maximum permissible time for transport to the laboratory to up to 96 h¹¹⁹.

Note: It is essential to follow the manufacturer's instructions on sample volume in boric acid containers⁵⁹.

For investigation of parasites see [BSOP 31 - Investigation of specimens other than blood for parasites](#).

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

Divide specimen on receipt for appropriate procedures such as investigation for viruses (boric acid samples are unsuitable for viruses) and *C. trachomatis* depending on clinical details.

4.2 APPEARANCE

N/A

4.3 MICROSCOPY OR ALTERNATIVE SCREENING METHODS

4.3.1 STANDARD

Microtitre tray with an inverted microscope

Mix the urine gently, to avoid foaming.

Using a pipette and disposable tips, dispense known volume (~60 µL see Note 2 below) of mixed urine to a numbered well in a flat-bottomed microtitre tray. Make sure that the specimen covers the whole bottom surface area (the use of a template will facilitate matching the specimen and well number).

Allow to settle for a minimum of 5 min, but preferably 10 – 15 min, before reading with an inverted microscope.

Scan several fields in each well to check for even distribution of cells and urine.

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Count the numbers or estimate the range of WBCs and RBCs per representative field and convert to numbers (or range) per litre.

Enumerate and record SECs.

Enumerate and record casts, if present, and state type.

Record if bacteria, yeasts, *Trichomonas vaginalis*, or significant crystals such as cystine are present.

All procedures for enumeration of cells should be carried out according to local protocols.

Note 1: This NSM contains a table of multiplicative factors to correct for variability in microtitre tray well size based on varying volumes of urine dispensed, diameter of well and field of vision diameter⁶¹ (refer to Appendix 1). The number of WBCs counted should be multiplied by the multiplicative factor to take into account all the variables. If the well size, volume of urine dispensed, diameter of well or field of vision diameter are altered then the multiplicative factor needs to be re-calculated.

Note 2: If the microtitre tray is also to be used for culture by multipoint inoculation it should be stored at 4°C until culture is performed (unless all specimens in the tray are preserved with boric acid when refrigeration is not necessary).

Note 3: Microscopy should not be performed on screening specimens sent exclusively for the isolation of *S. Typhi* and *S. Paratyphi* for safety reasons.

Other microscopy methods

Microscopy may be performed by use of a three coverslip counting chamber¹²⁰ or a commercial disposable counting chamber.

4.3.2 ALTERNATIVE METHODS

See introduction for a discussion on non-culture methods.

Screening by biochemical test strips may be performed in place of microscopy however these methods do not detect casts or abnormal cells such as dysmorphic cells.

Automated systems such as those using urine analysers must be validated and used in accordance with manufacturers instructions.

4.3.3 SUPPLEMENTARY

Microscopy for:

- **Dysmorphic RBCs**^{68,69} – Laboratories should consult with local urologists regarding the reporting of dysmorphic RBCs in urine. Fresh specimens (<30 minutes old) are essential.
- ***Mycobacterium* species** – (see [BSOP 40 - Investigation of specimens for mycobacterium](#))
- **Parasites** – (see [BSOP 31 - Investigation of specimens other than blood for parasites](#))

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

Standard

N/A

Supplementary

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Mycobacterium species (see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and for parasites (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

4.4.2 SPECIMEN PROCESSING (SEE INTRODUCTION FOR INTERPRETATION OF CULTURE RESULTS)

Choice of culture method is made locally.

Specimens with "negative" microscopy may be given a screening culture only, whereas treatment of those with "positive" microscopy may include direct susceptibility testing.

Calibrated loop/surface streak method

Mix the urine gently to avoid foaming.

Dip the end of a sterile calibrated loop (eg 1 µL, 2 µL or 10 µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank¹²¹.

Use this to inoculate CLED or chromogenic agar plate and spread according to the number of specimens (see [QSOP 52 - Inoculation of culture media \(formerly BSOP 54\)](#)). A maximum of four samples per 9 cm plate is recommended for this method with a 1 µL or 2 µL loop, or two samples if using a 10 µL loop.

If a 1 µL loop is used, one colony equals 1000 cfu/mL (ie 1 x 10⁶ cfu/L).

SPAs, other surgically obtained urine¹¹⁵, and urine samples with expected significant bacteriuria as low as 10⁵ cfu/L (increased inoculum sizes are required)

Inoculate 100 µL (0.1 mL) of specimen aseptically to a full CLED or chromogenic agar plate.

Spread inoculum over entire surface of plate with a sterile loop or a spreader. Do not use a sterile swab which will absorb much of the inoculum. To isolate individual colonies, spread inoculum with a sterile loop.

No. of cfu/L = No. of cfu on plate x 10⁴

This semi quantitative method is only sensitive for screening down to 10⁶ cfu/L if a 5 µL or 10 µL loop is used (eg 5 or 10 colonies), or 10⁷ cfu/L if a 1 µL or 2 µL loop is used (eg 10 or 20 colonies). (see table below).

Guidance on assessing colony counts (with the exception of filter paper strip method; see Introduction for the clinical interpretation results)

| No. cfu counted using inoculum of: | | | | | |
|------------------------------------|--------|------|------|------|-------|
| | 0.3 µL | 1 µL | 2 µL | 5 µL | 10 µL |
| 10 ⁶ cfu/L | - | - | - | 5 | 10 |
| 10 ⁷ cfu/L | 3 | 10 | 20 | 50 | 100 |
| 10 ⁸ cfu/L | 30 | 100 | 200 | 500 | 1000 |

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Multipoint methods

Using 96 pin head microtitre trays

Prepare microtitre tray and perform microscopy (see Section 4.3).

Label the microtitre tray containing chromogenic or CLED agar medium using the same template as for microscopy (see Section 4.4.1).

Sterilise the inoculating pins on the multipoint inoculator.

Inoculate the agar microtitre tray with urine (eg 0.3 µL, 1 µL, 2 µL, depending on pin size) from the 60 µL aliquots used for microscopy.

Note 1: The tray must be stored at 4°C until full culture is performed (unless all specimens in the tray are preserved with boric acid).

Note 2: To prevent the inoculated agar in the microtitre trays from drying out in the incubator overnight, place the microtitre trays either in a moist box or stack carefully with a lid on the top tray.

Note 3: This method is only sensitive for screening down to 10⁷ cfu/L. A larger inoculum may be required in selected patient groups or specimens when greater sensitivity is needed.

Using agar plates

Multipoint inoculation of no more than 20 specimens per 9 cm plate is recommended.

Prepare inoculum in sterile cupules, arranged according to the configuration of the inoculation head.

Label CLED/chromogenic agar plate to correspond to inoculation configuration.

Sterilise the inoculating pins on the multipoint inoculator.

Inoculate CLED/chromogenic agar plate.

Note: Detection of antimicrobial substances must be undertaken if a multipoint culture method is used with agar plates rather than microtitre trays as diffusion of antimicrobial substances from some urine samples may affect neighbouring inocula and give false negative results. (see section below: 'Detection of antimicrobial substances'). Any sample thought to be affected in this way should be retested.

Filter paper method⁷³

Dip the commercially prepared sterile filter paper strip in the urine up to the mark indicated.

Remove excess urine by touching the edge of the strip against the side of the specimen container. Allow the remaining urine to absorb into the strip before inoculating a CLED or chromogenic agar plate.

Bend the inoculated end of the strip and press flat against the agar for a few seconds.

Several specimens may be inoculated onto one CLED agar plate in this technique, although this is less effective than plating to chromogenic agar⁷² as mixed cultures are easier to detect.

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Note: This method is only sensitive for screening down to 10^7 cfu/L. A larger inoculum will be required in selected patient groups or for specimens where lower counts are expected.

Guidance on assessing colony count using the filter paper strip method

| No. cfu counted* | | Corresponding cfu/L (cfu/mL) |
|-----------------------|-----------|---------------------------------|
| Gram Negative Bacilli | Cocci | |
| 0 – 5 | 0 – 8 | $\geq 10^7$ ($\leq 10^4$) |
| 5 – 25 | 8 – 30 | $10^7 - 10^8$ ($10^4 - 10^5$) |
| ≥ 25 | ≥ 30 | $\geq 10^8$ ($\geq 10^5$) |

*Refer to individual manufacturer's instructions

Automated methods

Automated systems such as those using urine analysers must be validated and used in accordance with manufacturers instructions.

Other screening methods

Enteric fever screen

Enteric salmonellae may be recovered from urine following pre-enrichment in mannitol selenite, which can be prepared by carefully adding an equal volume of urine to mannitol selenite broth (see section: Safety Considerations)

Detection of antimicrobial substances^{122,123}

This method is performed most easily using multipoint systems, but inoculation of urine is possible with a sterile loop or pipette and disposable tips.

Surface seed plates or microtitre tray containing a defined susceptibility testing agar with a broth culture¹²² or spore suspension of *Bacillus subtilis* (NCTC 10400)¹²² diluted to give a semi-confluent growth. *B. subtilis* is the preferred organism as it is susceptible to a wider range of antimicrobials than either *E. coli* or *S. aureus*.

Dry before use.

Inoculate plate or microtitre wells with urine as described earlier, ensuring that the seeded plate is inoculated last to prevent contamination of other media with *B. subtilis*.

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4.4.3 CULTURE MEDIA⁷², CONDITIONS AND ORGANISMS FOR ALL SPECIMENS:

| Clinical details/ conditions | Standard media | Incubation | | | Cultures read | Target organism(s) |
|---|--|------------|-----------------------|-----------|------------------|---|
| | | Temp °C | Atmos | Time | | |
| UTI Screening in pregnancy for asymptomatic bacteriuria by culture | CLED agar or Chromogenic agar | 35 – 37 | Air | 16 – 24 h | ≥16 h | Enterobacteriaceae Enterococci Lancefield Group B streptococci Pseudomonads <i>S. saprophyticus</i> Other coagulase-negative staphylococci <i>S. aureus</i> |
| Enteric fever screen* | mannitol selenite broth subcultured to: XLD | 35 – 37 | air | 16 – 24 h | N/A | <i>S. Typhi</i> <i>S. Paratyphi</i> |
| | | 35 – 37 | air | 16 – 24 h | ≥16 h | |
| For these situations, add the following: | | | | | | |
| Clinical details/ conditions | Supplementary media | Incubation | | | Cultures read | Target organism(s) |
| | | Temp °C | Atmos | Time | | |
| Urine of patients in Intensive Care, Special Care Baby Units, Burns Units and any from a Transplant Unit or if yeast have been seen in microscopy | Sabouraud agar | 35 – 37 | Air | 40 – 48 h | ≥40 h | Fungi |
| Multipoint culture using agar plates | Susceptibility testing agar seeded with <i>B.subtilis</i> (NCTC 10400) | 35 – 37 | Air | 16 – 24 h | ≥16 h | Antimicrobial substances |
| Optional media | | Incubation | | | Cultures read | Target organism(s) |
| | | Temp °C | Atmos | Time | | |
| If sterile pyuria and no antimicrobials detected | Fastidious anaerobe agar | 35 – 37 | anaerobic | 40 – 48 h | ≥ 40 h | Anaerobes Streptococci |
| | Chocolate agar | 35 – 37 | 5-10% CO ₂ | 40 – 48 h | ≥40 h | Fastidious organisms |
| Susceptibility testing agar seeded with <i>B.subtilis</i> (NCTC 10400) (optional for all except multipoint agar plates) | | 35 – 37 | air | 16 – 24 h | ≥16 h | Antimicrobial substances |
| Other organisms for consideration – <i>C. trachomatis</i> , MRSA, <i>Mycobacterium</i> species, parasites and viruses (see relevant NSM) | | | | | | |
| * These samples are rarely received by the laboratory | | | | | | |

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

Note: All work on *S. Typhi* and *S. Paratyphi* must be performed in a microbiological safety cabinet under Containment Level 3 conditions.

| | |
|---|--|
| Anaerobes | "anaerobes" level BSOPID 14 - Identification of non-sporing, non-branching anaerobes BSOPID 8 - Identification of <i>Clostridium</i> species BSOPID 25 - Identification of anaerobic Gram-negative rods |
| β-haemolytic streptococci Enterobacteriaceae (except <i>Salmonella</i> species) | Lancefield group level "coliforms" level |
| Enterococci | genus level |
| Pseudomonads | "pseudomonads" level |
| S. saprophyticus | species level |
| Other coagulase-negative staphylococci | "coagulase-negative" level |
| S. aureus | species level |
| Salmonella | genus level |
| S. Typhi/Paratyphi | species level |
| Yeasts | "yeasts" level |
| Mycobacterium | BSOP 40 - Investigation of specimens for <i>Mycobacterium</i> species |
| Parasites | BSOP 31 - Investigation of specimens other than blood for parasites |
| Fungi (in urines from patients in Intensive Care, Special Care Baby Units, Burns Units and any from Transplant Units) | species level |

Organisms may be further identified if clinically or epidemiologically indicated.

4.5.2 REFERRAL TO REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

Isolates associated with outbreaks, where epidemiologically indicated and organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Refer to [BSOP 45 - Susceptibility Testing](#). Prudent use of antimicrobials according to local and national protocols is recommended

5 REPORTING PROCEDURE

(see Introduction for the clinical interpretation of culture results)

5.1 MICROSCOPY OR ALTERNATIVE SCREENING PROCEDURE

5.1.1 MICROSCOPY

Report on the actual numbers, or range of WBCs and RBCs per litre or per mL according to local protocol.

Report on the presence of bacteria, epithelial cells, casts, yeasts and *T. vaginalis*.

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Report on supplementary microscopy for dysmorphic RBCs, (see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

Note: Smaller colonies of *T. hominis* may also sometimes appear in urine microscopy.

5.1.2 CHEMICAL SCREENING

Report the results obtained together with a quantitative interpretation if applicable.

The following comments may be added:

“Culture not indicated – bacterial count below significant threshold”

“If symptoms persist or recur please submit a further sample indicating culture required”

5.1.3 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16 – 72 h.

5.2 CULTURE

Report bacterial growth in either imperial or SI units, according to local protocol (see section: Technical Information/Limitations at end of Introduction)

Including comments where appropriate (refer to Appendix 2) or

Report no significant growth or

Report absence of growth.

Report presence of antimicrobial substances, if detected.

Report results of supplementary investigations.

5.2.1 CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically.

Written report, 16 – 72 h stating that a further report will be issued if appropriate.

Supplementary investigations: *Mycobacterium* species (see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

6 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)¹²⁴

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Reporting to the CDR: A guide for laboratories"

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"Hospital infection control: Guidance on the control of infection in hospitals"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

Report all isolates of *Mycobacterium* species (clinically relevant), *Salmonella* species and *Schistosoma* species

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7 ACKNOWLEDGEMENTS AND CONTACTS

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For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

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APPENDIX 1: MULTIPLICATIVE FACTORS BASED ON VARYING VOLUMES OF URINE DISPENSED, DIAMETER OF WELL & FIELD OF VISION (FOV) DIAMETER⁶¹

| | | | | | | | | | | | | | | | | | | |
|--|------|------|------|------|------|------|------|------|------|------|------|------|-------|------|------|------|------|------|
| Volume of urine dispensed (µL) | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 | 80 |
| Diameter of well (mM) | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 6 | 6 |
| FOV diameter (mM) | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 |
| Multiplicative Factor | 0.8 | 1.0 | 1.3 | 1.6 | 2.2 | 3.2 | 0.6 | 0.8 | 1.0 | 1.3 | 1.7 | 2.5 | 0.5 | 0.6 | 0.7 | 0.9 | 1.3 | 1.8 |
| Multiplicative Factor (rounded) | 1 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 1 | 2 | 2 | 0.005 | 1 | 1 | 1 | 1 | 2 |
| Volume of urine dispensed (µL) | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 |
| Diameter of well (mM) | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 6 | 6 |
| FOV diameter (mM) | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 |
| Multiplicative Factor | 0.9 | 1.1 | 1.4 | 1.9 | 2.5 | 3.7 | 0.7 | 0.9 | 1.1 | 1.4 | 1.9 | 2.8 | 0.5 | 0.6 | 0.8 | 1.0 | 1.4 | 2.1 |
| Multiplicative Factor (rounded) | 1 | 1 | 1 | 2 | 3 | 4 | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| Volume of urine dispensed (µL) | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Diameter of well (mM) | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 6 | 6 |
| FOV diameter (mM) | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 | 1.00 | 0.90 | 0.80 | 0.70 | 0.60 | 0.50 |
| Multiplicative Factor | 1.1 | 1.3 | 1.7 | 2.2 | 3.0 | 4.3 | 0.8 | 1.0 | 1.3 | 1.7 | 2.3 | 3.3 | 0.6 | 0.7 | 0.9 | 1.2 | 1.7 | 2.4 |
| Multiplicative Factor (rounded) | 1 | 1 | 2 | 2 | 3 | 4 | 1 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 1 | 2 | 2 |

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APPENDIX 2: MULTIPLICATIVE FACTOR EQUATION⁶¹

Multiplicative Factor for Appendix 1

The Multiplicative factor = $1/V_o$

$$\frac{1}{\pi(\text{FOVr})^2 \times \text{fd}}$$

Where: fd – Fluid Depth
FOV – Field of Vision

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APPENDIX 3: GUIDANCE FOR THE INTERPRETATION OF URINE CULTURE

NOTE: This table is intended for guidance only - supplementation with local reporting policies may be necessary

| Growth cfu/L | No. isolates | Specimen type | Clinical details/microscopy influencing report* | Laboratory interpretation | Susceptibility testing recommended | Comments to consider | |
|---------------|--------------|---|---|---|--|--|--|
| $\geq 10^8$ | 1 | | Any | None | Probable UTI | Yes | If old specimen or no pyuria consider repeat to confirm Consider SPA or CCU if bag specimen |
| | 2 | Each org $\geq 10^8$ or $\geq 10^8$ and $\geq 10^7$ | MSU, CCU, SCU, BAG | WBC present Symptomatic | Possible UTI – colonisation, faulty collection or transport | Yes | Consider repeat to confirm |
| | | | CSU, (IL) | Indwelling catheter Neurogenic bladder | Probable colonisation | No – Consider keeping plates $\leq 5d$ in case patient becomes septic | Consider discuss if patient systemically unwell and therapy required |
| | 2 or 3 | 1 organism predominant at $\geq 10^8$ or 10^7 | Any | None | Possible UTI – ?colonisation, faulty collection or transport | Yes, predominant organism | If old specimen or no pyuria consider repeat to confirm Consider SPA or CCU if bag specimen |
| | ≥ 3 | Mixed growth - none predominant | Any | None | Faulty collection or transport | No | Heavy mixed growth – probable contamination. Consider repeat if symptomatic |
| $10^7 - 10^8$ | 1 | | Any | WBC present Symptomatic | Possible UTI – patient evaluation necessary | Yes | Consider repeat to confirm |
| | 2 | 1 predominant at $\geq 10^7$ | Any | WBC present Symptomatic Children | Probable UTI with predominant species. 2nd isolate probable contamination | Yes, predominant organism but suppress results | Consider repeat or SPA/CCU Sensitivities are available if required |
| | | 1 at $< 10^7$ or $10^7 - 10^8$ but not predominant | | None | Probable contamination | No | Mixed growth – probable contamination |
| | ≥ 3 | 1 organism predominant at $\geq 10^7$ | Any | WBC present Symptomatic | Possible UTI with predominant species. Others probable contamination | No – keep plates $\leq 5d$ if catheter specimen in case patient becomes septic | Mixed growth – consider repeat if symptomatic |
| | | Any combination | CSU | Indwelling catheter Neurogenic bladder | Colonisation | No – keep plates $\leq 5d$ in case patient becomes septic | Please discuss if therapy indicated |

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APPENDIX 3 (CONTINUED) – GUIDANCE FOR THE INTERPRETATION OF URINE CULTURE

NOTE: This table is intended for guidance only – supplementation with local reporting policies may be necessary

| Growth cfu/L | No. isolates | | Specimen type | Clinical details/microscopy influencing report | Laboratory interpretation | Susceptibility testing recommended | Comments to consider |
|--|--|---|-------------------|--|---|------------------------------------|---|
| 10 ⁶ – 10 ⁷ | 1 | | MSU, CCU, CSU, IL | Symptomatic female Prostatitis WBC present | Possible UTI – clinical evaluation necessary | Yes | Consider repeat to confirm |
| | 2 | Each org ≥10 ⁶ - including possible pathogen, eg <i>E. coli</i> or <i>S. saprophyticus</i> | Any | | Possible UTI – clinical evaluation necessary | Yes | Consider repeat to confirm |
| 10 ⁵ – 10 ⁸ | 1 | | SPA, CYS, (SCU) | None | Probable UTI | Yes | |
| | 2 | Each ≥ 10 ⁶ | | WBC present | Probable UTI – patient evaluation necessary | Yes | |
| | ≥3 | 1 organism predominant at ≥ 10 ⁶ | | WBC present | Probable UTI – patient evaluation necessary | Yes, predominant organism | Mixed growth: Consider repeat to confirm |
| No growth | ie: < 10 ⁶ if 1 µl loop used < 10 ⁵ if 10 µl loop used | | Any | None or asymptomatic | No UTI | | |
| | | | | Symptomatic Marked/persistent pyuria | <ul style="list-style-type: none"> • Patient on antibiotics? • Consider <i>Chlamydia</i>, AFB etc • Fastidious organism? • Consider bacteriuria < 10⁵ cfu/L | | As appropriate |
| <p>MSU -- Midstream specimen; CCU – clean catch; SPA – Suprapubic aspirate; IL– Ileal conduit; CSU -- Catheter; SCU – Single, intermittent catheter (“in and out”); CYS – Cystoscopy</p> <p>* - see Introduction for interpretation of culture results</p> | | | | | | | |

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www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. Official Journal of the European Communities 1998;1-37.
3. Sobel JD. Pathogenesis of urinary tract infections. Host defenses. Infect Dis Clin North Am 1987;1:751-72.
4. Van Kerrebroeck P, Abrams P, Chaikin D, Donovan J, Fonda D, Jackson S, et al. Nocturia. Neurourology Urodynamics 2002;21:179-83.
5. Meares EM, Jr. Urethritis, prostatitis, epididymitis, and orchitis. In: Gorbach SL, Bartlett JG, Blacklow NR, editors. Infectious Diseases. 2nd ed. Philadelphia: WB Saunders Company; 1998. p. 954-61.
6. Komaroff AL. Acute dysuria in women. N Engl J Med 1984;310:368-75.
7. Stamm WE. Measurement of pyuria and its relation to bacteriuria. Am J Med 1983;75:53-8.
8. Stamm WE, Wagner KF, Amsel R, Alexander ER, Turck M, Counts GW, et al. Causes of the acute urethral syndrome in women. N Engl J Med 1980;303:409-15.
9. Lipsky BA. Urinary tract infections in men. Epidemiology, pathophysiology, diagnosis, and treatment. Ann Intern Med 1989;110:138-50.
10. Warren JW. Clinical presentations and epidemiology of urinary tract infections. In: Mobley HLT, Warren JW, editors. Urinary Tract Infections. Molecular Pathogenesis Clinical Management. Washington D.C: American Society for Microbiology; 1996. p. 3-27.
11. Bahnson RR. Urosepsis. Urol Clin North Am 1986;13:627-35.
12. Sobel JD, Kaye D. Urinary tract infections. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 773-805.
13. Stamm WE, Stapleton AE. Approach to the patient with urinary tract infection. In: Gorbach SL, Bartlett JG, Blacklow NR, editors. Infectious Diseases. 2nd ed. Philadelphia: WB Saunders Company; 1998. p. 943-54.
14. Meares EM, Stamey TA. Bacteriologic localization patterns in bacterial prostatitis and urethritis. Invest Urol 1968;5:492-518.
15. NICE. Urinary tract infection in children: diagnosis, treatment and long-term management. National Institute for Clinical Excellence guidelines 2007.
16. McCracken GH. Diagnosis and management of acute urinary tract infections in infants and children. Paediatr Infect Dis J 1987;6:107-12.

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www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

17. Jakobsson B, Berg U, Svensson L. Renal scarring after acute pyelonephritis. *Arch Dis Child* 1994;70:111-5.
18. Krieger JN, Ross SO, Simonsen JM. Urinary tract infections in healthy university men. *J Urol* 1993;149:1046-8.
19. Nicolle LE. Urinary Tract Infection in the Elderly. How to Treat and When? *Infection* 1992;20:261-5.
20. Little PJ. The incidence of urinary infection in 5000 pregnant women. *Lancet* 1966;2:925-8.
21. Pedler SJ, Bint AJ. Management of bacteriuria in pregnancy. *Drugs* 1987;33:413-21.
22. Krieger JN. Complications and treatment of urinary tract infections during pregnancy. [Review] [55 refs]. *Urol Clin of North Amer* 1986;13:685-93.
23. Zhanel GG, Harding GK, Nicolle LE. Asymptomatic bacteriuria in patients with diabetes mellitus. *Rev Infect Dis* 1991;13:150-4.
24. Wheat LJ. Infection and diabetes mellitus. *Diabetes Care* 1980;3:187-97.
25. Prat V, Horcickova M, Matousovic K, Hatala M, Liska M. Urinary tract infection in renal transplant patients. *Infection* 1985;13:207-10.
26. Korzeniowski OM. Urinary tract infection in the impaired host. *Med Clin North Am* 1991;75:391-404.
27. De Pinho AM, Lopes GS, Ramos-Filho CF, Santos OR, De Oliveira MP, Halpern M, et al. Urinary tract infection in men with AIDS. *Genitourin Med* 1994;70:30-4.
28. Garibaldi RA. Catheter-associated urinary tract infection. *Curr Op infect Dis* 1992;5:517-23.
29. Stamm WE. Catheter-associated urinary tract infections: epidemiology, pathogenesis, and prevention. *Am J Med* 1991;91:65S-71S.
30. Warren JW, Tenney JH, Hoopes JM, Muncie HL, Anthony WC. A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. *J Infect Dis* 1982;146:719-23.
31. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob Chemother* 2003;51:69-76.
32. Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* 1991;4:80-128.
33. Andersson P, Engberg I, Lidin-Janson G, Lincoln K, Hull R, Hull S, et al. Persistence of *Escherichia coli* bacteriuria is not determined by bacterial adherence. *Infect Immun* 1991;59:2915-21.

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This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

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34. Johnson JR, Moseley SL, Roberts PL, Stamm WE. Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. *Infect Immun* 1988;56:405-12.
35. Leigh D. Urinary tract infections. In: Smith GR, Easmon CSF, editors. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Bacterial Diseases. 8th ed. Vol 3.* London: Edward Arnold; 1990. p. 197-213.
36. McNulty CAM, Richards J, Livermore DM, Little P, Chartlett A, Freeman E, et al. Clinical Relevance of laboratory-reported antibiotic resistance in acute uncomplicated urinary tract infection in primary care. *J Antimicrob Chem* 2006;58:1000-8.
37. Kunin CM. Urinary tract infections in females. *Clin Infect Dis* 1994;18:1-12.
38. Eisenstadt J, Washington JA. Diagnostic microbiology for bacteria and yeasts causing urinary tract infections. In: Mobley HLT, Warren JW, editors. *Urinary Tract Infections. Molecular Pathogenesis Clinical Management.* Washington D.C: American Society for Microbiology; 1996. p. 29-66.
39. Kunin CM, editor. *Principles of urinary bacteriology and immunology. 4th ed.* Philadelphia: Lea and Febiger; 1987. p. 125-93
40. Thomsen AC. Mycoplasmas in human pyelonephritis: demonstration of antibodies in serum and urine. *J Clin Microbiol* 1978;8:197-202.
41. Grenabo L, Hedelin H, Pettersson S. Urinary infection stones caused by *Ureaplasma urealyticum*: a review. [Review] [22 refs]. *Scand J Infect Dis - Supplement* 1988;53:46-9.
42. Soriano F, Aguado JM, Ponte C, Fernandez-Roblas R, Rodriguez-Tudela JL. Urinary tract infection caused by *Corynebacterium* group D2: report of 82 cases and review. *Rev Infect Dis* 1990;12:1019-34.
43. Maskell R. Are fastidious organisms an important cause of dysuria and frequency? - the case for. In: Asscher AW, Brumfitt W, editors. *Microbial Diseases in Nephrology.* Chichester: John Wiley & Sons; 1986. p. 1-18.
44. Brumfitt W, Hamilton-Miller JM, Ludlam H, Gooding A. Lactobacilli do not cause frequency and dysuria syndrome. *Lancet* 1981;2:393-6.
45. Brumfitt W, Hamilton-Miller JM, Gillespie WA. The mysterious "urethral syndrome". *BMJ* 1991;303:1-2.
46. Gillespie WA, Henderson EP, Linton KB, Smith PJ. Microbiology of the urethral (frequency and dysuria) syndrome. A controlled study with 5-year review. *Br J Urol* 1989;64:270-4.
47. Shah KV. Human polyomavirus (including the agent causing progressive multifocal leukoencephalopathy). In: Gorbach SL, Bartlett JG, Blacklow NR, editors. *Infectious Diseases. 2nd ed.* Philadelphia: WB Saunders Company; 1998. p. 2104-7.
48. General Information On Leptospirosis. 2009.
<http://www.hpa.org.uk/HPA/Topics/InfectiousDiseases/InfectionsAZ/1191942176636/>

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This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

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49. Lipsky BA, Inui TS, Plorde JJ, Berger RE. Is the clean-catch midstream void procedure necessary for obtaining urine culture specimens from men? *Am J Med* 1984;76:257-62.
50. Leisure MK, Dudley SM, Donowitz LG. Does a clean-catch urine sample reduce bacterial contamination? *N Engl J Med* 1993;328:289-90.
51. Prandoni D, Boone MH, Larson E, Blane CG, Fitzpatrick H. Assessment of urine collection technique for microbial culture. *Am J Infect Control* 1996;24:219-21.
52. Bradbury SM. Collection of urine specimens in general practice: to clean or not to clean? *J R Coll Gen Pract* 1988;38:363-5.
53. Dunne WM, Jr. Laboratory diagnosis of urinary tract infection in children. *Clin Microbiol Newslett* 1995;17:73-6.
54. Vernon S, Redfearn A, Pedler SJ, Lambert HJ, Coulthard MG. Urine collection on sanitary towels. *Lancet* 1994;344:612.
55. Porter IA, Brodie J. Boric acid preservation of urine samples. *Br Med J* 1969;2:353-5.
56. Johnston HH, Moss MV, Guthrie GA. The use of boric acid for the preservation of clinical urine specimens. In: Meers PD, editor. *The bacteriological examination of urine: Report of a workshop on needs and methods. Public Health Laboratory Service Monograph Series No. 10* ed. London: HMSO; 1978. p. 22-8.
57. Watson PG, Duerden BI. Laboratory assessment of physical and chemical methods of preserving urine specimens. *J Clin Pathol* 1977;30:532-6.
58. Meers PD, Chow CK. Bacteriostatic and bactericidal actions of boric acid against bacteria and fungi commonly found in urine. *J Clin Pathol* 1990;43:484-7.
59. Nickander KK, Shanholtzer CJ, Peterson LR. Urine culture transport tubes: effect of sample volume on bacterial toxicity of the preservative. *J Clin Microbiol* 1982;15:593-5.
60. McNulty C. Diagnosis of UTI - Quick Reference Guide. South West GP Microbiology Laboratory User Group. p. 1-3.
http://www.hpa.org.uk/infections/topics_az/primary_care_guidance/uti_guide_290404.rtf.
61. Shepherd ML. A revision of the microtitre tray method for urine microscopy. *PHLS Microbiol Dig* 1997;14:236-7.
62. Batchelor BI, Hunt AR, Bowler IC, Crook DW. Laboratory detection of leucocyte esterase and nitrite as an alternative to urine microscopy. *Eur J Clin Microbiol Infect Dis* 1996;15:663-4.
63. Vickers D, Ahmad T, Coulthard MG. Diagnosis of urinary tract infection in children: fresh urine microscopy or culture? *Lancet* 1991;338:767-70.
64. Pezzlo M. Detection of urinary tract infections by rapid methods. *Clin Microbiol Rev* 1988;1:268-80.
65. Brumfitt W. Urinary cell counts and their value. *J Clin Pathol* 1965;18:550-5.

INVESTIGATION OF URINE

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Reference no: BSOP 41i7

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

66. Kass EH. Bacteriuria and the diagnosis of infections of the urinary tract. *Arch Intern Med* 1957;100:709-14.
67. Robins DG, Rogers KB, White RH, Osman MS. Urine microscopy as an aid to detection of bacteriuria. *Lancet* 1975;1:476-8.
68. Kitamoto Y, Tomita M, Akamine M, Inoue T, Itoh J, Takamori H, et al. Differentiation of hematuria using a uniquely shaped red cell. *Nephron* 1993;64:32-6.
69. Simpson LO. Comment on: A new morphological classification of urinary erythrocytes by Tomita et al. *Clin Nephrol* 1993;39:229-30.
70. Kerr DNS. Investigation of renal function. In: Beeson PB, McDermott W, Wyngaarden JB, editors. *Cecil Textbook of Medicine*. 15th ed. Philadelphia: WB Saunders Company; 1979. p. 1336-46.
71. Piccoli G, Varese D, Rotunno M, editors. *Atlas of urinary sediments*. New York: Raven Press; 1984. p. 127-51
72. Fallon D, Ackland G, Andrews N, Frodsham D, Howe S, Howells K, et al. A comparison of the performance of commercially available chromogenic agars for the isolation and presumptive identification of organisms from urine. *J Clin Pathol* 2003;56:608-12.
73. Leigh DA, Williams JD. Method for the detection of significant bacteriuria in large groups of patients. *J Clin Pathol* 1964;17:498-503.
74. Faiers M, George R, Jolly J, Wheat P, editors. *Multipoint Methods in the Clinical Laboratory*. London: Public Health Laboratory Service (PHLS) and British Society for Microbial Technology (BSMT); 1991. p. 75-9
75. Aspevall O, Osterman B, Dittmer R, Sten L, Lindback E, Forsum U. Performance of four chromogenic urine culture media after one or two days of incubation compared with reference media. *J Clin Microbiol* 2002;40:1500-3.
76. Stevens M. Screening urines for bacteriuria. *Med Lab Sci* 1989;46:194-206.
77. Stevens M, Mitchell CJ, Livsey SA, MacDonald CA. Evaluation of Questor urine screening system for bacteriuria and pyuria. *J Clin Pathol* 1993;46:817-21.
78. Stevens, M., Mitchell, C. J., and Roberts, M. An evaluation of the Autotrak urine screening system. Report STD/88/28. Report STD/88/28. Department of Health. London. 1988. p. 1-25.
79. Stevens, M., Mitchell, C. J., Roberts, M., and Hardy, S. An evaluation of the Sensititre LeukoBact urine screen. MDD/92/03. Department of Health. London. 1992. p. 1-46.
80. Aliyu SH, Ludlum H, Abubakar I, Bentley N. What is the role of urine dipstick testing in the management of UTI? *Br J Gen Pract* 2002;52:414-5.
81. Van Nostrand JD, Junkins AD, Bartholdi RK. Poor predictive ability of urinalysis and microscopic examination to detect urinary tract infection.[comment]. *Am J Clin Path* 2000;113:709-13.

INVESTIGATION OF URINE

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Reference no: BSOP 41i7

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

82. Murray PR, Smith TB, McKinney TC, Jr. Clinical evaluation of three urine screening tests. *J Clin Microbiol* 1987;25:467-70.
83. Manoni F, Valverde S, Antico F, Salvadego MM, Giacomini A, Gessoni G. Field evaluation of a second-generation cytometer UF-100 in diagnosis of acute urinary tract infections in adult patients. *Clin Microbiol Infect* 2002;8:662-8.
84. Delanghe JR, Kouri TT, Huber AR, Hannemann-Pohl K, Guder WG, Lun A, et al. The role of automated urine particle flow cytometry in clinical practice. *Clin Chim Acta* 2000;301:1-18.
85. Zaman Z, Roggeman S, Verhaegen J. Unsatisfactory performance of flow cytometer UF-100 and urine strips in predicting outcome of urine cultures. *J Clin Microbiol* 2001;39:4169-71.
86. Hughes C, Roebuck MJ. Evaluation of the IRIS 939 UDx flow microscope as a screening system for urinary tract infection. *J Clin Pathol* 2003;56:844-9.
87. Lamchiaghase P, Preechaborisutkul K, Lomsomboon P, Srisuchart P, Tantiniti P, Ra N, et al. Urine sediment examination: a comparison between the manual method and the iQ200 automated urine microscopy analyzer. *Clin Chim Acta* 2005;358:167-74.
88. Hiscoke C, Yoxall H, Greig D, Lightfoot NF. Validation of a method for the rapid diagnosis of urinary tract infection suitable for use in general practice. *Br J Gen Pract* 1990;40:403-5.
89. Flanagan PG, Rooney PG, Davies EA, Stout RW. Evaluation of four screening tests for bacteriuria in elderly people. *Lancet* 1989;1:1117-9.
90. Barker BA, Ratcliffe JG, Turner GC. Urine screening for leucocytes and bacteria by dipstick and reflectance spectrophotometry. *Med Lab Sci* 1989;46:97-100.
91. Stevens, M., Mitchell, C. J., and Roberts, M. An evaluation of the Ames' multistix 8SG and the 'Clinitek 200' urine analyser. Report STD/90/39. Report STD/90/39. Department of Health. London. 1990. p. 1-41
92. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. *N Engl J Med* 1982;307:463-8.
93. Kunin CM, White LV, Hua TH. A reassessment of the importance of "low-count" bacteriuria in young women with acute urinary symptoms. *Ann Intern Med* 1993;119:454-60.
94. MacLean AB. Urinary tract infection in pregnancy. *Int J Antimicrob Agents* 2001;17:273-6.
95. Lipsky BA, Ireton RC, Fihn SD, Hackett R, Berger RE. Diagnosis of bacteriuria in men: specimen collection and culture interpretation. *J Infect Dis* 1987;155:847-54.
96. Bergqvist D, Bronnestam R, Hedelin H, Stahl A. The relevance of urinary sampling methods in patients with indwelling Foley catheters. *Br J Urol* 1980;52:92-5.
97. Stark RP, Maki DG. Bacteriuria in the catheterized patient. What quantitative level of bacteriuria is relevant? *N Engl J Med* 1984;311:560-4.
98. Glaister D. The detection of antimicrobial agents in urine: a simplified routine screening procedure. *Br Soc Microb Technol Newslett* 1994;23-7.

INVESTIGATION OF URINE

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This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

99. Neafie RC, Marty AM. Unusual infections in humans. *Clin Microbiol Rev* 1993;6:34-56.
100. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. *Official Journal of the European Communities* 1998;1-37.
101. Advisory Committee on Dangerous Pathogens 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
102. Health and Safety Executive, editor. *Biological Agents: Managing the risks in laboratories and healthcare premises*. 5 A.D.
103. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. *Safety Precautions: Notes for Guidance*. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
104. *Control of Substances Hazardous to Health Regulations 2002*. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
105. Health and Safety Executive. *5 steps to risk assessment: a step by step guide to a safer and healthier workplace*, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
106. Health and Safety Executive. *A guide to risk assessment requirements: common provisions in health and safety law*, IND (G) 218 (L). Suffolk: HSE Books; 2002.
107. Health Services Advisory Committee. *Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities*. 2nd ed. Suffolk: HSE Books; 2003.
108. NHS Estates. *Health Building Note 15. Accommodation for pathology services*. 1st ed. London: Her Majesty's Stationary Office (HMSO); 1991. (Out of print - 2nd edition in press).
109. BS EN 12469: 2000. *Biotechnology - performance criteria for microbiological safety cabinets*. London: British Standards Institution (BSI); 2000.
110. BS 5726: 1992. *Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation*. London: British Standards Institution (BSI); 1992.
111. BS 5726: 1992. *Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance*. London: British Standards Institution (BSI); 1992.
112. Advisory Committee on Dangerous Pathogens. *The management, design and operation of microbiological containment laboratories*. Suffolk: HSE Books; 2001.
113. Health and Safety Executive, editor. *Biological Agents: Managing the risks in laboratories and healthcare premises*. 5 A.D.
114. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. *Official Journal of the European Communities* 1998;1-37.
115. Clarridge JE, Pezzlo MT, Vosti KL. Laboratory diagnosis of urinary tract infections. *Cumitech* 1987;1-15.

INVESTIGATION OF URINE

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Reference no: BSOP 41i7

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

116. Hindman R, Tronic B, Bartlett R. Effect of delay on culture of urine. J Clin Microbiol 1976;4:102-3.
 117. Wheldon DB, Slack M. Multiplication of contaminant bacteria in urine and interpretation of delayed culture. J Clin Pathol 1977;30:615-9.
 118. Aliyu SH, Ludlum H, Abubakar I, Bentley N. What is the role of urine dipstick testing in the management of UTI? Br J Gen Pract 2002;52:414-5.
 119. Lum KT, Meers PD. Boric acid converts urine into an effective bacteriostatic transport medium. J Infect 1989;18:51-8.
 120. Hilson GR. A disposable counting chamber for urinary cytology. J Clin Pathol 1964;17:571-2.
 121. Haugen J, Strom O, Ostervold B. Bacterial counts in urine. 1. The reliability of the loop technique. Acta Pathol Microbiol Scand 1968;74:391-6.
 122. Cruickshank JG, Gawler AH, Hart RJ. Costs of unnecessary tests: nonsense urines. Br Med J 1980;280:1355-6.
 123. Klassen M. Measurement of antibiotics in human body fluids: Techniques and significance. In: Lorian V, editor. Antibiotics in Laboratory Medicine. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 391.
 124. Health Protection Agency. Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories. February. 2007.
- ^a *The requirements of the EU in vitro Diagnostic Medical Devices Directive¹¹⁴ (98/79/EC Annex 1 B 2.1) state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".*

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