# Aim

To isolate, quantify and permit presumptive identification and differentiation of the major microorganisms causing urinary tract infections (UTIs).

# Principle

All urines undergo a dipstick test and/or microscopy to look for the presence of white blood cells, red blood cells, nitrites and bacteria. All children under 3 years of age should have a microscopy performed since dipsticks can be unreliable in this age group due to frequent voiding.

A known volume of urine is cultured in order to allow quantification of the number of organisms in the original urine, although because of imprecisions in the method this is usually referred to as ‘semi-quantitative’ culture.

The UTI chromogenic medium (Oxoid *Brilliance*™ UTI Clarity™ agar) contains two specific chromogenic substrates. The different dyes produced by the organisms leads to different urinary isolates appearing as different coloured colonies after overnight incubation at 37ºC in air.

# Method

## Specimen collection

Urine specimens may be collected in several ways:

* Mid-stream specimen
* Clean catch specimen
* Bag specimen
* Catheter specimen (either from an in-out catheter or an indwelling catheter)
* Supra-pubic aspirate (needle directly into the bladder)

Wherever possible a specimen of urine should be collected aseptically directly into a sterile universal container following cleaning of the perineal area.

## Specimen transport and storage

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable.

## Specimen processing

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

### Pre-culture examination

Perform a urine dipstick and microscopy on all urine samples arriving in the microbiology laboratory.

#### Microscopy using the Kova slide technique

Tip the closed urine pot over to mix carefully then use a capillary tube to place unspun urine into one chamber of the Kova slide, leave the chamber on the bench for one minute for the cells to settle.

Using a low power microscope objective (x40), count the RBC or WBC cell numbers in 36 small grids, unless obviously >100 (i.e. >1 per small grid).Thirty six small grids is equivalent to four large squares on the Kova slide: therefore, count cells in all of the small grids form the four corner large squares.

**Multiply the average number of RBC or WBC cells per grid (total number cells / 36) by 90 to determine the number of cells per microlitre (**μL**).**

**Follow Table 1 for the cell numbers to report.**

**Table 1.** Calculating the number of cells per microlitre using the Kova slide

| **Total cells counted** | **Cells/μL** |  | **Total cells counted** | **Cells/μL** |
| --- | --- | --- | --- | --- |
| **1** | **1/36\*90 = 3** |  | **13** | **33** |
| **2** | **5** |  | **14** | **35** |
| **3** | **8** |  | **15** | **38** |
| **4** | **10** |  | **16** | **40** |
| **5** | **13** |  | **17** | **43** |
| **6** | **15** |  | **18** | **45** |
| **7** | **18** |  | **19** | **48** |
| **8** | **20** |  | **20** | **50** |
| **9** | **23** |  | **25** | **63** |
| **10** | **25** |  | **30** | **75** |
| **11** | **28** |  | **40** | **100** |
| **12** | **30** |  | **>40** | **>100** |

### Culture

**If the urine microscopy is positive or one of the following points are true, culture the urine:**

* Sample screened by counting chamber has over 10 WBC/µL (104 WBC/mL; i.e. ≥5 cells counted (in 36 small squares)
* Follow up of patients on treatment
* Urinary tract obstruction
* Follow up after removal of indwelling catheter
* Child of less than 3 years who has suspected urinary tract infection
* Suspected melioidosis patient: culture on Ashdown’s medium in addition to standard media

Describe the appearance of the urine (clear or cloudy) and colour: note this in the laboratory book and on the computer.

Turn the urine pot over to mix it carefully and remove the top of the container.

Dip the end of a sterile 1μL loop into the urine and remove it vertically making sure that there is no urine up the loop (as this would mean that a greater volume was cultured).

Spread the entire volume over the surface of a *Brilliance* UTI Clarity agar plate by making a single streak across the centre. Spread the inoculum evenly at right angles to the primary streak as shown in Figure 1. If many samples are being processed use half a plate per sample.

Incubate the plate aerobically at 35-37°C for at 18-24 hours.

After inoculation, estimate the number of bacteria by counting the number of colonies on the surface of the media. One colony = 1,000 cfu/mL (1x106 cfu/L).

**Figure 1.** Streaking the urine specimen onto a *Brilliance* UTI Clarity agar plate

# Interpretation

Culture results are categorised on the basis of quantity (Table 2) and purity (Table 4) of growth. Below a recognised threshold (105 cfu/mL) the likelihood is that the organisms grown are contaminants, particularly if more than one type of organism is present. Above the threshold it is more probable that a true urinary tract infection is occurring.

**Table 2.** Relationship between colony count and quantity of bacteria in the urine specimen

|  |  |
| --- | --- |
| **Colony count** | **Number organisms per mL of urine** |
| <10 | <104 cfu/mL |
| 10-100 | 104 – 105 cfu/mL |
| >100 | >105 cfu/mL |

If there is a pure growth of 10-100 or over 100 colonies, sub culture the isolate for identification and antimicrobial susceptibility testing.

For cultures that contain two organisms, one in low numbers (<100 colonies) and the other over 100 colonies, then only sub-culture the predominant organism because the organism of lower numbers is unlikely to be causing disease. If both are present at over 100 colonies, sub-culture both organisms.

If more than two organisms are isolated, then do not sub-culture / identify any of them since this is highly likely to be a contaminated specimen.

An exception is that all growth from a supra-pubic aspirate specimen must be fully identified and have sensitivity tests performed.

## Minimum level of identification in the laboratory

Presumptive identification can be made from the chromogenic agar plate (Figure 2).

**Figure 2.** Identification of bacterial species from the *Brilliance* UTI Clarity agar plate

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Confirmatory testing should be done on all organisms, if necessary make purity plates on blood agar first (Table 3).

**Table 3.** Confirmatory identification tests for urinary tract isolates

|  |  |
| --- | --- |
| **Organism** | **Identification methods** |
| Staphylococci | CatalaseCoagulase / StaphaurexDNase agarNovobiocin disc if coagulase negative (*S. saprophyticus* is resistant) |
| Streptococci | Catalase*Enterococcus* spp.: group and sub onto bile-aesculin agarOther streptococci: sub on blood agar (+optochin disc) and identify using conventional tests (haemolysis, group etc.) |
| GNB | *E. coli*: confirm with indole only (inoculate MIL medium)Other coliforms: ID using biochemistry short set +/- API 20E*Pseudomonas* spp.: confirm as *Pseudomonas aeruginosa* with oxidase and growth of green colonies on Columbia agar after incubation at 42°C. If not *P. aeruginosa* report as *Pseudomonas* sp. (ID with API 20NE only if clinically indicated) |
| Other organisms (e.g. yeasts) | Only if felt to be clinically significant |

## Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities determined according to SOP MIC-001.

## Reporting

## **Reporting of results from microscopy**

1. Report the number of WBC and RBC per mL in urine using the counting chamber.
2. Comment on the presence of epithelial cells, bacteria, casts or yeasts (see Appendix 1) following the following counts (using x40 objective and Kova slide):
	* No cells = none seen
	* <1 per field (not small grid) = +/-l
	* 1 – 10 per field = +
	* 11 – 25 per field = ++
	* >25 per field = +++
	* Seen under other cells (cannot count) = present
3. Report the presence of *Trichomonas vaginalis.*
4. Casts are solidified protein which are cylindrical in shape as they are formed by the kidney tubules
5. If culture is not indicated report "Culture not done because white blood cell count was below significant levels (104 WBC/mL)".

## Reporting results from culture

|  |  |
| --- | --- |
| **Culture result** | **Report** |
| ***No bacterial growth*** | No growth |
| ***Single organism*** |  |
| <104 CFU/ml | No significant growth |
| 104 -105 CFU/ml  | Growth of 104 -105 cfu/ml of *organism,* ?significanceReport antimicrobial sensitivities |
| >105 CFU/ml | Growth of >105 cfu/ml of *organism*Report antimicrobial sensitivities |
| ***Two organisms*** |  |
| Both <105 CFU/ml | No significant growth (please repeat if appropriate) |
| One >105 CFU/ml | Mixed growth including >105cfu/ml of *organism*, ?significanceReport antimicrobial sensitivities of the one >10 5 cfu/ml only |
| Both >105 CFU/ml | Mixed growth of >105cfu/ml of *organism1* and *organism2*, ?significanceReport antimicrobial sensitivities for both |
| ***>2 organisms*** | Mixed growth of >2 organisms (please repeat if appropriate) |

# Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP.

# Limitations

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Label plates with the date of preparation and store the prepared medium at 2-8°C. The shelf life after preparation is **two weeks after which plates should be discarded.**

Only use the media that has passed QC.

Organisms with atypical enzyme patterns may give anomalous results; e.g. white colonies may occasionally prove to be *E. coli* on further examination.

# References

1. NICE Guidelines. Urinary tract infection in children, diagnosis, treatment and long-term management. Clinical Guideline, August 2007.
2. Health Protection Agency, UK SOP B41: Investigation of Urine (Issue 7.1; December 2012).
3. Oxoid information page for *Brilliance™* UTI Clarity™ agar Code: CM1106.
4. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.
5. Kova slide manufacturer’s protocol.
6. Standard Operating Procedures from LOMWRU, SMRU and AHC.

# Synopsis / Bench aid



# Risk assessment

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| --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** |
| **Description of procedure**Culture of urine | **Substances used**Variable, depending on organism cultured (may include Gram stain reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents) |
| **Quantities of chemicals used**Small | **Frequency of SOP use**Daily |
| **Hazards identified**1. Autoclaved liquid2. Potentially infectious material in sample 3. Potentially pathogenic bacteria | **Could a less hazardous substance be used instead?** No |
| **What measures have you taken to control risk?** 1. Training in good laboratory practices (GLP)2. Appropriate PPE (lab coat, gloves, eye protection)3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) |
| **Checks on control measures**Observation and supervision by senior staff |
| **Is health surveillance required?**No | **Training requirements:**GLP |
| **Emergency procedures**:1. Report all incidents to Safety Adviser2. Use eyewash for splashes3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:1. Sharps discarded into appropriate rigid containers for incineration2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration3. Chemical waste disposed of according to manufacturer’s instructions |

# Appendix 1: Useful images for urine microscopy

**Red cells, White cells, and bacteria**



Bacteria

White Blood Cell

Red Blood Cell

**Epithelial cells** (indicate that the urine is not a clean catch)



**White cell casts** (found when there is inflammation of the kidney pelvis or tubules)



**Red cell casts** (indicate haemorrhage into the renal tubules or glomerular bleeding; orange red colour)



**Hyaline casts** (associated with damage to the glomerular filter membrane)

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**Crystals**

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