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## Supplemental Materials

for

### **Artificial Urine for Teaching Urinalysis Concepts and Diagnosis of Urinary Tract Infection in the Medical Microbiology Laboratory**

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#### **Table of Contents**

(Total pages 15)

- Appendix 1: Faculty instructions
- Appendix 2: Student instructions
- Appendix 3: Student assessment

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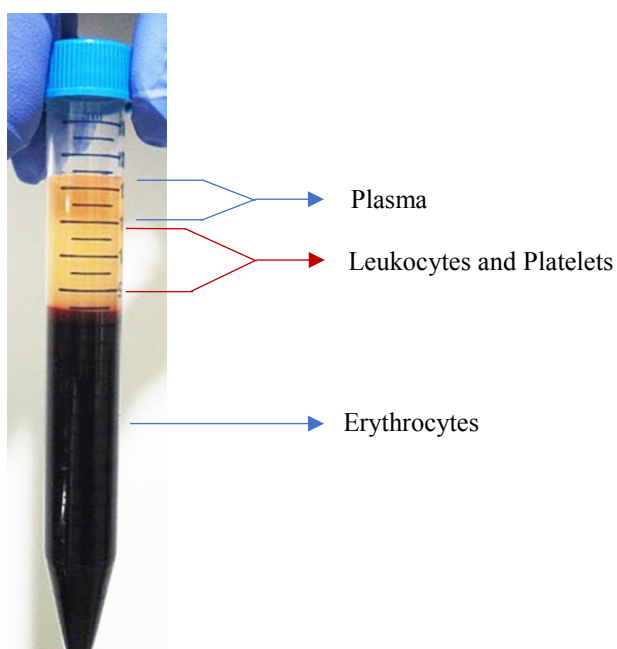
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### Preparation/extraction of WBCs/esterase from buffy coat:

Obtain blood sample [which is enriched with white blood cells (WBC) and platelets, also named as buffy coat] from the supplier. Most clinical teaching labs are probably located closely to human blood banks and will have very easy and cheap access to blood sample or buffy coat.

#### WBC/Esterase preparation

1. Spin ~50 ml of blood sample in 50 ml tube at 400 g for 10 min
2. Carefully remove the plasma with pipette and collect 5 ml of the buffy coat layer (Figure A1-I) in a separate tube
3. Mix 45 ml of lysis buffer (167 mM  $\text{NH}_4\text{Cl}$ , 10mM  $\text{KHCO}_3$ , 100  $\mu\text{M}$  EDTA, pH 7.2) with 5 ml of the buffy coat layer in a separate tube
4. Mix well and leave a maximum of 3 min at room temperature for the red blood cells (RBCs) to lyse
5. Mix and spin at 400 g for 6 min.
6. Remove supernatant and wash pellet with phosphate buffered saline (PBS) pH 7.0
7. Repeat wash 4-times to remove protein, platelets and erythrocytes
8. Re-suspend pellet in 3 ml phosphate buffered saline and store at  $-20^\circ\text{C}$  up to 3 weeks before use
9. This final suspension contains the white blood cells (WBC) and esterase. The preparation may contain some RBCs.



**Figure A1-I:** Buffy coat (after centrifugation).

**List of vendors for buffy coat or esterase:**

Product Name	Recommended Vendor	Catalogue Number
Buffy Coat (from Human Donor)	Lee Biosolutions, Inc. Tel.:1-314-968-1091 Email: order@leebio.com	993-01
Whole Leukocyte Esterase (from Human Leukocytes)	Lee Biosolutions, Inc.	341-10
Single Donor Human Buffy Coat Leukocytes	Innovative research, Inc Email: sales@innov-research.com	IPLA-WB5
Esterase from Rabbit Liver	Sigma-Aldrich	E0887-500UN
Esterase from Porcine Liver, Crude	Sigma-Aldrich	E3019-100KU

**Technical tips for teaching instructors:**

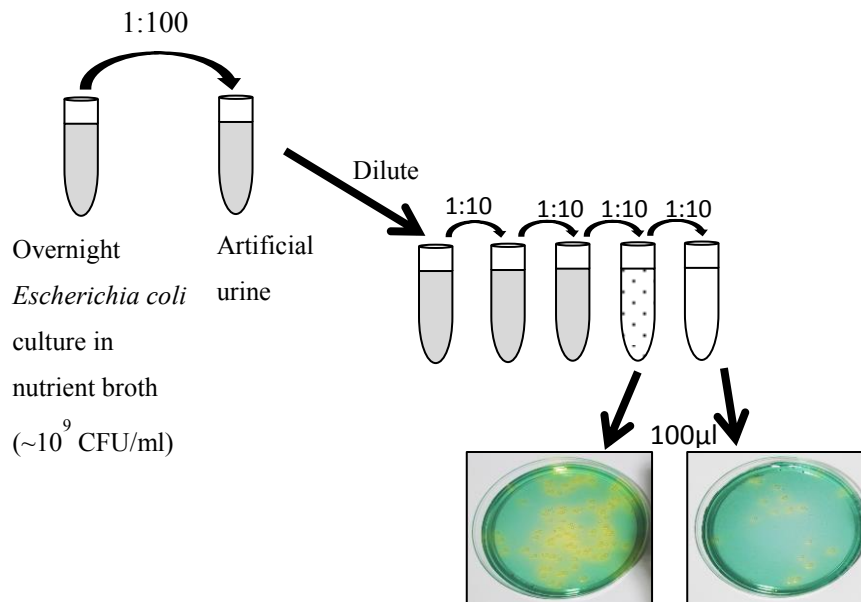
During dipstick urinalysis, teaching instructors need to make sure that:

- Roche Combur<sup>7</sup>-Test® strip containers are not left open, as extended exposure to air can lead to wrong test results.
- Students only briefly (about 1 second) dip the test strip into the artificial urine and drain excess fluid on a paper towel, as overexposure can lead to wrong test results.
- Results are not recorded after the recommended development time (60-120 seconds). Colourization appearing after more than 2 minutes are not clinically significant.

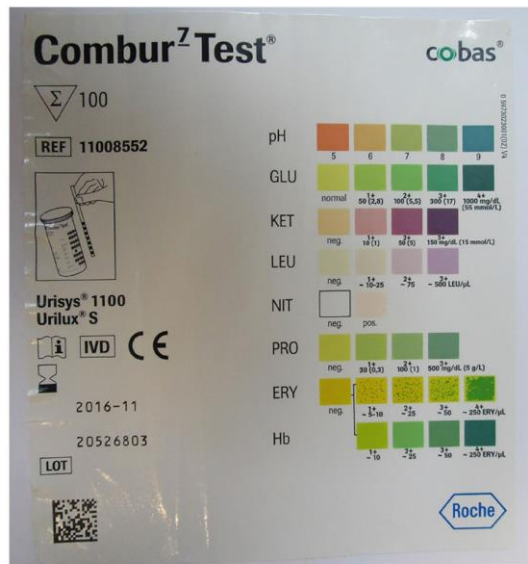
**Potential modification of the artificial urine sample:**

The dipstick exercise could be modified to suit courses teaching medical laboratory science students, nursing students or clinical microbiologists. We have found that addition of harmless laboratory strains, such as *Escherichia coli* K12 just prior to the lab does not affect the dipstick test results. This would allow an additional component to the UTI diagnostic exercise – students could dilute the artificial urine sample to learn serial dilution and plate onto agar plates for bacterial enumeration and identification (Figure A1-II). Cystine lactose electrolyte deficient (CLED) agar is commonly used for isolation and differentiation of uropathogenic bacteria. Our artificial urine sample can also be modified with different degrees of complexity to meet the goals of the urinalysis lesson for other systemic or renal diseases. For example by adding glucose (16 g l<sup>-1</sup>) for presence of glucose or milk powder (32 g l<sup>-1</sup>) for presence of

protein, to teach urinalysis lesson for diabetes or renal diseases (Figure. A1-III). The pH can be adjusted with 1M HCl or 1M NaOH, and also the concentration of protein and glucose can be adjusted to meet the needs of instructor. Artificial urine samples that require the addition of glucose, milk powder are stable ONLY for 5/7 days at 4°C to achieve the results as per Figure (A1-III).



**Figure A1-II:** Addition of bacterial culture to our artificial urine sample Case B. Serial dilution of this urine sample with saline and then plating onto Cystine Lactose Electrolyte Deficient (CLED) agar plates for bacterial enumeration and identification.



Reference colour scale



Combur Test Urine Base Protein (4+) Glucose + Strip

**Figure A1-III:** Possible modifications of artificial urine to mimic disease conditions. The urine base contained 32g l<sup>-1</sup> milk powder (protein test strip) or 16g l<sup>-1</sup> glucose (glucose test strip).

## Student laboratory protocol: “Urine Analysis from Patients with Suspected UTI”

**Perform in bench group (4 to 6 students). Suggested time: 15 minutes.**

### Urinary tract infection

Urinary tract infections (UTIs) are common and occur at all ages throughout life, with women more prone to UTI than men. UTIs cause significant morbidity. Apart from the anterior urethra, which is colonised by the normal microflora, the urinary tract is usually sterile. If microorganisms become established in the urinary tract, inflammation and invasion may follow.

Infection may be confined to the lower urinary tract where it causes cystitis (infection of the bladder) or urethritis (infection of the urethra) or the infectious agent may ascend to the upper urinary tract causing pyelonephritis. Asymptomatic bacteriuria is common, particularly in older men and women.

Bacteria usually invade the urinary tract by ascending via the urethra, but can also arise via the bloodstream. In women, the short urethra and the proximity to the vulva and perineum increase the risk of infection. Studies have shown that colonisation of the vaginal introitus is a predisposing factor in the development of UTI in women. Healthy flora, maintained by oestrogen, is an important defense against UTI.

### For reference only: Bacterial causes of urinary tract infection

Organism	Frequency %	Comment
<i>Escherichia coli</i>	>50	Most common cause of UTI
<i>Proteus</i> spp.	10-15	Most often associated with renal calculi
<i>Klebsiella</i> spp.	10	May be hospital or catheter associated
Enterococci	8-10	Usually low grade pathogens
<i>Staphylococcus saprophyticus</i>	5-8	Confined to young women
<i>Staphylococcus epidermidis/aureus</i>	2-6	Hospital acquired, associated with urinary catheters or bacteraemia ( <i>S. aureus</i> )
<i>Pseudomonas aeruginosa</i>	4	Often associated with underlying pathology

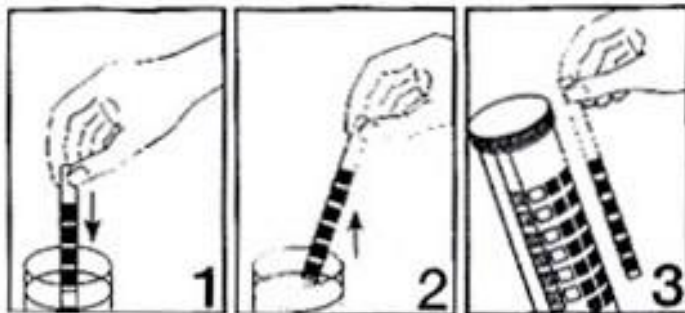
The most common bacterium causing UTI is *E. coli*, followed by other members of the *Enterobacteriaceae*. The species of bacteria isolated depends on several factors. Factors determining species of bacteria isolated:

- i. host factors, e.g. the age and health of the patient.
- ii. the place where the infection was acquired i.e. acquired in hospital or in the community.
- iii. some complication, e.g. an indwelling urinary catheter or abnormalities of the voiding mechanism.
- iv. host factors, e.g. immunosuppression, or neurological disease (incomplete bladder emptying).

Management of UTI includes prescribing an appropriate antimicrobial agent, adequate hydration of the patient and analgesics if necessary. When interpreting the results of microbiological examination of a urine specimen it is particularly important to note if urine leukocyte levels are raised (pyuria).

### Combur -7 Test®:

#### Technique



1. Use fresh urine that has not been centrifuged. Thoroughly mix the urine sample.
2. Briefly (no longer than 1 second) dip test strip into the urine. Wipe off excess urine onto the rim of the vessel.
3. After 60 seconds (leukocyte patch 60-120 seconds), compare reaction colours with the colour scales on the label.
4. Colourisations appearing after more than 2 minutes do not have any clinical significance.
5. The urine specimen must not stand for more than 2 hours before testing.

## Interpretation of results from Combur -7 Test®

### Urine pH

Urine pH is used to classify urine as either a dilute acid or base solution. Seven is the point of neutrality on the pH scale. The lower the pH, the greater the acidity of a solution; the higher the pH, the greater the alkalinity.

The glomerular filtrate of blood is usually acidified by the kidneys from a pH of approximately 7.4 to a pH of about 6 in the urine. Depending on the person's acid-base status, the pH of the urine may range from 4.5 to 8.

A highly acidic urine pH occurs in:

- Acidosis
- Uncontrolled diabetes
- Diarrhoea
- Starvation and dehydration
- Respiratory diseases in which carbon dioxide retention occurs and acidosis develops

A highly alkaline urine occurs in:

- Urinary tract obstruction
- Pyloric obstruction
- Salicylate intoxication
- Renal tube acidosis
- Chronic renal failure
- Respiratory diseases that involve hyperventilation (blowing off carbon dioxide and the development of alkalosis)

The formation of renal stones is related to the urine pH. Patients being treated for renal calculi are frequently given diets or medications to change the pH of the urine so that kidney stones will not form. Calcium phosphate, calcium carbonate, and magnesium phosphate stones develop in alkaline urine; when this occurs, the urine is kept acidic. Uric acid, cystine, and calcium oxalate stones precipitate in acidic urine; in this situation, the urine should be kept alkaline or less acidic than normal. Drugs such as streptomycin, neomycin, and kanamycin are effective in treating urinary tract infections if the urine is alkaline. During treatment with sulfa drugs, alkaline urine helps prevent the formation of sulphonamide crystals.



Here are important points to remember about urinary pH:

- An accurate measurement of urinary pH can be done only on a freshly voided specimen. If the urine must be kept for any length of time before analysis, it should be refrigerated.
- During sleep, decreased pulmonary ventilation causes respiratory acidosis. As a result, a first waking urine specimen is usually acidic.
- Bacteria causing a urinary tract infection or bacterial contamination will produce alkaline urine.
- A diet rich in citrus fruits, most vegetables and legumes will keep the urine alkaline.
- A diet high in meat and cranberry juice will keep the urine acidic.
- Urine pH is an important screening test for the diagnosis of renal disease, respiratory disease, and certain metabolic disorders.
- If urine pH is to be useful, it is necessary to use pH information in comparison with other diagnostic information.

### **Glycosuria**

Glycosuria refers to sugar in the urine. The renal threshold for glucose is approximately 160 to 190 mg/dl of blood: glucose does not appear in the urine until the blood glucose rises above this level.

### **Ketones in urine**

A negative test result is normal. When ketones are present in the urine, the results are usually listed as small, moderate, or large with these corresponding values:

Small: < 20 mg/dL; Moderate: 30 - 40 mg/dL; Large: > 80 mg/dL A positive test may indicate:

Abnormal nutritional conditions

- Anorexia
- Fasting
- High protein or low carbohydrate diets
- Starvation

Disorders of increased metabolism

- Acute or severe illness

- Burns
- Fever
- Hyperthyroidism
- Nursing a baby (lactation)
- Post-surgical condition
- Pregnancy

Metabolic abnormalities, including uncontrolled diabetes or glycogen storage disease.

Vomiting frequently over a long period of time.

### **Urinary Nitrites and Leukocyte Esterase**

Tests to detect nitrites and leukocyte esterase have become part of a routine urinalysis. Most species of bacteria that colonize in the urine cause nitrates, which are derived from dietary metabolites, to be converted into nitrites. In healthy people, both the urinary nitrite test and the leukocyte esterase (LE) tests are negative.

White cells in the urine usually indicate a urinary tract infection. The leukocyte esterase (LE) test detects esterase, an enzyme released by white blood cells. Positive test results indicate significant pyuria ( $>8-10$  WBC  $\times 10^6/L$ ).

These tests are indirect ways of detecting bacteria in the urine. Significant urinary tract infections may be present in patients who do not experience other symptoms. If undetected and untreated they can cause severe kidney disease. The urinary nitrite and LE tests are often ordered to screen patients at high risk for urinary tract infections, including pregnant women, school-age females, elderly patients, and persons with a history of urinary tract infections.

### **Proteinuria**

Proteinuria refers to the presence of protein in the urine. In healthy persons, the urine contains no protein or only a trace amount of protein. Proteins in the urine are comprised of albumin and globulin from the plasma.

Detection of protein in the urine, combined with a microscopic exam of urinary sediment, provides the basis for the differential diagnosis of renal disease.

## Urine haemoglobin

Free haemoglobin in the urine is referred to as haemoglobinuria. This finding is usually related to a condition outside the urinary tract and occurs when there is such extensive or rapid destruction (haemolysis) of red blood cells that the reticuloendothelial system cannot metabolize or store the excess free haemoglobin. Bleeding from the urinary tract (haematuria) may appear in the urine as intact red cells and/or as free haemoglobin, once the red cells breakdown in urine.

Note: It is normal to find red blood cells in the urine 3 days either side of menses. After physical activity, e.g. strenuous jogging, raised values for **erythrocytes** and **protein** may occur without being signs of disease.

Parameter	Visual Reading		
	Range	Practical detection limit	Accuracy
pH	5-9		>95% compared with pH meter
Glucose	Normal – 1000 mg/dL (55 mmol/L; 4+)	40 mg/dL (2.2 mmol/L)	>90% compared with hexokinase method.
Ketone bodies	Negative – 150 mg/dL (15 mmol/L; 3+)	For acetoacetic acid 5 mg/dL (0.5 mmol/L)	>85% compared with photometric enzymatic determination of acetate
Leukocytes	Negative – Approx. 500 WBC/ $\mu$ L (3+)	10-25 WBC/ $\mu$ L	>90 compared with counting chamber
Nitrite	Negative – Positive (1+)	0.05 mg/dL (11 $\mu$ mol/L)	>90% for $10^7$ Gram-positive organisms compared with Griess' test
Protein	Negative – 500 mg/L (5 g/L; 3+)	6 mg albumin/dL	90% compared with radial immunodiffusion
Blood and haemoglobin	Negative – Approx. 250 RBC/ $\mu$ L (4+)	Intact erythrocytes; 5 RBC/ $\mu$ L Hemoglobin or hemolysed erythrocytes: corresponding to 10 RBC/ $\mu$ L	>90% compared with counting chamber

**Materials provided in the class:**

Combur\_7-Test® urine test strips

Timer, Paper towel, Discard jar for used strips

A urine sample from each of the following patients (Cases A and Case B):

**Case A - Elevation of PSA:** A 65 year old Dutch male, a retired plumber, has a history of a slow stream of urine and needing to get up to void once or twice in the night. He has been referred to the Urology Outpatient Clinic after a blood test showed an elevated prostate-specific antigen.

**Case B - Dysuria:** An 18 year old European woman presents to the student health clinic with burning discomfort when she passes urine. She has recently started university and has a new boyfriend. They have been having sex without using condoms.

**Method**

1. Briefly (no longer than 1 second) dip test strip into the urine sample.
2. After 60 seconds (leucocyte patch 60-120 seconds) compare reaction colours with the reference colour scales
3. Colourations appearing after more than 2 minutes do not have any clinical significance.
4. Record your results on the table provided.

Test	Results (Case A)	Results (Case B)	Normal range
pH			approx. pH 6.0
Glucose			0 - 0.8 mmol/l (0 - 15 mg/dl)
Ketone			0
Leucocytes			0 - 10 leu/ul
Nitrite			0
Protein			0 - 150 mg/dl
Blood			less than 3 ery/ul
Haemoglobin			0

## Questions

- Which urine specimen is most likely to have been provided by the 18 year old student with dysuria?
- Briefly explain the significance of the results obtained by the dip strip method for each of the two patients.
  - What is the likely diagnosis in each case?
  - For Case B, what additional symptoms would you ask about?
- Describe an appropriate management plan for the student with dysuria. Are additional tests required? Are follow-up urine tests required? Which antibiotic is appropriate? (refer to the following table of bacterial susceptibility data derived from the testing of community laboratory isolates in Auckland).



**CUMULATIVE ANTIMICROBIAL SUSCEPTIBILITY REPORT-AUCKLAND AND NORTHLAND  
COMMUNITY ISOLATES 2016**

Organism	<i>E. coli</i>	ESBL producing <i>E. coli</i> (4% of <i>E. coli</i> )	Enterobacteriaceae group	ESBL producing Enterobacteriaceae group	<i>Prateus, Providencia, Morganella</i> group	<i>H. influenzae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	Methicillin resistant <i>S. aureus</i> (13% of <i>S. aureus</i> )	<i>S. saprophyticus</i>	<i>S. pyogenes</i>	<i>S. pneumoniae</i> †	Enterococci
Number tested#	38260	1581	5916	498	2156	1632	2729	38980	5184	2351	2245	553	2694
Amoxicillin	49	0	0	0	72	73	R		R	96	S		98
Cefalexin	94	0	0	0	85		R		R	50	S		R
Ciprofloxacin	91	40	91	39	97	100	92	94	90	100		0	
Clindamycin								89	86		96	87	
Co-amox/Clav(Augmentin)	88	71	69	46	87	95	R		R				
Cotrimoxazole	72		94			74	R	99	99			73	R
Erythromycin								88	80		96	81	
Fludoxacillin								87	0	97			
Fosfomycin*	98	97		91	30								72
Fusidic acid								74	40	100			
Gentamicin	72	66	93	46	87		94						R
Mecillinam	89	93		74	61								
Mupirocin								91	92				
Nitrofurantoin*	99	95		56	0		R	99	100	100			99
Norfloxacin*	92	35			96		95	92	70	100			94
Penicillin							R	R	R		S	87	
Tetracycline					0	98	R	98	98	94	86	84	
Trimethoprim*	71	29		7	76		R	94	89	91			

S Predictable susceptibility

R Predictable resistance

# All organisms were not tested against all antibiotics

\* Tested against urinary isolates only

\*\* Enterobacteriaceae group: *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia* spp.

† In patients without meningitis 87% of *S. pneumoniae* isolates were susceptible to oral penicillin

In Northland 3% of *E. coli* are ESBL producers and 15% of *S. aureus* are methicillin resistant

Author: Dr Gary McAuliffe, Clinical Microbiologist, Labtests March 2016

**Post laboratory test questions and answer key:**

- 1) Which of these specimens is most likely to culture *Escherichia coli*?
- A. midstream urine from a 18 year old with dysuria, urinary frequency and pyuria
  - B. a stool sample from a patient with diarrhoea, fever and abdominal cramping
  - C. a blood culture from a central venous catheter from a 56 year old man with leukaemia and fever
  - D. a bone sample from the tibia of a 12 year old with osteomyelitis
  - E. cerebrospinal fluid from a 14 month old boy with meningitis

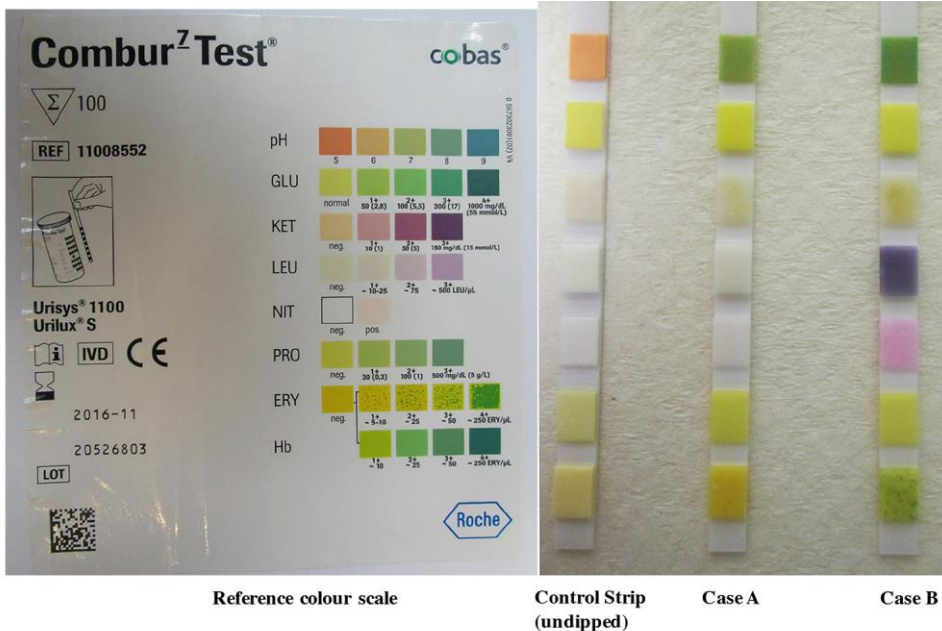
**Answer key:** A, *Escherichia coli* is the most common cause of cystitis.

- 2) Which of these specimens is most likely to culture *Salmonella enterica*?
- A. midstream urine from a 18 year old with dysuria, urinary frequency and pyuria
  - B. a stool sample from a patient with diarrhoea, fever and abdominal cramping
  - C. a blood culture from a central venous catheter from a 56 year old man with leukaemia and fever
  - D. a bone sample from the tibia of a 12 year old with osteomyelitis
  - E. cerebrospinal fluid from a 14 month old boy with meningitis

**Answer key:** B, In New Zealand, *Salmonella enterica* is most commonly associated with enterocolitis.

- 3) Which of these specimens is most likely to culture *Pseudomonas aeruginosa*?
- A. midstream urine from a 18 year old with dysuria, urinary frequency and pyuria
  - B. a stool sample from a patient with diarrhoea, fever and abdominal cramping
  - C. a blood culture from a central venous catheter from a 56 year old man with leukaemia and fever
  - D. a bone sample from the tibia of a 12 year old with osteomyelitis
  - E. cerebrospinal fluid from a 14 month old boy with meningitis

**Answer key:** C, *Pseudomonas aeruginosa* is commonly associated with healthcare associated infections, including those that occur in patients undergoing treatment for leukaemia. It is also a cause of cystitis in older people, men with enlarged prostates and people with urinary catheters or other devices. It is a very, unusual cause of cystitis in healthy young women.



**Figure A3-I:** Combur-<sup>7</sup> Test® strips dipped into artificial urine samples for Case A and Case B. Control strip not dipped into urine sample, Case A and Case B strips dipped into relevant urine samples.

- 4) Which of these urine dipstick results is likely to have come from an 18 year old woman, who has dysuria and urinary frequency? A or B (see Figure A3-I)

**Answer key: B**

- 5) Which of the following is the most appropriate treatment plan for an 18 year old woman, who has dysuria and urinary frequency and the dipstick urine result shown above?
- A. send a urine specimen for culture and antimicrobial susceptibility testing and start treatment with nitrofurantoin
  - B. send a urine specimen for culture and susceptibility testing, and await the results prior to starting treatment
  - C. start treatment with nitrofurantoin and only send a urine for culture if symptoms do not resolve after 5 days
  - D. start treatment with nitrofurantoin and send a urine for culture after completion of treatment to see if the infection has been cured
  - E. start treatment with cranberry juice and only give antibiotics if symptoms don't resolve after 5 day

**Answer key: C**