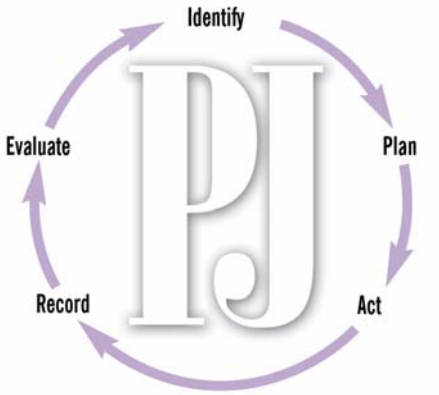
Tests on specimens of urine or stools

In this fourth article in a series on clinical testing, **Pamela Mason** focuses on common urine and faecal tests that pharmacists may encounter



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Identify knowledge gaps

1. List three substances that can be measured in urine.
2. Why is mid-stream urine collected?
3. List two tests performed on stools.

Before reading on, think about how this article may help you to do your job better. The Royal Pharmaceutical Society's areas of competence for pharmacists are listed in "Plan and record", (available at: www.rpsgb.org/education). This article relates to "diagnostic tests" and "therapeutic drug monitoring" (see appendix 4 of "Plan and record").

Examation of urine for signs of disease has been a diagnostic practice for many centuries. For example, according to Hippocrates blood or pus in the urine indicated ulceration either of the kidneys or of the bladder. Today, urine and stools can provide information not only about the kidneys, bladder and gastrointestinal tract, but also about a wide range of other conditions.

Urinalysis

Urine is produced by the kidneys to remove soluble waste substances from the body. These can be detected using dipstick methods (eg, tests for pregnancy or diabetic ketoacidosis) or, if more detailed information is required, urine can be sent for laboratory analysis.

Some substances can be measured in blood or urine (eg, glucose). The main advantage of tests on urine is that they are relatively non-invasive compared with blood tests. However, if the urine specimen is collected incorrectly, this can affect test results. The correct procedure for collection needs, therefore, to be explained clearly. It is important that any container used to collect a urine specimen is clean and contains no traces of detergent or disinfectant because these can affect the results of some tests. For example, a container contaminated with chlorhexidine can result in false positive results for protein. It is, therefore, best for the patient to be supplied with a container specifically for the purpose of specimen collection.

Pharmacists need to be sensitive to the fact that people can have different attitudes towards handling or talking about body fluids and it is important for privacy to be maintained.

Midstream specimens Most tests require that the urine specimen is collected "midstream" (also known as "clean-catch" specimens). This is so that the specimen is not contaminated by bacteria surrounding the urethra — often, these bacteria can be the same as those causing a urinary tract infection (UTI), so can result in a false positive result. First, the skin around the urethra must be cleaned. The patient then urinates, pauses, then urinates again into the specimen container.

Not everyone is able to collect their own specimen, particularly if a midstream specimen is required. Parents may need to help a child hold the container (urine collection bags are used for babies) and people with disabilities or illness may need the help of a carer. Gloves should be provided to protect the person collecting the specimen as well as to prevent cross contamination. In hospital, specimens may need to be collected from catheter tubing, using a sterile syringe. Urine from catheter bags can be several hours old and should not be used for testing.

Visual examination The appearance and odour of urine can also suggest health problems. Normal urine is a clear, straw-coloured fluid and, often, simply examining a specimen visually can provide evidence of an infection or disease. Like other bacterial infections, UTIs are associated with the recruitment of the white blood cells to the site of infection. Pus is formed, which causes urine to become cloudy. However, cloudy urine is not always a sign of a UTI and clear urine does not always rule out infection.

Patients who are jaundiced will have dark yellow, orange or brown urine because of the excess bile (often accompanied by pale stools and yellow skin). Blood can colour urine red (indicating disease) as can the rare, inherited disorder porphyria. Some foods, such as beetroot and asparagus, can also affect the colour of urine. Drugs that change urine colour include: levodopa (red), rifampicin (red), triamterene (blue-green) and vitamin B complex (dark yellow). Freshly voided urine has almost no smell, whereas infected urine has a fishy odour. Urine from people with anorexia can smell of pear drops.

Pharmacists asked for advice on abnormal urine colour should check that it is not diet-

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or drug-related. Change that cannot be clearly attributed to foods or medicines, or that is accompanied by unexplained symptoms, should be referred to a GP.

Dipstick and colour chart must be closely compared

Dipstick tests Reagent strips, or "dipsticks", are strips of plastic, with test areas treated with chemical reagents. For example, a Clinistix strip is impregnated with glucose oxidase and an indicator substance (*o*-toluidine) which is oxidised to various shades of blue-green, depending on the amount of glucose present. Most pharmacists will be familiar with the dipsticks used by people with diabetes who find blood glucose monitoring difficult (eg, Diabur-test 5000), dipsticks used to detect diabetes and those popular with people on the Atkins diet (eg, Ketostix, see *PJ,* 27 July 2002, pp135–7). Dipstick testing is simple, convenient and offers quick results. Tests can be performed at home, in surgeries or hospitals and in pharmacies.

Various dipsticks are available to detect substances such as glucose, protein and blood. Multiple reagent strips are also available. For example, Multistix 10 SG strips test for bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity and urobilinogen, to give a comprehensive urinalysis profile. However, not all of these reagent strips are prescribable on NHS prescriptions.

Pharmacists can advise patients, who need to test their urine regularly to follow the manufacturer's instructions on the storage of reagent strips as well as use. These should be kept in their original container (which often contains a dessicant) and the expiry date should be checked. Usually, reagent strips should be discarded six months after the container is opened. General instructions for testing are given in Panel 1.

If urine cannot be tested within an hour of urinating, it should be refridgerated. Prolonged exposure to room temperature can result in microbial contamination and this can affect test results (eg, bacterial consumption of glucose can give a false negative). However, samples should be allowed to return to room temperature before testing. Pharmacists can also advise patients on keeping records of their test results.

In addition to contamination, diet and medicines can also sometimes cause false negatives or false positives. For example, a high ascorbic acid concentration in urine can give false negative results with Clinistix. Drugs containing azo dyes (eg, nitrofurantoin) can also affect the readability of the reagent area.

Urinary tract infections Assuming that the urine has been collected appropriately (ie, it is not contaminated with the bacteria normally present in the lower third of the urethra or anogenital area), bacteria in the urine indicates a UTI. The dipstick method for detecting UTIs is based on the fact that all common bacteria causing UTIs convert nitrate to nitrite. Thus, an increase in urinary nitrite concentration indicates bacterial infection. In addition, the enzyme leucocyte esterase indicates the presence of white cells and detection of this enzyme provides further evidence of infection. Dipsticks for UTIs usually consist of at least two reagent squares, one for detecting nitrite and the other for detecting the enzyme.

Although convenient, these dipstick methods are limited by the number of false positives they generate so positive results should always be submitted for urine culture (which can take at least two days) to confirm, reliably, the presence of bacteria. A negative dipstick result, however, is strong evidence that the patient does not have a UTI.

Other conditions that dipstick tests can indicate are shown in Panel 2.

pH The body's control of urine pH is not as strict as its control of blood pH. Conditions associated with high or low pH are listed in Panel 2. The efficacy of some medicines is affected by acidic or alkaline environments so pH can be used to select the most appropriate treatment for a UTI. For example, strep-

Panel 1: Dipstick procedure

- Dip the reagent strip into the urine, making sure that the reagent area is completely immersed
- Take the strip out immediately, removing excess urine by gently tapping the strip against the side of the specimen container
- At a prescribed time after dipping the strip into the specimen (eg, 30 seconds for Diastix and 10 seconds for Clinistix), closely compare the colour of the test area with the colour chart provided
- After testing, urine can be disposed of in a toilet

Panel 2: Dipstick test results

Bilirubin The presence of bilirubin in urine can suggest biliary disease.

Blood Blood is not usually present in urine. Haematuria (blood in the urine) can be caused by renal conditions (such as glomerulonephritis), carcinoma and vasculitis (eg, endocarditis, systemic lupus erythmatosus or other connective tissue diseases). It can also be caused by infection (eg, cystitis, prostatitis and urethritis), bladder catheterisation, calculi and the use of cyclophosphamide.

Urine can also be examined for the presence of red and white blood cells under a microscope.

Glucose Glycosuria (sugar in the urine) can be caused by diabetes mellitus, pregnancy, sepsis or renal tubular damage (ie, abnormal renal absorption).

Ketones Ketones are breakdown products of fats and their presence in urine can indicate anorexia, dieting or diabetes that is poorly controlled.

Leucocytes A significant increase in white cell numbers is evidence of infection, which can be confirmed by urine culture.

Nitrites Nitrites in urine indicate infection. The first morning urine, or urine passed at least four hours after last urinating, is the best specimen to use.

pH Bacteria will usually increase the pH of urine because they break down urea to ammonia, which combines with hydrogen ions. In terms of monitoring acid-base imbalance in the body, serum pH is generally a better measure than urine pH.

Acidic urine is associated with uric acid and calcium oxalate stones. Alkaline urine is associated with calcium carbonate, calcium phosphate, and magnesium phosphate stones. Risks can, therefore, be lowered by modifying urine pH accordingly.

Ideally, pH tests should be performed immediately after urine collection. The specimen container must be covered to prevent the escape of carbon dioxide.

Protein Proteinuria (protein in the urine) can be caused by urinary tract infection, diabetes mellitus, glomerulonephritis, nephrosis, pyrexia and pregnancy. A morning specimen is best for detecting levels outside the reference range.

Specific gravity Increased urine specific gravity can indicate dehydration, diarrhoea, glucosuria, heart failure (decreased blood flow to the kidneys) or renal arterial stenosis. Decreased urine specific gravity can suggest excessive fluid intake, diabetes insipidus, glomerulonephritis or pyelonephritis.

Urobilinogen Small amounts of urobilinogen are usually present in urine, but raised levels suggest liver disease.

tomycin and neomycin are more effective in treating UTIs when urine is alkaline.

Specific gravity The specific gravity of urine is a measure of the amount of substances dissolved in the urine (g/ml). It primarily indicates how well the kidneys are functioning to adjust the amount of water in urine.

24-hour urine tests Urine is sometimes collected over 24 hours in order to measure a number of metabolites, such as calcium, creatinine, nitrogen, oxosteroids, potassium, sodium, urea and urate. The accuracy of such measurements depends, mainly, on the accuracy of the urine collection.

For a 24-hour urine collection required from 9am on Tuesday, the patient should be asked to empty the bladder completely at 9am and discard this specimen. Urine in the bladder at the start of the test must not be included in the collection. All urine passed after this point is collected. At 9am on Wednesday the bladder should be emptied completely and this final specimen added to the collection, which is given to the health care professional carrying out the analysis. Analysis must be performed within one hour of collection.

Stool tests

A lot of information about a person's diet and general state of health can be gained from stools. For example, a change in dietary habits can lead to an increase in the amount of gas produced by gastrointestinal (GI) bacteria, as can acute GI infections (increased gas in the intestines due to the rapid movement of food through the gut) and excessive flatus and stools that float are generally associated with these conditions. Floating stools can, however, also be associated with malabsorption syndromes. Increased levels of nutrients in the stool (due to malabsorption in the GI tract) are supplied to GI bacteria, which produce more gas.

Bile salts (made by the liver) give stool its a normal brown colour. Cholestasis or liver infections like viral hepatitis may produce clay coloured stools. Pharmacists asked for advice on a change in stool characteristics should refer patients to a GP if the change has persisted for more than two weeks or if blood, fever, or dizziness accompanies these changes. It is likely that the patient will be asked to collect a stool sample for further investigation.

Commonly, stool tests look for blood, urobilinogen and fat or infection. For example, a faecal occult blood (FOB) test can be requested if a patient has unexplained anaemia. If a parasitic infestation is suspected (eg, in prolonged diarrhoea of unknown cause or other intestinal symptoms) stools are examined for parasites and ova.

Less common stool tests include the detection of proteolytic enzymes (trypsin and chymotrypsin) in young children suspected of having cystic fibrosis. Another example is a stool test to detect toxins produced by *Clostridium difficile*, particularly in hospital patients when there is a suspicion that diarrhoea is the result of antibiotic use. This bacterium grows excessively when antibiotics alter the balance of colonic bacterial flora and can cause pseudomembranous colitis.

Recently, a stool antigen test for *Helicobacter pylori* infection has become available.

Microscopy and stool cultures Stool specimens can be examined for the presence of GI infective organisms, such as those causing food poisoning (eg, *Salmonella* species, *Staphylococcus aureus* and *Clostridium botulinum*), shigella, cholera and giardia. A small amount of stool is smeared on to a microscope slide and a Gram stain is performed. The stained smear is then examined under the microscope for the presence of bacteria. The colour, size and shape of cells allow identification of infecting organisms.

Infections can also be identified by placing a small stool sample in culture media and observing bacterial growth.

Faecal occult blood Blood in the stool can come from anywhere along the digestive tract, from the mouth to the anus. Some conditions, such as GI ulcers, are associated with heavy bleeding. This can result in stools being black or "tarry". A black stool usually means that the blood has come from the upper part of the GI tract (ie, the oesophagus, stomach or duodenum) because exposure to digestive juices turns the blood black. Stomach or duodenal ulcers caused by non-steroidal anti-inflammatory drugs are common causes of upper GI bleeds. Other causes include gastritis and oesophageal varices. Taking iron supplements or bismuth-containing medicines (such as Pepto-Bismol) can also blacken stools.

Bleeding in the lower GI tract can result in maroon or bright red, bloody stools. Causes include haemorrhoids, anal fissures, diverticular bleeding, bacterial enterocolitis, inflammatory bowel disease, colon polyps and colon cancer. People with such symptoms must be referred to their GP.

Small amounts of blood in stools may not be visible but an FOB test should detect this. FOB tests are mainly used to screen for colorectal cancers and polyps. Such tests are also used in people with persistent abdominal symptoms (eg, pain). There are two types of FOB test. The traditional guaiac smear test involves smearing a sample onto a card, adding a testing solution and observing a colour change (green). Flushable reagent pads are also now available. These are convenient for home use and there is no stool handling. Many health care providers, however, favour guaiac tests because these were used in the large studies that have shown the benefits of colon cancer screening.

Although safe and inexpensive, FOB tests are limited by the fact that colorectal cancers bleed intermittently so tests can give false negatives. Large amounts of vitamin C can also cause false-negative results. Moreover, other lesions can cause blood to be present in stools (even bleeding gums following a dental procedure), so the test is non-specific for colorectal cancer and positive results are bound to cause anxiety. Oral iron preparations can also give false positive results. In addition to NSAIDs, drugs that can cause GI bleeding include anticoagulants, colchicine and corticosteroids. Other factors that can cause inaccurate FOB test results include eating red meat, fish, turnips or horseradish within three days of the test.

Screening of asymptomatic people over 45 years of age shows that 2 per cent of people test positive. Of these, one in 10 will have a carcinoma and one in three an adenoma. A series of two or three samples taken over several days may be a more definite way of detecting bleeding in the gut, so sometimes a patient might be asked to supply three stool samples for laboratory testing. It should be remembered that the earlier that diseases, like bowel cancer, are detected the better the prognosis. Some health care professionals recommend that all people over 50 years old should have an annual test for FOB.

Faecal urobilinogen Urobilinogen is produced in the small intestine by the action of the intestinal bacteria on bile and it is the compound which gives the stool its brown colour. Increased faecal urobilinogen levels are found where there is increased haemolysis of red blood cells. Decreased levels suggest obstructive biliary disease.

Faecal fat Tests for faecal fat are used to help diagnose malabsorption syndromes (eg, pancreatic disease with a deficiency of lipase and biliary obstruction). If there is steatorrhoea (excess fat in the stools), stools will be frothy, foul smelling and greasy. This indicates that fats are not being digested.

It is a misconception that floating stools are caused by an increase in the fat content of the stool.

Action: practice points

Reading is only one way to undertake CPD and the Society will expect to see various approaches in a pharmacist's CPD portfolio.

1. Familiarise yourself with the range of dipsticks available and how they should be used.
2. Revise the symptoms of bowel cancer and consider how you would advise a patient with a change in bowel habit or stool appearance.
3. Make a list of the drugs that can alter the colour of urine or stools and be sure to counsel patients about this.

Evaluate

For your work to be presented as CPD, you need to evaluate your reading and any other activities. Answer the following questions: What have you learnt? How has it added value to your practice? (Have you applied this learning or had any feedback?) What will you do now and how will this be achieved?