

ACTIVITY BRIEF

Microbiology and the pathology service

The science at work

The NHS pathology service is an essential organisation that assists doctors to diagnose and treat illness. Clinical pathologists test tissue and body fluid taken from patients for abnormalities and infection. Examples include cancer, HIV and food poisoning. Other kinds of tests include checking a patient's blood group for an emergency blood transfusion, testing for a suspected drugs overdose or a heart attack. The pathology department can also help monitor the treatment of patients.

Scientists and technicians working for the pathology service have to be trained in and employ a wide range of practical skills. It is essential that their work is carried out to the highest standards of accuracy and with the strictest regard to health and safety issues. With lives at stake, they must be highly technically competent.

Your brief

You will conduct some research to find out about and report on the role and activities of the NHS pathology service. You will then carry out practical microbiological investigations based closely on those carried out by microbiologists working in the pathology service.

Task 1 Investigating the pathology service

You will find out about how the pathology service is structured, the kind of work the scientists do and how you might train to work in a pathology department.

Use *Study sheet: The pathology service*.

Task 2 Identifying bacteria

In this practical you will find out about the basic concepts of identifying bacteria.

Use *Practical sheet: Identifying bacteria* and *Information Sheet: Working without contamination*

Task 3 Diagnosing bacterial infections

You will find out how microbiologists routinely test patient samples for bacterial infections.

Use *Practical sheet: General bacterial infections* and *Information Sheet: Working without contamination*

STUDY SHEET

The pathology service

Use this study sheet to find out about the pathology service. Write notes as you carry out your research. You will need to write up a report from your findings.

Useful resources

CPA (Clinical Pathology Accreditation): <http://www.cpa-uk.co.uk/>

Health Professionals Council: <http://www.hpc-uk.org/>

Association of Clinical Microbiologists: <http://www.aclinmicrobiol.org.uk/>

Standard procedures for processing different specimens can be downloaded from the Health Protection agencies website: http://www.hpa-standardmethods.org.uk/pdf_sops.asp

The Institute of Biomedical Science (IBMS):
<http://www.ibms.org/index.cfm?method=site.home>

Data protection:
<http://www.dh.gov.uk/en/Policyandguidance/PolicyAtoZ/index.htm?indexChar=D>

NHS Careers for Scientists in Health Care:
<http://www.nhscareers.nhs.uk/details/Default.aspx?Id=268>

The structure and role of the pathology service

NHS pathology laboratories are usually based in hospitals, but sometimes smaller specialist laboratories outside hospitals are also used. Occasionally, private laboratories are contracted to do work for the NHS.

Find a local pathology laboratory. You might search your local district hospital's website. Smaller hospitals may have limited provision, so choose somewhere with a large department. Alternatively try searching the CPA (Clinical Pathology Accreditation) website <http://www.cpa-uk.co.uk/>.

Find out the information required by the unit specification. This includes:

- What departments are found in the pathology laboratory?
- What is the role of each department?
- What types of specimens does each department analyse?
- What stages of processing do the specimens go through?
- What standard procedures are in common use in each department?
- What is involved in carrying out these procedures?
- What safety measures and legislation are in force?

Use the tasks in the following sections to get you started.

Departments

There are many disciplines within pathology and almost all pathology laboratories have several different departments. Four key departments are Biochemistry, Haematology, Microbiology and Pathology. There are others and you will find some variation in the way different hospital laboratories are organised and run.

Question 1

Department functions

(a) Draw lines to match each of these disciplines to its area of expertise.

Discipline	Area of expertise
Biochemistry	micro-organisms
Blood transfusion	matching blood groups
Cytology	poisons and their effect on living organisms
Endocrinology	chemistry of living organisms
Haematology	hormones
Histology	biological phenomena at the molecular level
Immunology	blood and its formation
Microbiology	detailed structure of living tissue
Molecular biology	viruses
Toxicology	structure, functions and life history of cells
Virology	the immune system

(b) Construct a table for the pathology department that you are investigating, for example:

Department	Disciplines	Specimens tested	Tested for
Biochemistry			
Haematology			
Microbiology			
Histopathology			

(i) In your table, list the disciplines found in each department.

HINT: you can use the CPA's (Clinical Pathology Accreditation) website to see which disciplines belong to which department.

(ii) Which of these specimens are tested by each department? Add your answers to the table. Include brief descriptions about what is tested for in each specimen and the purpose of the test (the kind of things that can be determined).

· blood · urine · faeces · sputum · cerebrospinal fluids · serum · biopsy tissue · blood proteins

(NB: Different departments may test the same specimens, but for different purposes.)

Question 3

Processing

Read the four case studies of patients:

Case study 1

Jo goes to her local GP because she is finding it painful to urinate. The doctor suggests she may have a bladder infection and asks her for a specimen of urine.

Case study 2

Phil is booked in for a knee operation. A week before the operation is due, the hospital takes a sample of blood to check his blood type.

Case study 3

Hannah's doctor is concerned that her diabetes is under control. She undergoes a glucose tolerance test at the hospital.

Case study 4

An elderly man has been found dead in his home. The cause of death is unknown, but a heart attack is suspected. A pathologist is asked to perform an autopsy. They take a sample of artery tissue to check for atheroma (hardening/furring of artery walls that may indicate he died from a heart attack/stroke).

For each case study, describe how the specimen mentioned is processed. Include:

- health and safety considerations
- how the specimen is collected and stored/transported,
- the tests that are carried out on the specimen
- how the specimen is recorded/tracked through the process
- how results are processed
- the safeguards in place to protect patient information

Standard procedures for processing different specimens can be downloaded from the Health Protection Agency website.

http://www.hpa-standardmethods.org.uk/pdf_sops.asp

The scientists

The pathology service employs many kinds of healthcare scientists. Most are registered biomedical scientists, but there are also laboratory assistants and support workers.

The Institute of Biomedical Science (IBMS) is the professional body for biomedical scientists in the United Kingdom. All biomedical scientists must be registered with the Health Professionals Council (<http://www.hpc-uk.org/>).

Using the websites below find out how you might become a:

- biomedical scientist
- medical laboratory assistant
- clinical support worker.

Make sure you include:

- a description of how you could become qualified for each job
- some of the kinds of knowledge and skills required for each role
- a list of some of the day to day procedures that would be carried out by each scientist.

Useful websites

The Institute of Biomedical Science (IBMS):

http://www.ibms.org/index.cfm?method=education_and_careers.careers

NHS Careers:

<http://www.nhscareers.nhs.uk/details/Default.aspx?Id=213>

<http://www.nhscareers.nhs.uk/details/Default.aspx?Id=251>

Reporting your findings

This work will have helped you to find useful sources and to gather information for parts of this unit. To gain accreditation you will need to write a full report to meet unit criteria.

Check with your teacher what you need to do next.

Portfolio report

Write a report including:

- a description of the structure of the pathology service
- the role of different departments
- how specimens are processed including, health and safety considerations, how the specimen is collected and stored/transported, the tests that are carried out on the specimen, how the specimen is recorded/tracked through the process, how results are processed, the safeguards in place to protect patient information
- a brief outline of careers for scientists working in a pathology laboratory.

PRACTICAL SHEET

Identifying bacteria

Introduction

Microbiologists identify bacteria based upon a number of physical characteristics. They usually compare unknown samples with known reference cultures. A scientist's experience of specimens is usually the main aid to identification. For example, pathologists know which types of bacteria are normally associated with infections at a particular site of the body.

Identification

The most important investigations which help scientists identify bacteria include Gram staining, growth in the presence or absence of air, growth on various types of culture media and catalase and oxidase tests. This is normally enough to identify which medically important group the organism fits into.

Normally, identification relies heavily on the experience of the investigator, but a step-by-step approach can also be used. The first test that is normally carried out is a Gram stain to find out if the bacterium is **Gram-negative** or **Gram-positive**.

Use the standard procedure to identify:

Bacillus subtilis: a Gram-positive bacillus (rod shaped)

Escherichia coli K12: a Gram-negative bacillus (rod shaped)

Staphylococcus albus: a Gram-positive coccus (spherical)

Standard procedure: Gram's stain

1 Introduction

Bacteria can be classified into two groups according to their Gram-staining reaction based on the ability to retain a stain (positive or negative). First a preliminary stain (crystal violet) is added which turns all bacteria purple. Iodine is then added. Gram-positive bacteria have thicker and denser peptidoglycan layers in their cell walls. This means that when iodine is added, the preliminary stain is inhibited from leaving the Gram-positive cells. The stain is retained when a decolourising agent is added.

Gram-negative cells do not retain the preliminary stain. They are stained when a contrasting counterstain is added. Neutral red, safranin or carbol fuchsin may be used as the counterstain.

2 Health and safety

A risk assessment must be carried out before starting work. Wear disposable gloves and eye protection. Wash your hands with hot soapy water before the practical work. When the Bunsen burner is not being used, close the air hole so you can see a yellow flame and turn it off when you have finished the experiment.

Dispose of any waste that has come into contact with micro-organisms as directed by your teacher.

3 Gram's method for examination of smears

- a Prepare a smear.

Using aseptic technique, use an inoculation loop to spread a very small amount of inoculum on the centre of a microscope slide. You want a sparse single layer of bacteria, so do not add too much. Allow to air dry.

- b Heat gently to fix. This prevents the bacteria from washing away and kills the bacteria.

Holding the slide inoculum side up, pass the slide through a gentle blue Bunsen flame 2-3 times. Allow the slide to become warm, but not hot, to the touch.

- c Use a pipette to flood the slide with 0.5% crystal violet and leave for 30 seconds. This dye is taken up by both Gram-positive and Gram-negative bacteria, so at this point both types of bacteria are purple.
- d Hold the slide over a beaker. Tilt and gently add sufficient (1%) Lugol's iodine solution to wash away the stain. Cover with fresh iodine and allow to act for 30 seconds.

The Lugol's iodine solution (1% iodine, 2% potassium iodide in water) acts as a mordant and fixes the dye.

- e Tilt the slide over a beaker and using a pipette, gently wash off the iodine with 95-100% ethanol [HIGHLY FLAMMABLE] [HARMFUL] or acetone [HIGHLY FLAMMABLE] [IRRITANT] until colour ceases to run out of the smear. This washes away all the unbound crystal violet dye and leaves Gram-positive organisms stained purple and Gram-negative organisms unstained (colourless).

- f Rinse gently with distilled water immediately to prevent over-decolourisation.
- g Add a few drops of 0.1% counterstain [HIGHLY FLAMMABLE] (neutral red, safranin or carbol fuchsin) and leave to act for about 2 minutes.

This stain is taken up by both Gram-positive and Gram-negative organisms, but does not alter the colour of Gram-positive organism much, as they are already purple. It does, however, make the Gram-negative organisms pinkish-red.

- h Wash with distilled water, blot gently with filter paper and leave to dry. Do not smear.
- i View under the microscope.

4 Interpretation

Positive: Gram-positive organisms stain deep blue/purple.

Negative: Gram-negative organisms stain pink/red.

5 Quality control organisms

A culture containing known Gram-positive and Gram-negative organisms may be used for quality control to make sure you have done the staining correctly.

6 Results

Complete the table below to record your results

Sample	Shape of bacterium	Colour of bacteria after Gram stain	Gram-positive or Gram-negative	Identity of bacterium

Questions

- 1 Draw an annotated diagram of Gram-negative and Gram-positive bacteria to show the difference between them.
- 2 Why did the Gram-negative and Gram-positive bacteria stain differently during this procedure?

Portfolio report

Write a report including:

- details of the standard procedure used
- risk assessments undertaken
- how health and safety principles were adhered to including, avoiding contamination, sterility, work procedures, healthy and safe handling and disposal of specimens

- all observations and measurements
- an interpretation, explanation and evaluation of the results obtained.

Portfolio report

Compare the procedure you used with how it would have been undertaken in a hospital laboratory.

The method you used here was adapted from the following Health Protection Agency Standard Operating procedures:

BSOP ID 1 Introduction to the preliminary identification of medically important bacteria

BSOP TP 39 Staining procedures

Both can be downloaded from http://www.hpa-standardmethods.org.uk/pdf_sops.asp using the search function at the top of the page.

Look at these procedures and, with other considerations, compare differences between colleges and professional laboratories in terms of:

- health and safety guidelines
- equipment
- number of samples that have to be tested.

PRACTICAL SHEET

General bacterial infections

Introduction

Microbiologists working in the pathology service routinely test patient samples for bacterial infections. For most patients it is enough to identify the general type of bacterium, for example *E. coli* or *Streptococcus sp.* but the specific strain isn't important. The patient's samples are then checked for antibiotic sensitivity so that treatment can be recommended. In a hospital laboratory, microbiologists will test many samples at the same time. In this case study you will carry out the procedure for one sample.

Resources

MISAC The Microbiology in Schools Advisory Committee:

<http://www.microbiologyonline.org.uk/misac.html>

Health protection agency:

http://www.hpa-standardmethods.org.uk/pdf_sops.asp

British Society for Antimicrobial Chemotherapy (BSAC), Susceptibility Testing Guide:

http://www.bsac.org.uk/susceptibility_testing/guide_to_antimicrobial_susceptibility_testing.cfm see Chapter 5, *BSAC standardized disc susceptibility testing method* (Version 6) April 2007

Investigation of throat swabs

Geraldine has been suffering from a sore throat for several days; she has a temperature and feels really ill. The doctor takes a throat swab and sends it to the local pathology laboratory for testing.

Here is an outline of the method used in the pathology laboratory:

- 1 Inoculate a nutrient agar plate with the throat swab and incubate for 12-24 hours.
- 2 Subculture a colony in nutrient broth and incubate for 12-24 hours.
- 3 Carry out an antibiotic susceptibility test and incubate for 24 – 48 hours.
- 4 Complete a report to send back to the doctor.

You will be provided with a swab of a 'safe bacteria' prepared by your technician/teacher. However, you should treat the swab as though you were receiving a genuine patient specimen.

Standard procedure: Susceptibility testing

1 Health and safety

A risk assessment must be carried out before starting work. It is important you work in as sterile a manner as possible. Wear eye protection and gloves. When the Bunsen burner is not being used, close the air hole so you can see a yellow flame and turn it off when you have finished the experiment. All micro-organisms present in a sample multiply during incubation, including any harmful ones.

Dispose of any waste that has come into contact with micro-organisms as directed by your teacher.

2 Specimen processing

Media preparation

Prepare nutrient broth and nutrient agar.

Both nutrient agar and nutrient broth are general purpose media used in the cultivation of micro-organisms. Some more sensitive micro-organisms require more specialist media. The main ingredients of nutrient agar and broth are Beef extract and Peptone which supply a quantity of carbon, nitrogen and vitamins for the growth of most non-fastidious micro-organisms (organisms without special nutrient requirements).

Preparation of nutrient broth and nutrient agar

Nutrient agar and broth can be purchased from a number of suppliers including Philip Harris and Scientific and Chemical. You should follow the instructions that come with the media, but a general method for preparing 1.5% nutrient agar or broth from powder is as follows.

- a Suspend 1.5 g of powder in 100 cm³ of distilled/deionised water. Dissolve the agar medium by heating to a boil and stirring. **WARNING:** Agar can easily boil over and is liable to spurt. Heat in a water bath, NOT over a Bunsen flame. Never add agar to boiling water.
- b Dispense about 25 cm³ of the medium into sterile Universal/McCartney bottles ready to dispense onto plates (agar) or use directly (broth).
- c Sterilise the medium in an autoclave at 121 °C for 15 minutes. Do not sterilise agar for longer than recommended by the supplier as it will adversely affect the agar.

STORAGE

- Store dry medium at room temperature (15-30 °C). The dehydrated powder should be a light tan colour, homogeneous and free-flowing. Deterioration of powdered medium may be recognized by (a) granulation, clumping, or particulate matter throughout the powder, (b) pH change, (c) inability to promote growth when properly used.
- Prepared plates: 1 month at 2-8 °C.
- Prepared tubes and flasks: 3 months at 2-8 °C.

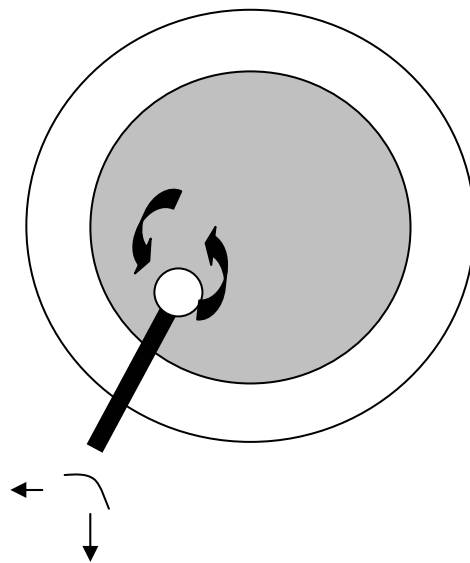
Preparation of agar plates

To prepare nutrient agar plates you need work using aseptic technique. The information sheet *Working without contamination* provides information on how to do this.

- a Arrange your workspace so you have a Bunsen burner with a blue flame and a sterile Petri dish in easy reach (do not open the Petri dish yet).
- b Collect a bottle of sterile liquid agar from the water bath. Dry the outside as you remove it.
- c Flame the glass rim of the bottle before pouring the molten agar into the Petri dish to give a depth of about 4 mm (about 25 cm³ agar). Open the dish for as little time as possible and hold the lid above the base.
- d Gently rotate the dish on the work top to spread the agar and allow to set on a level surface. Thin or uneven plates will affect your experiments.
- e Dry the plates until the surface of the agar is free of visible surface moisture but do not over dry. If not immediately required, store plates in sealed sterile bags to prevent over drying.

Inoculating agar plates

- a All culture plates and containers must be labelled to identify the patient name or laboratory number or barcode. Include the date of this primary culture.
- b Identify the area of a plate to inoculate:



- c Using aseptic technique, roll the swab over the inoculation area to maximise transfer of organisms, taking care to avoid the edges of the plate.
- d Incubate your plate for 12-24 hours at 20-25 °C.

To carry out your experiment you need to grow the bacteria evenly over the agar plate. Bacteria which grow so that they form a continuous layer over the plate will stop growing and are called confluent. You do not want this to happen, they need to be semi-confluent. In other words, evenly distributed but still with space to grow.

Sub-culturing

With the exception of urine, direct susceptibility testing (testing for resistance to antibiotics) from a specimen is not recommended. This is because pure semi-confluent

growth is unlikely to be obtained. Instead a bacteria colony from the specimen grown on the initial agar plate is selected for sub-culturing. One colony is grown in nutrient broth. The bacteria in the nutrient broth are then plated again for the susceptibility test.

- a Select a representative colony or colonies of the organism to be sub-cultured. Transfer to nutrient broth with a sterile wire or loop, using aseptic technique. Gently agitate before incubation to distribute the organisms throughout the broth.
- b Incubate overnight (12-24 hours at 20–25 °C).

Inoculation for susceptibility testing

- a Dip a dry sterile cotton swab in the broth suspension and remove excess fluid by turning the swab against the inside of the tube.
- b Spread the inoculum evenly over the surface of the agar plate by swabbing in three different directions. Make sure you go close to the edges, but do not touch the Petri dish with the swab. You need to make sure that you will get even growth without streaks on the plate. Before applying discs, allow the plate to dry until there is no visible surface moisture. This will probably take 5-10 minutes, but if it takes more than 15 minutes you have added too much fluid.

Antibiotic application

- a Use cooled flamed forceps to place a mast ring (or antibiotic discs) on plates. Apply to plates within 15 minutes of inoculation, before organisms have begun to grow too much. All parts of the mast disc should make even contact with the plate. Use the forceps to press down discs if necessary.

NB: The antibiotics will begin to diffuse from the disc into the agar as soon as contact is made, so do not move the discs once they have touched the agar.

- b Incubate plates at 25 °C for 24-48 hours.

3 Reading results

It is not necessary to remove the lid of plates to read results. If the bacteria were susceptible to an antibiotic there will be a circular zone of inhibited growth around the antibiotic disc.

Measure zones of inhibition from the underside of the plate using a ruler.

If zones of inhibition are not circular, it is possible that adjacent antibiotics interfered with each other. The test will need to be repeated.

Record your findings appropriately.

4 Reporting

Report as: susceptible, intermediate or resistant

5 Write up your findings

You need to include:

- 1 details of the standard procedure used
- 2 risk assessments undertaken

- 3 how health and safety principles were adhered to including, avoiding contamination, sterility, work procedures, safe handling and disposal of specimens
- 4 all observations and measurements
- 5 an interpretation, explanation and evaluation of the results obtained.

6 Comparison with professional practice

You also need to compare the procedure you used with how it would have been undertaken in a hospital laboratory. The method you used here was adapted from the following Health Protection Agency Standard Operating procedures:

BSOP 54 Inoculation of culture media

BSOP 9 Investigation of throat swabs

BSOP 45 Susceptibility testing

All can be downloaded from http://www.hpa-standardmethods.org.uk/pdf_sops.asp using the search function at the top of the page.

Look at these procedures and also think about differences between colleges and professional laboratories in terms of:

- health and safety guidelines
- equipment
- number of samples that have to be tested.

Questions

- 1 How is a throat swab normally collected, stored and transported to the laboratory?
- 2 Why is the sample sub-cultured before the susceptibility test?
- 3 How does a pathologist decide which antibiotics to test against a patient's sample?
- 4 For a patient that is on a long term course of antibiotics, why might it be necessary to test different concentrations of an antibiotic?
- 5 This procedure probably took you about five days to complete. In a hospital laboratory, results can be completed quicker because incubation times are less. Why is this, and why could you not have followed the same method?

INFORMATION SHEET

Working without contamination

Aseptic technique is the most important skill a microbiologist needs to learn. Using aseptic technique makes it unlikely that samples are contaminated with micro-organisms from the environment (in the air or on surfaces) and the micro-organisms being studied do not escape to cause infection.

Basic guidelines

- Wear gloves and a clean laboratory coat (one which stays in the laboratory).
- Before beginning work make sure your work place is clean (use an appropriate disinfectant spray or wipe over surfaces using a suitable disinfectant cleaner).
- Work quickly but carefully to reduce the time specimens are open/out of incubators.
- Avoid working in draughts or next to open windows, where micro-organisms may blow across your work place.

Aseptic technique

- Sterilise equipment in an autoclave before use or use disposable sterile equipment.
- Once removed from its packet, avoid contaminating sterile equipment (for example do not put down on the laboratory bench or walk around the room with it).
- If sterile equipment accidentally touches a non-sterile surface (such as the outside of a bottle) discard it and start again with a sterile item.
- When removing caps and lids from containers they should not be placed on the workbench, but retained in the hand whilst the sample is being processed, taking care not to contaminate the hand or cap. If possible, hold the lid the right way up. Caps and lids should be replaced as soon as possible.
- If the work is being carried out on the open bench, a Bunsen burner should be in close proximity to flame loops, wires or bottle necks.
- When opening culture containers, keep samples away from the face.

Airborne contamination should be minimised by:

- opening the caps slowly as the contents are sometimes under pressure
- avoiding vigorous swirling or shaking of the sample prior to opening
- cooling loops that have been heated before use (to prevent spluttering)
- avoiding expelling the last drop from a pipette
- if forceps or scissors are used when handling specimens, they should be heated in a Bunsen flame and allowed to cool before use
- loops and wires should be heated in the Bunsen flame with the loop end downwards until red-hot, then removed and allowed to cool. Care should be taken to see that the loop

does not contain fluid or large particles of matter that may 'splutter' when placed into the flame, and that the entire loop and wire up to the loop holder is heated to red-heat.

- Petri dish lids should only be removed when necessary and for the minimum time only. Lids should be held over the dish during transfer operations.
- Seal agar plates with four pieces of sticky tape placed vertically over the rim. Do not totally seal around the rim as this can lead to the growth of more dangerous anaerobic bacteria. Store the plates upside down to prevent moisture build up.

Disposal

Treat all cultures as potentially pathogenic. All waste that has come into contact with micro-organisms should be classed as a biological hazard. It will need to be sterilised before disposal. Follow your laboratory guidelines for disposal.

Teacher notes

This activity links to **AQA A2 Unit 15 The Role of the Pathology Service**. Relevant sections of the specification include:

You will need to produce a portfolio of evidence which considers the work undertaken by the following departments in the pathology service: biochemistry, haematology, microbiology and histopathology. You will then undertake a microbiological analysis, and either a chromatographic or electrophoresis analysis.

Your portfolio of evidence should comprise:

A. a report on the work of the following departments in the pathology service: biochemistry, haematology, microbiology and histopathology, including:

- *an overview of the role of each department*
- *the types of specimens tested by each department, for example urine, faeces, blood, sputum, cerebrospinal fluids, serum and tissues*
- *details of the stages involved in the processing of specimens, for example the sending and receipt of specimens, recording, sorting, storage, testing, noting of results, interpretation of results, dissemination of results, use of computers and the Data Protection Act*
- *the importance of health and safety principles relating to, for example, avoidance of contamination (self, others, environment, specimens), sterility (equipment, medium, work environment), training, work procedures (testing, maintenance of equipment, cleaning of equipment), hazard analysis, risk assessment, safe handling of specimens and safe disposal*
- *the role of legislation in maintaining health and safety principles*
- *details of the knowledge and skills used by people employed in the biochemistry and microbiology departments of the pathology service.*

B. a report of:

- *a microbiological analysis,*
- *and either a chromatographic or electrophoresis analysis that you have undertaken, which includes:*

- *details of the standard procedure followed*
- *details of the risk assessments undertaken*
- *how health and safety principles were adhered to including, where appropriate, avoidance of contamination, sterility, work procedures, safe handling and disposal of specimens*
- *a record of qualitative observations and quantitative measurements*
- *an interpretation, explanation and evaluation of the results obtained*
- *an evaluation of the procedure you followed in each case. This should include comparing how each procedure was undertaken in a school/college laboratory and how it would have been undertaken in a hospital department.*

Organisation

Study sheet: The pathology service can be used to help students begin collecting evidence for part A of their portfolio evidence on the work of the different pathology departments. Students might work in teams to research different areas. However, they will each need to write up their findings separately. They will need additional guidance to write a report to meet the assessment criteria in full.

There are two practicals: *Practical sheet: Identifying bacteria* and *Practical sheet: General bacterial infections* which can be used by students to provide evidence for part B (a report of a microbiological analysis).

Both these practicals assume students have prior knowledge of aseptic techniques. *Information sheet: Working without contamination* provides guidance to remind students about aseptic technique. If they have no prior knowledge of aseptic technique you will need to demonstrate the techniques and let them practice before they begin these practicals. There is very detailed guidance in the CLEAPSS laboratory handbook on how students should carry out aseptic technique (Edition: Autumn 2006 pg 1522 – 1526).

Task 1 Investigating the pathology service

Students can start the unit by getting a feel for what the pathology service is and how it works. Most general hospital websites have a webpage for their pathology service, which can provide useful information. They may like to look at a few different ones around the country.

A virtual tour of Derby Hospital's pathology laboratory can be downloaded from:

http://www.bbc.co.uk/derby/content/panoramas/derby_hospital_pathology_lab_360.shtml

Some pathology departments may allow small groups of A-level students to visit their laboratories. To avoid alienation of potentially useful contacts, students should be advised that all contact with external bodies must be made through their teacher. A directory of microbiology laboratories along with contacts can be downloaded from the Department of Health website:

http://www.dh.gov.uk/en/Policyandguidance/Healthandsocialcaretopics/Microbiologyandinfectioncontrol/DH_4135669

Laboratories with connections to universities can sometimes be more amenable to student visits.

Departments

Although the specification states that students should find out about the biochemistry, haematology, microbiology and histopathology departments of a pathology laboratory, local organisations vary and some work may be undertaken by outside specialist laboratories. The examination board can be approached for advice if there are any difficulties meeting the requirements of the specification verbatim.

Answers

Students will need to give detailed answers for portfolio assessment. The answers given here are only a starting point for their research.

Department	Disciplines	Specimens
Biochemistry	Biochemistry (<i>the chemistry of living organisms</i>) Toxicology (<i>poisons and their effect on living organisms</i>) Endocrinology (<i>hormones</i>) Immunology* (<i>immune system</i>)	Blood and urine (identification of proteins/enzymes/antibodies in the blood, which may indicate a particular disease; measurement of glucose, cholesterol, sodium and potassium levels)

	Molecular biology* (<i>biological phenomena at the molecular level</i>)	
Haematology	Blood transfusion (<i>matching blood groups</i>) Haematology (<i>blood and its formation</i>)	Blood and serum (cross-matching blood groups; checking components of the blood are present in the correct amounts)
Microbiology	Virology (<i>viruses</i>) Microbiology (<i>micro-organisms</i>)	Blood, urine, faeces and sputum (screened for micro-organisms)
Histopathology	Cytology (<i>structure, functions and life history of cells</i>) Histology (<i>detailed structure of living tissue</i>)	Cerebrospinal fluids, biopsy tissue and blood (checked for abnormalities such as cancer cells)
*sometimes found in other departments		

The scientists

Students need to know about the skills and knowledge used by scientists employed in the biochemistry and microbiology departments of the pathology department. By looking at careers in these areas they will begin to piece together information.

After carrying out some initial research you might ask students to collect together information and discuss what they found out in class.

Students might like to discuss topics such as:

- which disciplines they find most interesting
- the positives and negatives of such a career
- how difficult they think it is to obtain the necessary skills and knowledge

Other resources

On the Institute of Biomedical Science (IBMS) website, under careers, under *Further information*, there is a link to 'Careers in biomedical science and pathology: leaflets and publications'. This power point presentation might be downloaded for student use.

Task 2 Identifying bacteria

This activity provides students with an opportunity to write a report of a microbiological analysis for their portfolio assessment. The procedure is based upon standard operating procedures (SOP's) from the Health Protection Agency:

BSOP ID 1: Introduction to the preliminary identification of medically important bacteria

BSOP TP 39: Staining procedures

Both can be downloaded from http://www.hpa-standardmethods.org.uk/pdf_sops.asp

These SOP's are written as national standards for clinical microbiologists (i.e. those working in pathology laboratories). To gain experience, ideally students will carry out this practical individually, but they might work in pairs to share microscopes if equipment is limited.

Students might test and distinguish three unknown samples such as:

Bacillus subtilis: a Gram-positive rod

Escherichia coli K12: a Gram-negative rod

Staphylococcus albus: a Gram-positive coccus

Nutrient agar containing bacterial cultures of *Bacillus subtilis*, *Escherichia coli* K12 and *Staphylococcus albus* will need to be prepared at least 24 hours prior to the lesson.

Health and safety

A risk assessment should be carried out before starting work. Students should wear gloves and clean lab coats. Health and safety guidelines should be followed from the CLEAPSS Laboratory Handbook: see Table 15.2 of section 15.2 Microbiology available on CLEAPSS Science Publications CD-ROM (updated annually).

Equipment and materials

- microscope with high power (x400)
- microscope slides
- prepared slides of stained Gram-negative and -positive bacteria (control, for comparison)
- cover slips
- tweezers
- Bunsen burner
- heat mat
- inoculation loop
- gloves
- 4 pipettes
- beakers
- bacterial cultures of *Bacillus subtilis*, *Escherichia coli* K12 and *Staphylococcus albus*
- 0.5% methyl/crystal violet dye
- 1% Lugol's iodine
- 95% ethanol [HIGHLY FLAMABLE] [HARMFUL]
- distilled water
- 0.1% neutral red , safranin or carbol fuchsin [solutions may be HIGHLY FLAMMABLE]
- filter paper
- tissues
- disinfectant spray for cleaning surfaces
- disinfectant (VirKon)
- beakers
- bags for collecting contaminated waste

Task 3 Diagnosing bacterial infections

This activity provides students with an opportunity to write a report of a microbiological analysis for their portfolio assessment. The procedure is based upon several standard operating procedures (SOP's) from the Health Protection Agency:

BSOP 54 Inoculation of culture media

BSOP 9 Investigation of throat swabs

BSOP 45 Susceptibility testing

Available from http://www.hpa-standardmethods.org.uk/pdf_sops.asp

These SOP's are written as national standards for clinical microbiologists (i.e. those working in pathology laboratories) to use. Ideally students will carry out this practical individually. This helps avoid contamination and is how microbiologists would realistically work. The practical assumes students already have a high level of knowledge and skill for working aseptically.

Students are given a throat swab that you should prepare containing bacteria from a known culture (one that is recommended for use in school/college). However, the experiment can be run with students imagining they are dealing with a real patient sample.

Health and safety

A risk assessment should be carried out before starting work. Students should wear gloves and clean lab coats.

Health and safety guidelines should be followed from the CLEAPSS Laboratory Handbook section 15.2 Microbiology (available on the CLEAPSS Science Publication CD-ROM which is updated annually). This practical should ONLY be used with POST-16 students who have a high level of skill in aseptic technique. It is advisable for all students to practise aseptic technique before this practical and if necessary receive training to get them to a high standard.

See also:

Practical Microbiology and Biotechnology for Schools, The Society for General Microbiology (SGM), MacDonald Educational

Microbiology On-line The Microbiology in Schools Advisory Committee (MISAC)
<http://www.microbiologyonline.org.uk/misac.html>

Micro-organisms for investigations in schools and colleges MISAC / SGM
<http://www.microbiologyonline.org.uk/forms/BPMList.pdf>

The greater potential hazard during this procedure is that a contaminated plate or bottle is opened. At each stage of the practical, all plates or bottles must be checked by a teacher or technician for contamination. If contamination is suspected they should NOT be given back to students to open. Such cultures should be autoclaved and disposed of unopened.

Advice for arranging the classroom:

- keep windows and doors shut throughout the lesson to avoid draughts

- make sure all students have everything at their desks before anyone begins work to avoid people walking around the classroom
- classrooms should not be overcrowded
- make sure the workspaces are cleaned before and after the lesson
- work surfaces should be cleaned with a disinfectant cleaner.

Possible timescale

Day 1	Prepare nutrient agar and nutrient broth Pour agar plates Inoculate plates with patient swab (swab really contains known bacteria from culture)
Day 2	Subculture a colony grown on agar into nutrient broth.
Day 3	Inoculate agar plates with bacteria grown in nutrient broth. Apply mast ring/antibiotic disc
Day 4/5	Read results

Suitable bacteria

Several bacterial cultures that are generally used in schools such as *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus albus* will be inhibited by most antibiotics available to you. Therefore, they are not suitable if you want to compare susceptibility and resistance.

E. coli strains K12 or B are both safe to use in school and are resistant to some of the antibiotics available from the educational suppliers (Philip Harris or Scientific and Chemical). Please see the below for their antibiotic resistance (growth seen even when antibiotic present) and susceptibility (growth inhibited).

Mast ring antibiotics	<i>Escherichia coli</i> K12	<i>Escherichia coli</i> B
Chloramphenicol	Susceptible	Susceptible
Erythromycin	Resistant	Susceptible
Fusidic acid	Resistant	Resistant
Methicillin	Resistant	Resistant
Novobiocin	Resistant	Resistant
Penicillin G	Resistant	Resistant
Streptomycin	Susceptible	Susceptible
Tetracycline	Susceptible	Susceptible

Equipment and materials

Equipment and materials can be purchased from Beecroft and Partners, Philip Harris or Scientific and Chemical.

- nutrient agar (powder)
- nutrient broth (powder)
- distilled/deionised water
- antibiotic mast rings
- *E. coli* K12 or B bacterial cultures (Available from Beecroft and partners <http://www.beecroft-science.co.uk/home/>)
- refrigerator
- autoclave or pressure cooker and autoclave tape
- balance (accurate to 0.1 g)
- water bath
- incubator (or suitable area for incubation)
- Universal or McCartney bottles (glass)
- weighing boats
- Petri dishes
- Bunsen burner
- sterile cotton wool swabs (these can be wrapped individually in silver foil or placed in a Universal bottle and sterilised in an autoclave/pressure cooker). Sterile swabs can be bought. Cosmetic cotton buds are not suitable as they often contain anti-microbial agents.
- disposable gloves (preferably nitrile)
- tissues
- disinfectant spray for cleaning surfaces
- disinfectant (VirKon)
- beakers
- inoculation loops
- conical flasks
- forceps
- ruler
- bags for collecting contaminated waste

Notes on the procedure

Patient sample

These should be prepared just before the start of the lesson.

Use a sterile swab to collect a sample of bacteria from culture and place in a sterile Universal bottle. Label the bottle with a fictional patient's name, reference number and the date.

Media preparation

Students should be aware of how agar and nutrient broth are prepared, However you might decide that it is more practical in terms of expense, safety and time for a laboratory technician to prepare the nutrient agar and nutrient broth into Universal/McCartney bottles.

Preparation of agar plates

If students have no prior experience of working aseptically, additional lessons prior to this practical will be needed to teach them aseptic techniques. This is best done via a demonstration followed by students practicing individually. *Information sheet: Working without contamination* provides guidelines to students on aseptic technique.

Inoculation

For this case study, the scenario is that of a patient with a suspected throat infection. Students begin the practical with a 'sample' collected from the patient.

The sample is actually pre-prepared bacterial sample of an organism that is safe to use in schools. See technical guidance for preparation. Actual throat/saliva samples must not be used.

Once plates have been inoculated with swabs they should be left for about 24 hours at room temperature (20-22 °C) until colonies of bacterial growth can be seen. They must then be carefully examined to check for contaminants. If they have been contaminated, they should not be given back to students to open (although they may be sealed with tape and shown to students).

It is advisable to prepare some spare plates yourself so that, if a student does contaminate their work, they will still be able to continue with the next stage of the practical.

Students can then select a colony to culture in nutrient broth. These should be left for about 12 - 24 hours at room temperature (20-22 °C), until bacterial growth can be seen. Again a teacher or technician should carefully examine the broth for contaminants before allowing students to open the bottle and plate out the bacteria.

Antibiotic application

The easiest way for students to test the bacteria against a number of antibiotics is to use a mast ring. However, separate antibiotic discs could also be used. These can be spread farther from each other so that it is easier to measure inhibition zones, but it can be difficult to get hold of several different suitable antibiotics.

Comparison with professional practice

This antibiotic application deviates from what is actually done in a modern pathology laboratory. In reality the process is often semi-automated. A number of agar plates, each containing a different antibiotic (and a control plate) are used. An automatic dispenser is used to put a drop of a patient's broth culture onto the plates in a specified place (several patients are tested on the same plate). After they have been incubated, the plates are read in a machine which uses light diffraction to identify where colonies have grown on the plate. The computer then records which plates the patient's broth culture grew on.

Reading results

Students should determine the susceptibility of the bacteria to each antibiotic by comparing the zones of inhibition. They can then write up their findings in a manner suitable for their portfolios.

Students can compare the standard procedure they used with those designed for hospital laboratories. You should make students aware of how health and safety restrictions differ between schools/colleges and a hospital laboratory.

Before students start writing their report you might ask them to read through the Health Protection Agency standard procedures highlighting words and phrases they do not understand. With the whole class you could then discuss the words and phrases with which they have difficulty.

Answers

- 1 A sterile throat swab is wiped across the infected area by a medical practitioner and the swab placed in a tube containing a growth medium. This is then sent by a specialist courier to the pathology laboratory.
- 2 To make sure that the susceptibility test is carried out on a single type of bacterium.
- 3 The pathologist first identifies the general type of bacterial infection each patient has, for example *Staphylococcus*. All *Staphylococcus* samples are then tested with about 12 antibiotics that are known to be likely to prevent the growth of *Staphylococcus*.
- 4 When a patient has been taking an antibiotic for a long period of time the bacteria may start to become resistant to the antibiotic. Therefore, it is important that the correct dosage of antibiotic is given to the patient. The microbiologist tests different concentrations of the antibiotic against the patient's bacteria. They can then establish a concentration that effectively inhibits the bacterial growth.
- 5 In a pathology laboratory samples are incubated at 35 °C so the samples grow quicker. This is close to body temperature and is promotes the growth of organisms that infect the human body. In school, health and safety rules prevents students incubating their samples at this temperature, for fear of growing harmful organisms.