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How to Pass SQA ADVANCED HIGHER Biology

Contents

Introduction

Welcome to *How to Pass SQA Advanced Higher Biology*. The Advanced Higher Biology course is split into three areas of study – cells and proteins, organisms and evolution, and investigative biology – and an individual project.

How to use this book

The knowledge content is covered in Key Areas 1.1 to 1.5 and 2.1 to 2.5. The skills content is largely covered in Key Areas 3.1 to 3.3.

Key points

Each Key Area starts with a list of success criteria called key points. Each point has a check box into which you can traffic light your confidence level for that information: green for 'confident', amber for 'needs more work' and red for 'need teacher help'.

Summary notes

The main text provides concise, illustrated expansions of the key points. These should be read carefully several times and should be revisited throughout the course. Hints and tips are given in the margins, while key links cross-reference to ideas that appear in other areas of the book – you should follow these.

Check-up questions

The text is split into handy chunks by numbered check-up questions. These should be answered as you progress through a key area and self-marked to provide feedback to help in traffic lighting the key points. Answers are given at the end of the book.

Key words

Scoring marks in SQA exams requires knowledge of technical terms – these will appear regularly throughout the question paper and, most importantly, its marking instructions. The key words appear in **bold** in the key points and are further defined in key word boxes. You should study the key words carefully. We recommend that you make a set of flashcards for each key area – index cards with the term on one side and the definition on the other.

Exam-style questions

After each Key Area are some SQA-style structured and extended-response exam-style questions that bring together many of the ideas in the Key Area. These should be completed soon after finishing the study of a Key Area. It is probably better to self-mark these and use your score as feedback for revision of your weaker areas.

Answers

These provide easily accessible and understandable answers to all check-up and exam-style questions, and give mark breakdowns.

Practice course assessment

At the end of each of the three areas is a test with a sample of questions that will allow you to evaluate your overall progress in that area and provide feedback for your revision. The tests for Areas 1 and 2 are for 50 marks and you could mark and grade your work:

- 25 for a C pass
- 30 for a B pass
- 35 for an A pass.

The Area 3 test is for 30 marks:

- 15 for a C pass
- 18 for a B pass
- 21 for an A pass.

Practice course assessment answers are given on pages 152, 158–159 and 163–164.

Your project and exam

The *Your project* section on pages 138–141 has more information and helpful hints for your project. Part 4 has more information and helpful hints for your exam.

WWW

- SQA course specification, past papers and project information: **www.sqa.org.uk**
- SQA's examples of candidate evidence with marks and commentaries: **www.understandingstandards.org.uk**
- Scottish Schools Education Research Centre (SSERC) additional project support: **www.sserc.org.uk**
- Information on course assessment and grading, general exam advice and a downloadable record of progress and evaluation: **www.hoddergibson.co.uk/ah-biology-extras**

Area 1 Cells and proteins

Key Area 1.1
Laboratory techniques for biologists

Key points

1 Substances, organisms and equipment in a laboratory can present a **hazard**. ☐
2 Hazards in the lab include toxic and corrosive chemicals, heat and flammable substances, **pathogenic** organisms, and mechanical equipment. ☐
3 Risk is the likelihood of harm arising from exposure to a hazard. ☐
4 Risk assessment involves identifying possible risks and the control measures to minimise them. ☐
5 Control measures used to minimise risk include using appropriate handling techniques, protective clothing and equipment, and **aseptic technique**. ☐
6 Dilutions in a **linear dilution series** differ by an equal interval, for example 0.1 M, 0.2 M, 0.3 M, and so on. ☐
7 Dilutions in a **log dilution series** differ by a constant proportion, for example 10^{-1}, 10^{-2}, 10^{-3}, and so on. ☐
8 A **standard curve** is produced by plotting measured values for known concentrations; it is used to determine the concentration of an unknown solution. ☐
9 **Buffers** are used to control pH; the addition of acid or alkali only has a very small effect on its pH, allowing the pH of a reaction mixture to be kept constant. ☐
10 A **colorimeter** can be used to quantify the concentration and **turbidity** of a solution. The colorimeter is calibrated using an appropriate blank as a baseline. The measurement of absorbance is used to determine the concentration of a coloured solution using suitable wavelength filters. The measurement of percentage transmission is used to determine turbidity. ☐
11 **Centrifugation** is a technique used to separate substances of differing density. More dense components settle in a pellet; less-dense components remain in the **supernatant**. ☐
12 Paper and thin layer **chromatography** can be used for separating different substances such as amino acids and sugars. ☐
13 The speed that each solute travels along the chromatogram depends on its solubility in the solvent used. ☐
14 **Affinity chromatography** is a separation technique in which soluble target proteins with a high **affinity** in a mixture become attached to specific molecules as the mixture passes down a column. Non-target molecules with a weaker affinity are washed out. ☐
15 **Gel electrophoresis** is a technique that can be used to separate proteins and nucleic acids. ☐
16 In gel electrophoresis, charged macromolecules move though an electric field applied to a gel matrix. ☐
17 **Native gel electrophoresis** separates proteins and nucleic acids by their shape, size and charge. ☐
18 Native gels do not denature the molecules, so the separation is by shape, size and charge. ☐
19 **SDS–PAGE** gives all the molecules an equally negative charge and denatures them, separating proteins by size alone. ☐
20 Proteins can be separated from a mixture using their **isoelectric points (IEPs)**. ☐

⇨

21 The IEP is the pH at which a soluble protein has no net charge and will precipitate out of solution. ☐
22 If the solution is buffered to a specific pH, only the protein(s) that have an IEP of that pH will precipitate. ☐
23 Soluble proteins can be separated using an electric field and a pH **gradient gel**. ☐
24 A protein stops migrating through the gel at its IEP in the pH gradient because it has no net charge. ☐
25 Proteins can be detected using antibodies. ☐
26 **Immunoassay** techniques are used to detect and identify specific proteins. These techniques use stocks of antibodies with the same specificity, known as **monoclonal antibodies**. ☐
27 An antibody specific to the protein **antigen** is linked to a chemical 'label'. ☐
28 The label is often a **reporter enzyme** producing a colour change, but chemiluminescence, fluorescence radioactivity and other reporters can be used. ☐
29 In some cases, the assay uses a specific antigen to detect the presence of antibodies. ☐
30 **Western blotting** is a technique used after SDS–PAGE electrophoresis. In western blotting, the separated proteins from the gel are transferred (blotted) on to a solid medium. ☐
31 The proteins can be identified using specific antibodies that have reporter enzymes attached. ☐
32 **Bright-field microscopy** is commonly used to observe whole organisms, parts of organisms, thin sections of dissected tissue or individual cells. ☐
33 **Fluorescence microscopy** uses specific **fluorescent** labels to bind to and visualise certain molecules or structures within cells or tissues. ☐
34 Aseptic technique eliminates unwanted microbial contaminants when culturing micro-organisms or cells. ☐
35 Aseptic technique involves the sterilisation of equipment and culture media by heat or chemical means, and subsequent exclusion of microbial contaminants. ☐
36 A microbial culture can be started using an **inoculum** of microbial cells on an agar medium, or in a broth with suitable nutrients. ☐
37 Many **culture media** exist that promote the growth of specific types of cells and microbes. ☐
38 Animal cells are grown in medium containing **growth factors** from **serum**. ☐
39 Growth factors are proteins that promote cell growth and proliferation. ☐
40 Growth factors are essential for the culture of most animal cells. In culture, **primary cell lines** can divide a limited number of times, whereas tumour cell lines can perform unlimited divisions. ☐
41 Plating out of a liquid microbial culture on solid media allows the number of colony-forming units to be counted and the density of cells in the culture estimated. ☐
42 Serial dilution is often needed to achieve a suitable colony count. ☐
43 **Haemocytometers** are used to estimate cell numbers in a liquid culture. ☐
44 **Vital staining** is required to identify and count viable cells. ☐

Health and safety

Hazards

Biology laboratories often have substances, organisms and equipment that can present hazards to the people that work there. Figure 1.1 shows some of the main hazard warning signs you may see in biology laboratories.

Toxic chemicals

Toxic chemicals are substances that are harmful when inhaled, ingested, injected or absorbed. Essentially, they are poisons. Some are inorganic and others are produced by living organisms. The degree of toxicity depends on the concentration of the substance. Substances that are toxic to humans may not necessarily harm other species.

Corrosive chemicals

A corrosive chemical is a reactive substance that can damage living tissue. They act either directly, by chemically destroying part of the body, or indirectly, by causing inflammation. Acids and bases are corrosive substances commonly found in biology laboratories.

Heat and flammable substances

Sources of heat that laboratory workers should be aware of to prevent burns and scalds include lighted Bunsen burners, electric ovens, hotplates and steam baths. Flammable substances are those that can be easily ignited at room temperature.

Pathogenic organisms

Pathogens are organisms that can cause disease. They are a biohazard.

Mechanical equipment

Mechanical equipment includes machines that may have moving or vibrating parts, hot surfaces, or sharp or heavy components. The main physical risks from mechanical equipment include noise; hand, arm and whole-body vibration; and heat stress. The main mechanical risks include cuts, lacerations, needle punctures and crushing. Different hazard symbols warn of the specific danger posed by mechanical equipment.

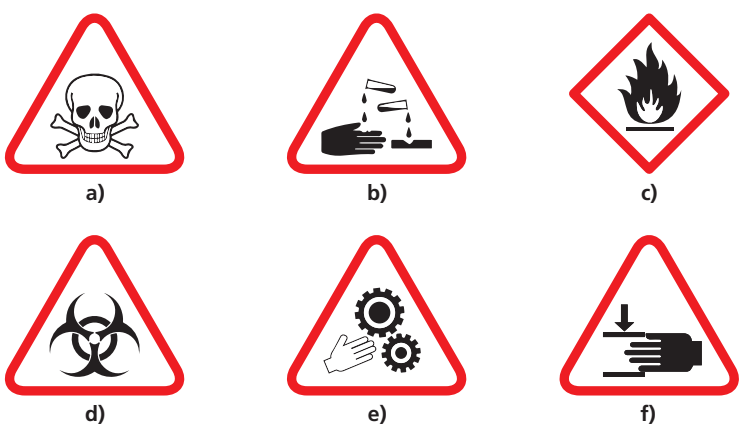


Figure 1.1 Some of the main hazard warning signs: a) toxic, b) corrosive, c) flammable, d) biohazard, e) and f) specific mechanical warning signs

Risk and risk assessment

Risk is the likelihood of harm arising from exposure to a hazard. Risk assessment involves identifying risk levels, their likely severity and the control measures that can be used to minimise these risks. Control measures include:

- using appropriate handling and disposal techniques
- using appropriate masses, volumes and concentrations of substances
- use of protective clothing, such as laboratory coats and gloves
- use of protective equipment, such as goggles and masks
- use of aseptic technique in microbiology.

Key links

You may have to carry out a risk assessment when planning experimental procedures for your project – see the *Your project* section in Area 3.

A grid for carrying out a risk assessment is shown in Figure 1.2.

Hazard identified	Risk level (low, medium, high)	Severity of risk (low, medium, high)	Control measures to be used (handling techniques, equipment and clothing, concentrations of substances, disposal methods, etc.)

Figure 1.2 Headers of a risk assessment grid – there are many more examples of control measures

Check-up 1

1 List five hazards found in biology laboratories. **5**
2 Describe what is meant by a risk assessment. **2**

Liquids and solutions

Making and using linear and log dilutions

Linear dilution is often used when the substance being diluted is the independent variable in an experiment. Linear dilutions differ from each other by an equal interval. To make a linear dilution of a substance, for example glucose, start with a stock solution of a known concentration of glucose in the solvent (distilled water). Add increasing, stepped volumes of that solution to separate tubes, then add pure solvent (distilled water) to each tube so that an equal volume of each dilution is produced, as shown in Figure 1.3.

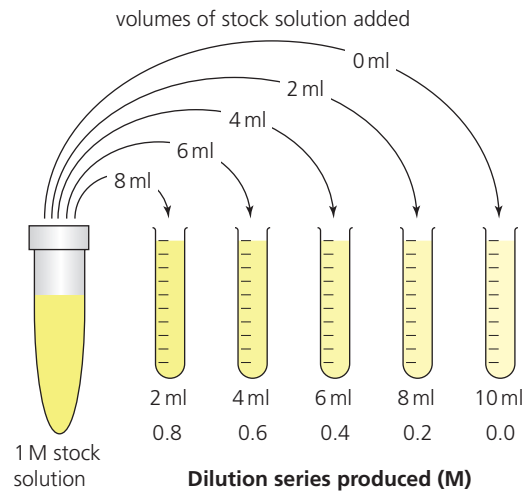


Figure 1.3 Making a linear dilution from 1 M glucose solution – various volumes of the stock are added to separate tubes and diluted up to the same volume with solvent (distilled water) to produce the linear dilutions from 0.0 M to 0.8 M

Log dilution is often used in microbiology to estimate the concentration or density of cells in a stock culture. Dilutions in a log dilution series differ by a constant proportion, for example 10^{-1}, 10^{-2}, 10^{-3}. They are created by diluting a stock solution by a factor then further diluting the dilution produced by the same factor, and so on, as shown in Figure 1.4. This serial dilution produces low concentrations of cells that can be cultured on an agar plate, producing a number of easily countable colonies. From this result, the estimated number of cells in the original stock can be calculated.

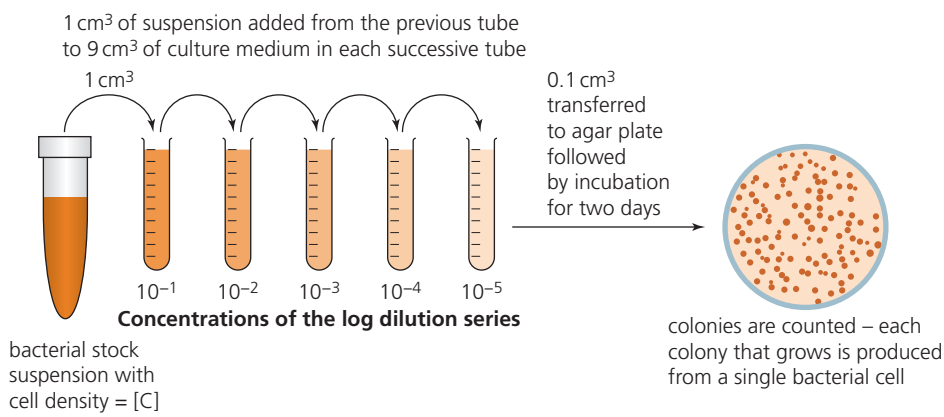


Figure 1.4 Making a log dilution from a cell culture – each individual bacterium grows to a colony that can be counted, then a calculation can be carried out to give a value for bacteria per unit of culture volume (the cell density)

Hints & tips

Notice in Figures 1.3 and 1.4 that liquid volumes can be expressed in millilitres (ml) or in cubic centimetres (cm^3); these are essentially the same.

Check-up 2

1 Describe how you would make a linear dilution series of a 1 M glucose solution. **3**

2 Describe how you would estimate the bacterial cell density in a sample of *Escherichia coli* in a culture of stock solution. **4**

Colorimetry

A colorimeter can be used to determine the concentration of solutions that have been coloured by an indicator reagent by measuring how much light they absorb. Light is passed through a sample of the solution contained in a small tube called a cuvette; an electronic sensor detects how much light has been absorbed as it passed through the solution or culture suspension, as shown in Figure 1.5. A typical school colorimeter is also shown.

Colorimeters can also be used to determine the turbidity of a cell culture. Turbidity is proportional to the density of cells in the culture. Light is passed through a sample of the culture in a cuvette; an electronic sensor detects how much of light has been transmitted through the culture suspension.

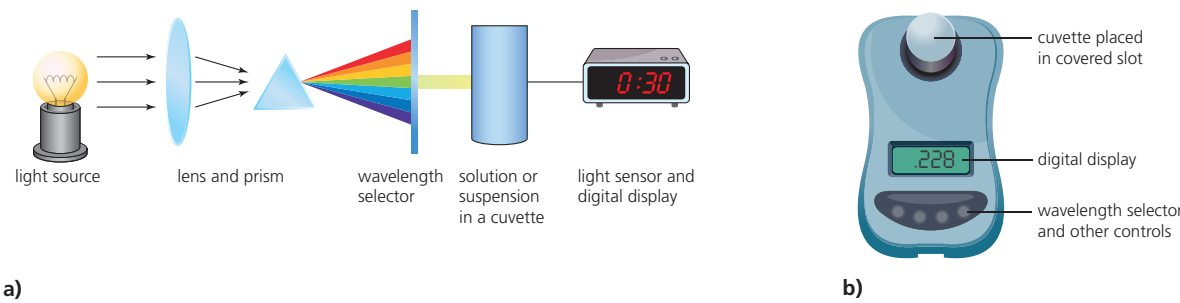


Figure 1.5 a) Components of a colorimeter; b) a typical school colorimeter with controls to set the device to read either absorbance or transmission of specific wavelengths

Before measuring concentration or turbidity with a colorimeter, the instrument must be calibrated using a blank as a baseline. A blank is a cuvette containing only the solvent used when making the dilutions or a sample of the medium used in the cell culture.

Standard curves

A linear dilution series of a substance such as glucose can be used to produce a standard curve. Each dilution of a glucose dilution series has a reagent added that reacts with the glucose to give a coloured product; the colour is proportional to the glucose concentration. The absorbance of the different coloured glucose solutions can then be measured with a colorimeter and the results used to plot a standard curve, as shown in Figure 1.6a).

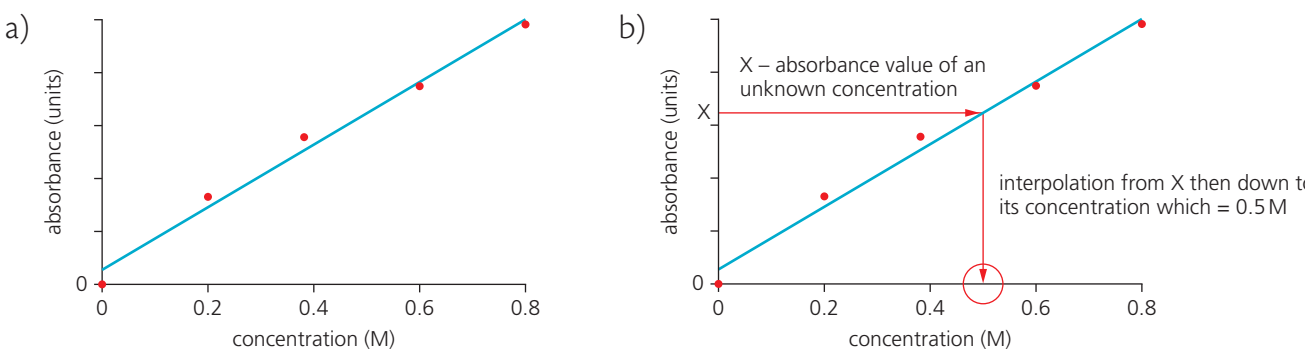


Figure 1.6 a) Standard curve of concentration of a glucose solution; b) using the curve to find the concentration of a glucose solution of unknown concentration

A glucose solution of unknown concentration can be added to the same reagent to produce a coloured solution; its concentration can then be determined by interpolation to the standard curve, as shown in Figure 1.6b).

This general method can also be used to determine unknown cell culture densities.

Use of buffers to control pH

Buffers are solutions that can resist changes of pH even although acid or alkali is added. This allows the pH of a reaction mixture to be kept constant in spite of the production of acidic or alkaline products.

Most biological reactions are dependent on pH, so buffer solutions are often used in *in vitro* experiments on these reactions so that changes in pH during the reaction don't act as confounding variables and cause a mistaken association between the independent and dependent variables.

Check-up 3

1 Describe how you could use colorimetry and a standard curve to identify the glucose concentration of an unknown solution. **4**

2 Explain what buffers are and why they are used in experiments. **2**

Key links

There is more about confounding variables and *in vitro* procedures in Key Area 3.2a.

Separation techniques

Centrifugation to separate substances of differing density

Centrifugation is used to separate components of a suspension that have a different density. For example, different components of cells can be separated by homogenisation of tissue followed by centrifugation. The cell homogenate is placed in a centrifuge tube, which is then spun in a centrifuge machine at between 200 and 120,000 revolutions per minute (rpm). After a time, the denser components of the cells are separated into a pellet while less-dense components remain suspended in the supernatant.

The homogenisation of potato cells and the separation of starch grains from enzyme-rich cytoplasm is shown in Figure 1.7.

Hints & tips

Homogenisation means using a mortar and pestle, sieve or liquidiser to break open all cells.

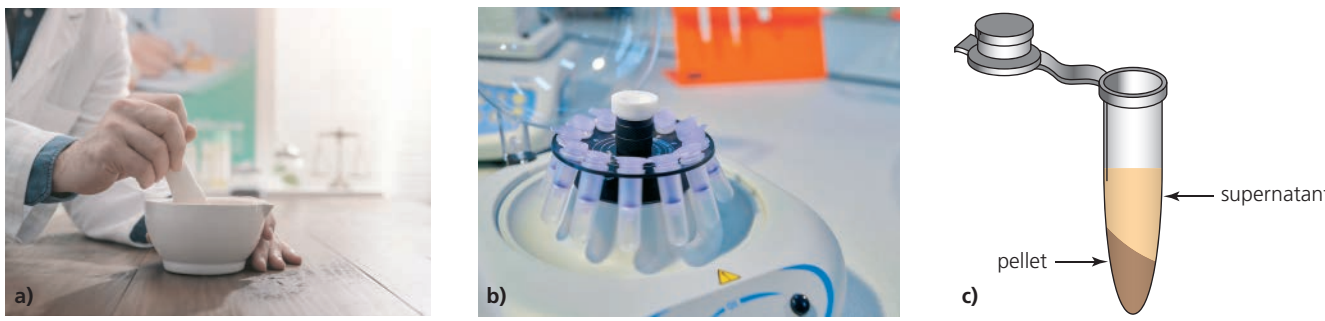


Figure 1.7 a) Homogenisation of potato cells using a mortar and pestle; b) spinning homogenate in a centrifuge; c) the separation of dense starch grains in a pellet from less-dense cytoplasm in the supernatant

Paper and thin layer chromatography

Chromatography can be used to separate different solutes such as amino acids and sugars. Mixtures of these substances dissolved in a solvent can be added to a paper strip or to a metal foil strip with a thin layer of silica or cellulose bonded to it. The speed that each solute travels along the strip depends on its differing solubility in the chromatography solvent used, and its differing affinity for the paper or thin layer. If the substances being separated are colourless, like amino acids, they must be made visible on the paper or thin layer using a developing agent, as shown in Figure 1.8.

Hints & tips

The solubility and affinity of an amino acid is dependent on its R group.

Key links

There is much more about R groups in Key Area 1.2.

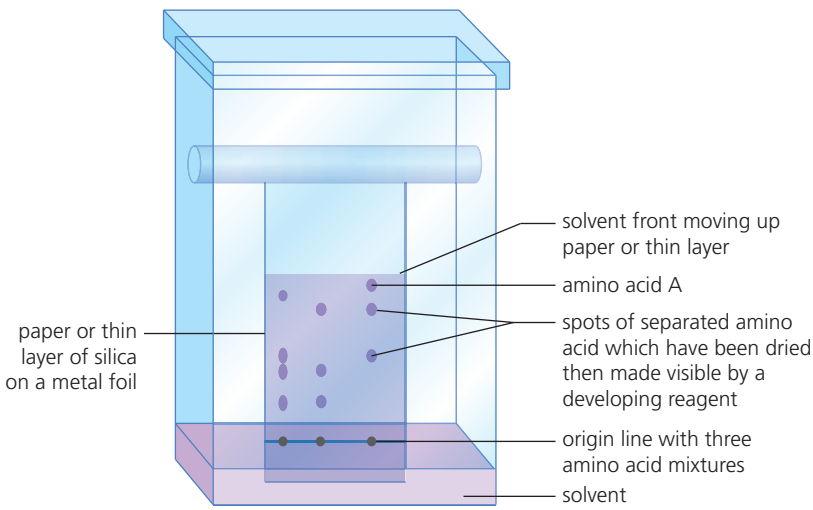


Figure 1.8 Separation of amino acids by paper or thin layer chromatography – amino acid A travels the furthest up the paper because of the properties of its R group, which determine its solubility and affinities

Affinity chromatography

Affinity chromatography can be used to separate target proteins from a mixture of proteins. A solid gel column in a glass tube is produced with specific molecules, such as antibodies or ligands, bound to the gel. Soluble target proteins with a high affinity for these specific molecules become attached to them as a mixture of proteins passes down the column. Non-target proteins with a weaker affinity or no affinity are washed out. The target protein can then be washed out separately and collected, as shown in Figure 1.9.

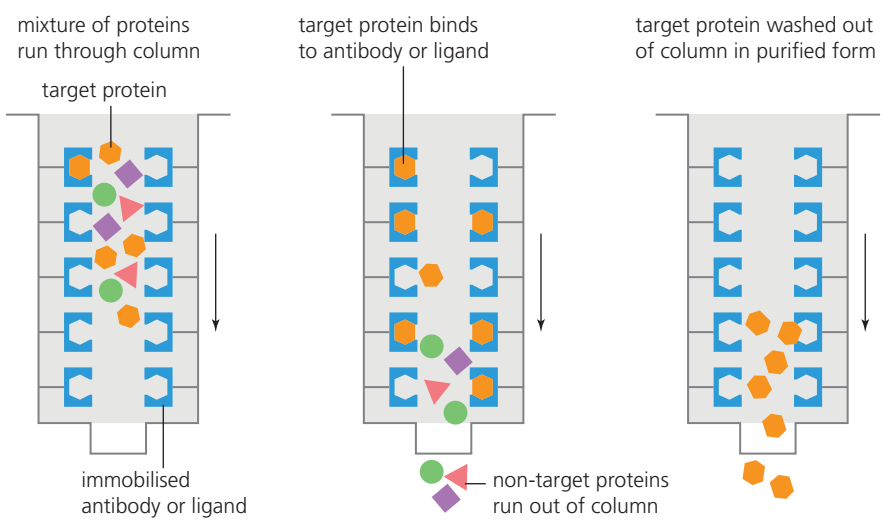


Figure 1.9 Separating a target protein by affinity chromatography

Check-up 4

1 Explain how centrifugation achieves separation of a suspension into a pellet and a supernatant. **2**

2 Describe how a solution with three different amino acids can be separated. **3**

3 Describe how proteins in a mixture can be separated by affinity chromatography. **3**

Key links

There is more about ligands in Key Area 1.2, and more about antibodies in Key Area 2.5.

Gel electrophoresis (PAGE)

In electrophoresis charged macromolecules, such as proteins or nucleic acids, move through an electric field applied to a buffered gel matrix (polyacrylamide gel electrophoresis, or PAGE) as shown in Figure 1.10.

Native PAGE gels do not denature the molecules being separated – they preserve their structure and function – so the separation is by shape, size and charge, but it is tricky to carry out.

SDS–PAGE gels contain sodium dodecyl sulfate (SDS), which denatures molecules passing through it. It gives all the molecules present an equally negative charge, separating proteins by size alone. It is simple to carry out, but the structure and function of any separated protein is lost.

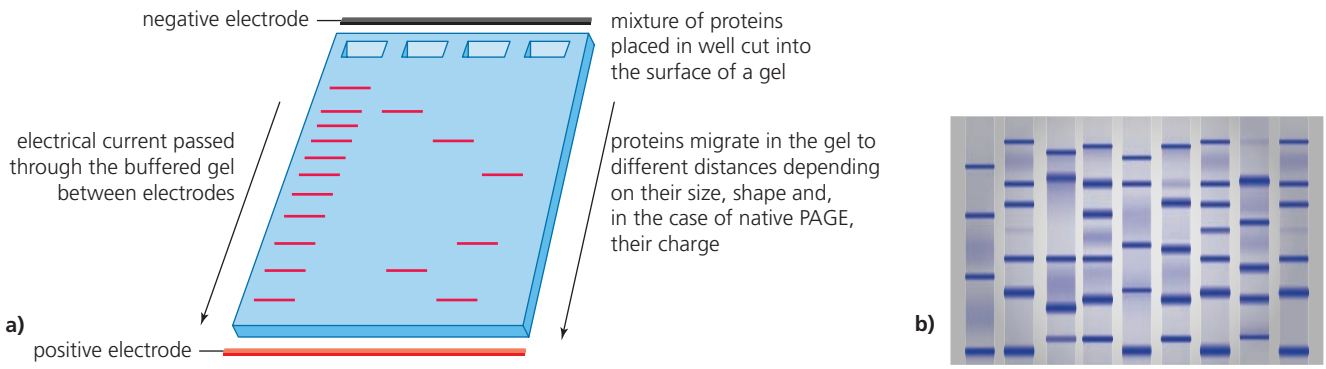


Figure 1.10 a) Separation of proteins by gel electrophoresis; b) photograph of a completed electrophoresis gel

Isoelectric point (IEP)

Proteins have net charges caused by the R groups of their hydrophilic surface amino acids. The isoelectric point (IEP) is the pH value at which a protein is electrically neutral, with surface charges that are balanced as shown in Figure 1.11. At pHs below the IEP the net charges are positive, and at pHs above the IEP the net charges are negative, allowing the protein to remain in solution or suspension. At IEP, the protein loses solubility in water and starts to solidify and precipitate out of solution.

Key links

There is more about hydrophilic amino acids in Key Area 1.3.

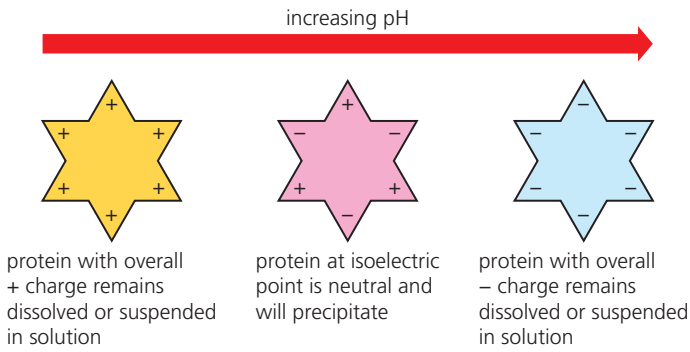


Figure 1.11 Protein molecule with surface charges, which change depending on the pH of its surroundings – at IEP there is no net charge and the protein precipitates

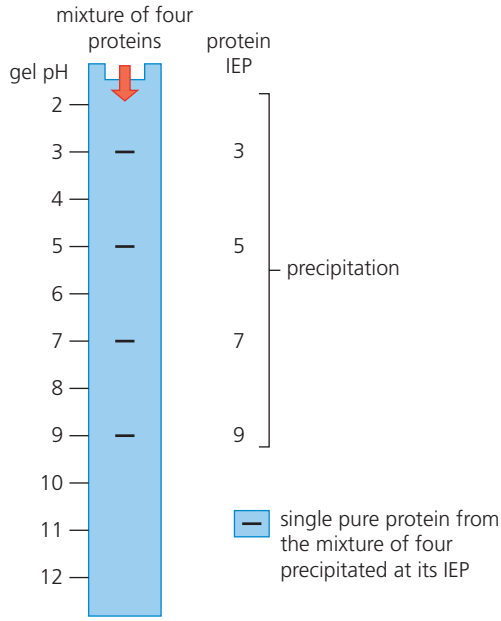


Figure 1.12 Four proteins of different isoelectric point being separated in a pH gradient gel electrophoresis procedure

IEP can be used to separate a mixture of proteins by a type of electrophoresis in which the mixture is passed through a gel with a built-in pH gradient. The individual proteins reach their IEPs one by one and are collected as precipitates, as shown in Figure 1.12.

Check-up 5

1 Explain the difference between native and SDS–PAGE techniques. **2**

2 Explain how isoelectric point can be used to separate proteins. **3**

Detecting and identifying proteins using antibodies

Immunoassay techniques

Immunoassay techniques are used to detect and identify specific proteins. These techniques use stocks of antibodies with the same specificity, known as monoclonal antibodies. The antibodies can be linked to a chemical 'label', often a reporter enzyme that produces a colour change, but reporters that show by chemiluminescence, fluorescence or radioactivity can also be used.

In the simple example in Figure 1.13, a sample to be assayed has been added to a container and antigenic material adheres to the container surface. To identify if a specific antigen is present, a labelled antibody specific to the antigen to be detected or identified is added and then washed out. In the case of a reporter enzyme label, its colourless substate is then added, which will change to a coloured product if the enzyme has been trapped on the antibody bound to the antigen.

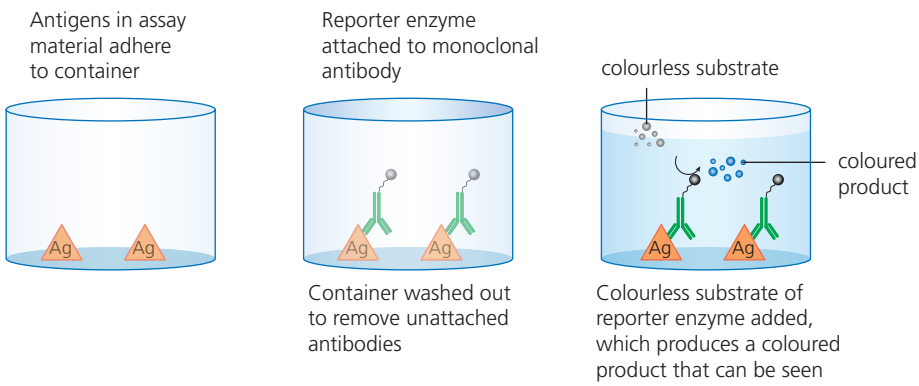


Figure 1.13 Detection of a specific antigen in assay material – in this example, the colour change happens if the reporter is bound, and it can only be bound if the specific antigen is present

Other immunoassay techniques involve the antibody being pre-attached to the container and the sample being analysed added. If the antigen is present in the sample, it will specifically bind to the

antibody in the container and can't be washed away. A second antibody with a reporter that binds to the first can then be added and the reporter signal looked for.

Hints & tips

Make sure you know why washing occurs in immunoassays, and the implication for the result if it is not done properly.

Western blotting

Western blotting is a technique used after SDS–PAGE electrophoresis. The separated proteins from the gel are transferred (blotted) on to a solid medium or membrane and dried. The proteins can be labelled by soaking the blot with specific antibodies. The antibodies bind to specific proteins. A second antibody with reporter enzymes – radioactive tags or fluorescent markers attached – are then added. The presence of the protein can then be visualised by eye following the addition of the reporter enzyme's substrate, which changes colour, or by seeing fluorescence in UV light or by using photographic paper, which is sensitive to radioactivity, as shown in Figure 1.14.

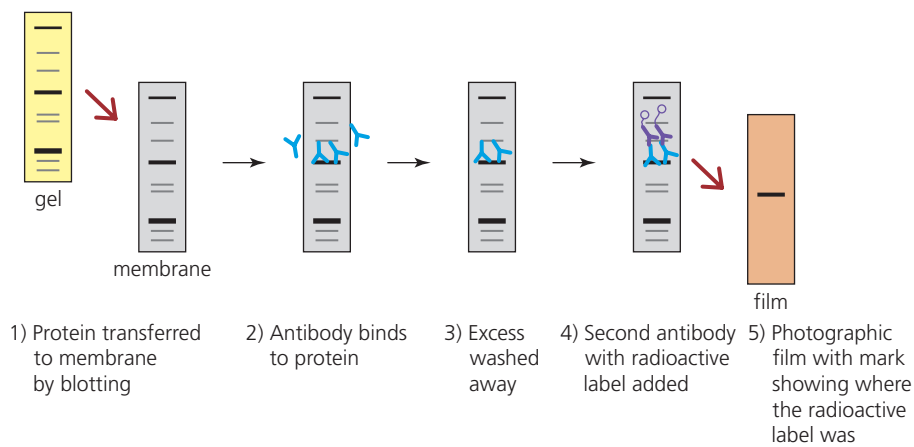


Figure 1.14 Western blotting – 1) proteins separated by gel electrophoresis are pressed on to a membrane; 2) membrane is treated with antibodies specific to a target protein; 3) excess is washed away but some antibody binds specifically to target; 4) a second antibody with a radioactive reporter is added and binds to any antibody already present; 5) membrane placed against a piece of photographic film – the radioactivity leaves a mark showing the presence of the target protein

Microscopy

Bright-field microscopy is used to examine whole organisms, parts of organisms, thin sections of dissected tissue or individual cells. Figure 1.15 shows some onion cells undergoing cell division by mitosis. The cells have been stained with a pink stain to make them more visible.

Fluorescence microscopy uses UV light to detect specific fluorescent stains, which bind to and visualise certain molecules or structures within cells or tissues.

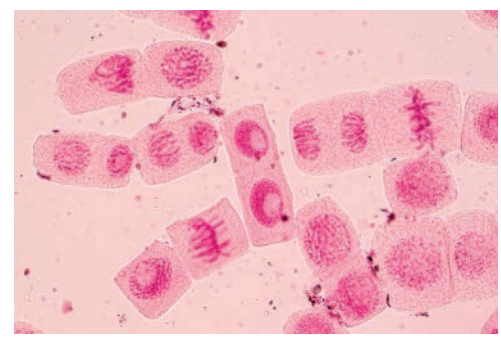


Figure 1.15 Stained onion cells undergoing mitosis captured using bright-field microscopy

Key links

An example of an image captured using fluorescence microscopy is shown in Figure 1.47a) on page 51.

Key links

There is more about mitosis in Key Area 1.5.

Aseptic technique and cell culture

Aseptic technique eliminates unwanted microbial contaminants when culturing micro-organisms or cells. Aseptic technique involves the sterilisation of equipment and culture media by heat or chemical means, and the subsequent exclusion of microbial contaminants, as shown in Figure 1.16.

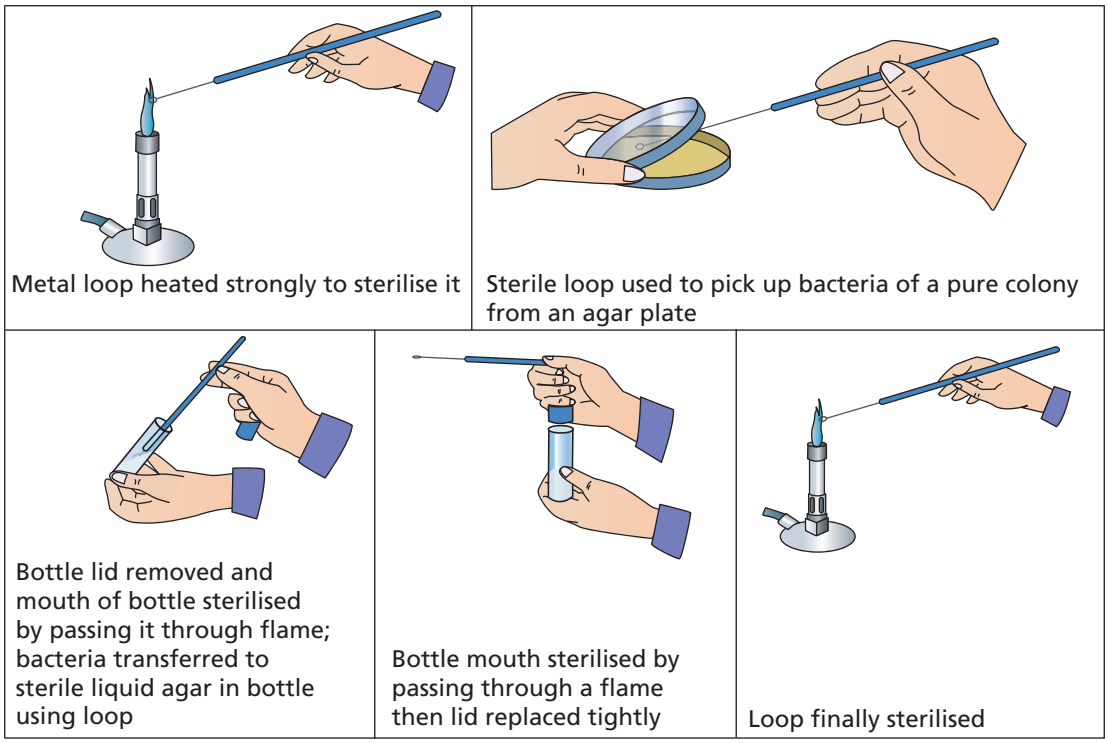


Figure 1.16 Simple example of the use of aseptic transfer technique in a biology laboratory – an inoculum is taken from a sterile culture growing on agar in a Petri dish using an inoculating loop, added to sterile liquid agar in a labelled culture bottle and sealed

A microbial culture can be started using an inoculum of microbial cells on an agar medium, or in a broth with suitable nutrients.

Many culture media exist that promote the growth of specific types of cells and microbes. Animal cells are grown in medium containing growth factors from serum. Growth factors are proteins that promote cell growth and proliferation, and they are essential for the culture of most animal cells. Plating out of a liquid microbial culture on solid media allows the number of colony-forming units to be counted and the density of cells in the culture to be estimated. Serial dilution is often needed to achieve a suitable colony count.

Haemocytometers

Haemocytometers are microscopic grids used to estimate cell numbers in a liquid culture, as shown in Figure 1.17a). When counting cells, a protocol is needed to deal with cells lying across the grid boundaries – often, cells over the right-hand and bottom boundaries of a grid are counted, but those on the top or left-hand boundaries are not. Often, the four corner and central grids in an array of grids are counted and averaged.

Vital staining is required to identify and count viable cells because the stain can distinguish between cells that are alive or dead, as shown in Figure 1.17b).

Hints & tips

Note that the stain in Figure 1.17b) colours the dead cells.

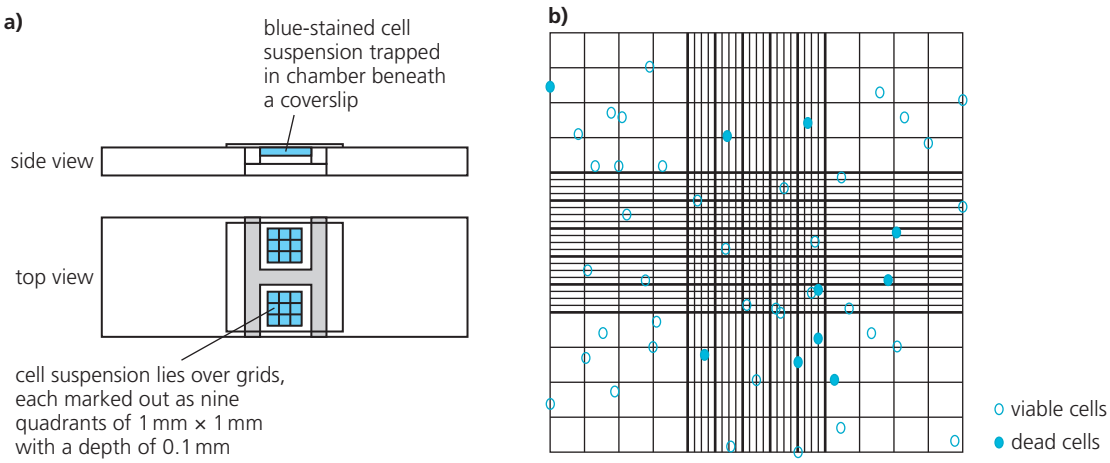


Figure 1.17 a) Haemocytometer chamber with stained culture trapped against the grids using a coverslip; b) haemocytometer grid with a sample of cells from a culture stained using trypan blue, a vital stain

Check-up 6

1 Explain what is meant by a monoclonal antibody. **1**
2 Describe how a reporter enzyme works. **3**
3 Describe western blotting. **4**
4 Explain why aseptic technique is used when working with cell cultures. **2**
5 In Figure 1.17, the volume of the haemocytometer chamber is 0.001 ml. Use this information and the cell number in the whole chamber to calculate:
a) the number of viable cells
b) the number of dead cells in 1 ml of the culture. **2**

Hints & tips

Note that vital stains may stain live cells but others stain dead cells.

Key words

Affinity – the degree to which a substance is attracted and tends to bind to another
Affinity chromatography – a technique used to separate and purify proteins based on a specific binding interaction between an immobilised ligand and its binding partner
Antigen – a specific protein with which antibodies can bind in an immune response
Aseptic technique – procedures in place to prevent contamination, including sterilising equipment and work surfaces
Bright-field microscopy – microscopy commonly used to observe whole organisms, parts of organisms, thin sections of dissected tissue or individual cells
Buffer – a solution used to set and maintain a particular pH
Centrifugation – a process that uses centrifugal forces to separate components of different densities in a mixture
Chromatography – a technique used to separate different substances; it has a stationary phase (for example, paper or gel) that the mobile phase (for example, a solvent) moves through, carrying the substance being examined; different distances are moved by substances of different solubility
Colorimeter – a device used to measure the absorbance of a specific wavelength of light by a solution
Culture media – a nutrient-rich growth medium providing the basic requirements for cell growth (amino acids, glucose, salts, water, as well as specific growth factors for animal cell lines)
Fluorescence – the emission of light of a different wavelength to that which was absorbed
Fluorescence microscopy – microscopy technique that uses specific fluorescent labels to bind to and visualise certain molecules or structures within cells or tissues
Gel electrophoresis – technique used to separate samples of nucleic acid and protein by size; introduced to a gel, they move through it due to an electric current; smaller fragments move further than larger fragments
Growth factors – proteins that promote cell growth and proliferation

Haemocytometer – microscopic grid used to estimate the total number of cells within a sample (originally used to count blood cells)
Hazard – anything that poses a potential risk or threat to an individual or the environment
Immunoassay – technique used to detect and identify specific proteins; antibodies linked with reporter enzymes, for example, cause a colour change in the presence of a specific antigen
Inoculum – starting material used to grow a culture from, for example a bacterial culture
Isoelectric point (IEP) – the pH at which a soluble protein has no net charge and will precipitate out of solution
Linear dilution series – a series of dilutions that differ by an equal interval, for example 0.1 M, 0.2 M, 0.3 M, and so on
Log dilution series – a series of dilutions that differ by a constant proportion, for example 10^{-1}, 10^{-2}, 10^{-3}, and so on
Monoclonal antibodies – stocks of identical antibodies that are specific to a particular antigen
Native gel electrophoresis – does not contain SDS and does not denature the molecule, so proteins are separated by their shape, size and charge
Pathogenic – causing disease
Primary cell lines – a culture of cells isolated directly from animal or plant tissues; they have a finite lifespan and limited expansion capacity
Reporter enzyme – an enzyme linked to an antibody specific to a protein antigen; they are used in immunoassay techniques
SDS–PAGE – electrophoresis in which the gel contains SDS, which gives all the molecules an equally negative charge and denatures them, separating proteins by size alone
Serum – vitally important as a source of growth factors, hormones, lipids and minerals for the culture of cells
Standard curve – a graph that can be used to determine the concentration of an unknown solution
Supernatant – the liquid that lies above a solid residue or pellet in centrifugation
Turbidity – a measure of the degree to which a fluid loses its transparency due to the presence of suspended particles or cells in suspension
Vital staining – a technique in which a harmless dye is used to stain either living tissue cells or dead cells for microscopical observation to allow a viable cell count to be made
Western blotting – an analytical technique used to identify and locate specific proteins in a sample of tissue homogenate or extract based on their ability to bind to specific antibodies

Exam-style questions

Structured questions

1 The **diagram** below shows a haemocytometer counting chamber containing a sample from a culture of animal cells prepared by an aseptic technique and stained with a vital stain. The depth of the chamber is 0.01 cm.

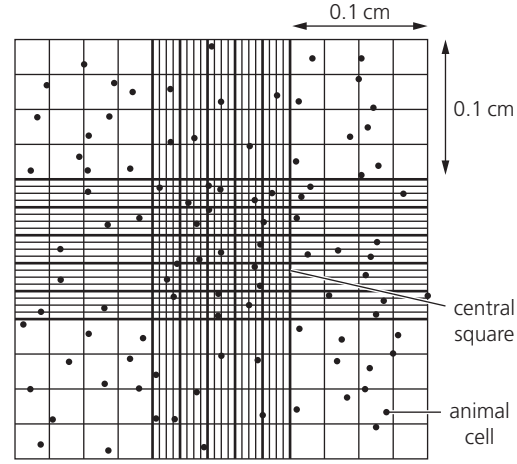
a) Calculate the density of cells per cm^3 in this culture based on the central square. **1**

b) The cells have been stained using a vital stain. Explain what is meant by a vital stain in this example. **1**

c) Describe the components of the culture medium that could ensure the normal growth and proliferation of cells in the culture. **2**

d) The culture was prepared using an aseptic technique. Explain what is meant by an aseptic technique. **2**

Diagram



2 In a procedure to purify an enzyme, a tissue sample was taken through a number of stages.

The table below describes the purification stages and shows the total mass of protein present and the enzyme activity in the sample following each stage in the purification procedure.

Stage	Description of purification stage	Total protein (mg)	Enzyme activity (units)
1	Liquidise tissue sample	10 000	2 000 000
2	Precipitation by salts	3 000	1 500 000
3	Separation by isoelectric point	500	500 000
4	Separation by affinity chromatography	30	42 000

a)

(i) Calculate the percentage of the protein that had been removed from the liquidised tissue by the end of Stage 4. **1**

(ii) Enzyme purity in a sample can be calculated using the formula below:

$$\text{Enzyme purity} = \frac{(\text{enzyme activity})}{(\text{total protein})}$$

Use the formula to calculate the number of times by which enzyme purity had been increased between the liquidised sample and the end of Stage 4. **1**

b) Explain how separation by isoelectric point, as in Stage 3, occurs. **2**

c) In affinity chromatography at Stage 4, a ligand specific to the enzyme being purified was bonded to agarose gel beads packed into a column.
Describe how this method can improve the purity of the enzyme. **2**

Extended response

3 Give an account of laboratory separation techniques used in the separation of amino acids and proteins. **7**

4 Give an account of immunoassay techniques. **7**

Answers are given on pages 147–148.

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£14.99
ISBN 978-1-398-31217-3
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