# Guidance for Option B – Practical 1

## *Immobilised enzymes*

### Safety

Although great care has been taken in checking the accuracy of the information provided in this guidance, Cambridge University Press shall not be responsible for any errors, omissions or inaccuracies.

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You should carry out the practical yourself before presenting it to students. Make sure you are comfortable with the procedures, and can anticipate any difficulties any of your students may encounter.

### Guidance

This practical enables student to learn how immobilised enzymes are prepared and used, on a small scale. It can be used as a starting point to examine how flow rates, size of alginate beads and concentration of substrate affect the activity of the enzyme.

Note that this practical may instead be used during study of Topic **2**, *Molecular Biology*.

### Apparatus and materials

Each student or pair will need:

• 2 cm3 lactase (or invertase)

• 30 cm3 5% lactose found in full fat milk (or sucrose if invertase is used)

• 8 cm3 2% sodium alginate made in distilled water

• 100 cm3 2% calcium chloride solution

• two 10 cm3 syringes

• three 100 cm3 beakers

• glass rod or spatula

• small sieve

• distilled water bottle

• 10 cm3 syringe without plunger

• small piece of muslin or nylon gauze

• screw clip

• retort stand

• glucose test sticks (proprietary medical brand such as Clinisticks or Diastix)

### Setting up the practical

Full fat milk can be used as a source of lactase. To prepare the 2% sodium alginate made in distilled water, add the alginate slowly to warm distilled water, stirring constantly, and then allow the mixture to cool. The concentration of the enzyme varies with the age and storage of the enzyme, so trials should be carried out to find the most suitable concentration.

### Supporting the practical

Students may need help establishing a suitable flow rate. It may be beneficial organise the class so that different groups collect data from different flow rates and then share their results.

### Clearing up

Alginate beads and used test sticks can be safely disposed of in normal waste.

### Answers to questions

**1** Students might suggest producing a range of substrate concentrations by diluting the original lactose or sucrose solution. They might discuss that in a commercial factory it is important to maximise production and reduce costs and wastage. This can be achieved by adjusting concentration and flow rates.

**2** The sizes of the alginate beads are likely to be variable due to the method of production. Instead, an automated dispenser could be used to form the alginate beads and eliminate human error in this process. An even bead size ensures that flow through the column is consistent.

Flow rate is dependent on the pressure from the liquid above and will therefore vary as the volume of solution remaining in the column decreases. To overcome this problem, readings of product concentration could be taken at a specific time as the substrate flows through, so that for all replicates of the experiment the flow rate values at that time will be comparable.

# Guidance for Option B – Practical 2

## *Gram staining of bacteria*

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### Guidance

This practical introduces students to an important laboratory technique, which historically has been a valuable diagnostic tool: the Gram stain is invariably the first step in the identification of a bacterium.

### Apparatus and materials

Each student or pair will need:

• cultures of *Escherichia coli* and *Staphylococcus albus* in nutrient broth in McCartney bottles (if these are unavailable other bacteria such as *Bacillus megaterium* and *Micrococcus luteus* can be used, or bacteria can be obtained from ‘live’ yoghurt)

• bacteriological loop

• Bunsen burner or spirit lamp

• microscope slides

• a few drops of crystal violet stain (≤5 cm3)

• a few drops of Gram’s iodine (≤5 cm3)

• a few drops of basic fuchsin (≤5 cm3)

• laboratory alcohol (95% ethanol) (approximately 10 cm3)

• distilled water

• tissue or blotting paper

• microscope with ×400 magnification and oil immersion lens

• lens tissue

• antiseptic wipes and hand soap for cleaning benches and hands after the practical

### Setting up the practical

Cultures should be fresh, inoculated the day before the practical for best results.

### Supporting the practical

It is unlikely that many students will have prior knowledge of basic aseptic technique so it may be helpful to demonstrate this before the practical gets underway.

Students will need to be reminded to cover up cultures once they have been used. The organisms suggested are not dangerous but safety discipline is a good one to establish.

It will be helpful if reference material about the structure of the two types of bacterial cell wall is available if it has not been studied previously.

### Clearing up

Students should wipe down their work areas with antiseptic wipes and wash their hands carefully with warm soapy water.

### Answers to questions

**1** Students’ findings will depend on their research.

**2** In a Gram-positive bacterium, the layers are arranged in this order (from interior to exterior): plasma membrane, thick peptidoglycan layer.

In a Gram-negative bacterium, the layers are: plasma membrane, thin peptidoglycan layer, outer membrane made of lipopolysaccharide.

# Guidance for Option B – Practical 3

## *Investigating the action of bactericides*

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### Guidance

Claims of antibacterial properties are made for many commonly used household products. In this practical students can assess the validity of these claims. It may be difficult to obtain similar formulations of different products that are sold as gels, liquids or creams but students can suggest how they might attempt to control this variable.

The instructions to students direct them to pour their own agar plates containing bacterial culture and then to use them for the experiment. In this way, they develop their own handling skills and dexterity.

The experiment can lead to further work to quantify the optimum strength of a chosen antibacterial agent.

### Apparatus and materials

Each student or pair will need:

• 20 cm3 culture solution of *Micrococcus luteus*

• Bunsen burner

• eight sterile Petri dishes

• dropping pipettes

• disposal beaker containing antiseptic solution, for used pipettes

• 250 cm3 bottle of nutrient agar in a water bath at 60 °C

• forceps

• small discs of absorbent paper (filter paper or blotting paper)

• seven common antibacterial products, e.g. toothpaste, handwash, disinfectant, cleansing products

• distilled water

• sticky tape

• marker pen

• incubator at 25 °C

• antiseptic wipes

### Setting up the practical

Aseptic techniques should be followed and fresh cultures of *M. luteus* used.

Students should not seal the Petri dishes all the way round, just a couple of strips of sticky tape at either side of the plate are required, to join the lid and base.

### Supporting the practical

It may be helpful to demonstrate aseptic technique to inexperienced students.

Teachers may like to lead the discussion about the formulations of the products used and whether they are comparable here.

Students should be reminded throughout about safety issues, and the disposal of equipment they have used in containers of antiseptic solutions provided.

### Clearing up

After the practical, students should wipe down their work areas with antiseptic wipes and wash their hands thoroughly.

After incubation, Petri dishes should not be opened and must be disposed of using an autoclave or pressure cooker.

### Answers to questions

**1** Students’ findings will depend on their experiments.

**2** Students may suggest using different dilutions of the best antibacterial agents. They may also understand that a serial dilution can produce quantitative data.