Cambridge Assessment International Education

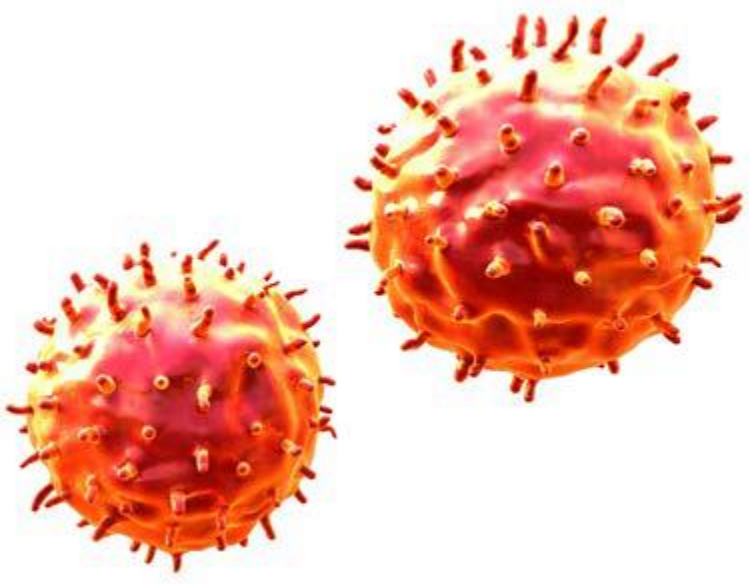
Teaching Pack

Investigating the use of biological washing powders that contain enzymes

Cambridge IGCSE™

Biology 0610

This *Teaching Pack* can also be used with the following syllabuses:
- Cambridge IGCSE® (9–1) Biology **0970**
- Cambridge IGCSE® Combined Science 0653
- Cambridge IGCSE Co-ordinated Sciences (Double Award) 0654
- Cambridge IGCSE (9–1) Co-ordinated Sciences (Double Award) 0973
- Cambridge O Level Biology **5090**
- Cambridge O Level Combined Science 5129



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Introduction

This pack will help you to develop your learners' experimental skills as defined by assessment objective 3 (AO3 Experimental skills and investigations) in the course syllabus.

Important note
Our *Teaching Packs* have been written by **classroom teachers** to help you deliver topics and skills that can be challenging. Use these materials to supplement your teaching and engage your learners. You can also use them to help you create lesson plans for other experiments.

This content is designed to give you and your learners the chance to explore practical skills. It is not intended as specific practice for Paper 5 (Practical Test) or Paper 6 (Alternative to the Practical Test).

There are two options for practising experimental skills. If you have laboratory facilities this pack will support you with the logistics of running the experiment. If you have limited access to experimental equipment and/or chemicals, this pack will help you to deliver a virtual experiment.

This is one of a range of *Teaching Packs*. Each pack is based on one experiment with a focus on specific experimental techniques. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:

Briefing lesson (1 hour*)
This lesson introduces the focus experimental skills to be developed. It also introduces any content needed for your learners to understand the experiment being carried out in the *Lab lesson*.

↓

Lab lesson (1 hour*)	
Option 1 – run the experiment	**Option 2 – virtual experiment**
This lesson allows the experiment to be run with your learners, providing an opportunity to practise the experimental skills introduced in the *Briefing lesson*.	This lesson allows your learners to complete a virtual experiment, providing an opportunity to practise the experimental skills introduced in the *Briefing lesson*.

↓

Debriefing lesson (1 hour*)
This lesson consolidates and builds on the progress learners have made. In some cases, it will also provide the opportunity to practise extended writing skills.

** the timings are a guide only; you may need to adapt the lessons to suit your circumstances.*

In this pack will find the lesson plans, worksheets for learners and teacher resource sheets you will need to successfully complete this experiment.

Experiment: Investigating the use of biological washing powders that contain enzymes

This *Teaching Pack* focuses on an investigation into the effectiveness of…

Washing powders are sometimes called biological. This means that… Starch… Protein…

This experiment has links to the following syllabus content (see syllabus for detail):

- 21.2: Investigate and describe the use of biological washing powders that contain enzymes

The experiment covers the following experimental skills, adapted from **AO3: Experimental skills and investigations** (see syllabus for assessment objectives):

- demonstrate knowledge of how to safely use techniques, apparatus and materials (including following a sequence of instructions where appropriate)
- plan experiments and investigations
- make and record observations, measurements and estimates
- interpret and evaluate experimental observations and data
- evaluate methods and suggest possible improvements.

Prior knowledge

Knowledge from the following syllabus topics is useful for this experiment.

- 4.1 Biological Molecules
- 5.1 Enzymes

Going forward

The knowledge and skills gained from this experiment can be used for when you teach learners about genetic engineering, because the enzymes included in washing powders are produced by genetically modified organisms.

Briefing lesson: Applications of enzymes

Resources	• Teacher instructions 1 and 2 • Worksheet A – F • Petri dish (without agar) • Petri dish (with agar) • Cork borer • Overhead projector
Learning objectives	By the end of the lesson: • **all learners** will be able to outline how the activity of an enzyme can be investigated in a quantitative experiment. • **most learners** will be able to select equipment and describe how the activity of an enzyme can be investigated in a quantitative experiment. • **some learners** will be able to evaluate, using specific terminology, an approach that can be used to investigate the activity of an enzyme in a quantitative experiment.

Timings	Activity
10 min	**Starter/Introduction** Learners can sometimes find the modes of enzyme activity difficult to imagine. Modelling can help. In this activity, host a 'molecular puppet show' by setting up an overhead projector (OHP) to display a series of cardboard shapes on the wall. These are provided as cut-out shapes on Teacher Instructions 1. Explain that this task gives them the opportunity to construct a visual representation of a mechanism that cannot be seen with the naked eye, and its purpose is to remind them of the mechanism of enzyme-catalysed reactions. Alternatively, photocopy enough sheets and provide them to students to cut out and demonstrate within pairs how enzymes lower the activation energy of a biological reaction – by forming enzyme-substrate complex. If you decide to host a learner-led activity, circulate around the class to give support and to stretch learners' thinking by asking further questions (listed on Teacher Instructions 1). These questions place an emphasis on observations that could be made during an enzyme-catalysed reaction.
40 min	**Main lesson** Through a class discussion, remind learners of the difference between qualitative measurements and quantitative measurements. Provide learners Worksheet A. Ask them to explain if the table shows quantitative or qualitative data. Agree that although the table shows numerical data (sample times, temperature) the recorded data are observations not measurements, so the data is qualitative. Arrange the class into groups of 3–4, with each group ideally comprising learners of mixed ability, gender and cultural backgrounds. Give each group Worksheet B,

	which includes a range of laboratory equipment. Tell learners that in their next lesson they are going to investigate the effectiveness of two different brands of washing powder. Biological washing powders contain a range of enzymes, including amylase, proteases and lipases. These help break down some of the molecules found in blood, food and plant-based stains. Explain that they need to use what they have learned so far in the course to help them devise a method to follow. Learners should be provided with at least 5 minutes to discuss their thoughts, and then should be provided with a piece of A3 paper to produce a rough labelled diagram. During this activity, circulate to provide support and guidance. If learners find it difficult to make a start, provide some hints – e.g. 'mix the substrate with agar gel,' and 'use the cork borer to make a well in the agar gel' (Questions will vary depending on the choices learners make.) Show learners some key items of equipment that they will use in the investigation – especially the Petri dishes Give each learner Worksheet C, which includes an unlabelled diagram of the actual experimental set-up that learners will prepare in the practical lesson. They should be challenged to think carefully about how the arrangement has features that are similar and different to their approach, and to evaluate their approach accordingly. Give each learner Worksheet D, which outlines the method they will use in the practical lesson. They are asked to identify the activity that should be undertaken in each step, or the rationale for taking a step. Learners should attempt this individually. Ask them to compare their answers with a partner once they have finished. They should discuss how they could ensure that the method improves the likelihood of obtaining valid, reliable and accurate data and then answer the questions at the bottom of the worksheet. Finally, after discussion, provide Worksheet E1, which provides a blank space into which they should design a table for homework. Learners should be directed to consider this in advance of the next lesson.
	**Plenary** Hand out Worksheet F, which is a game of 'bingo.' The purpose of this activity is to reinforce the key terms relevant to this lesson and their meanings. Learners choose 6 terms at random from their 3x2 grid from a choice of at least 20 that are listed. Ensure that learners choose 3 terms from the 'scientific terminology' table and 3 terms from the 'investigation terminology' table. The teacher then defines the terms, at random, and the learners tick off their terms if the definition matches. Teacher Instructions 2 lists the definitions for these words. The first learner to have all terms ticked shouts 'bingo' to win.

Lab lesson: Option 1 – run the experiment

Resources	• Worksheets E1 (from last lesson), E2 and G1 • All practical equipment listed on the next page
Learning objectives	By the end of the lesson: • **all learners** should be able to investigate the ability of enzymes found in washing powder to digest their substrates. • **most learners** should be able to evaluate the effectiveness of a method used to investigate the ability of enzymes found in washing powder to digest their substrates. • **some learners** will be able to suggest how an investigation into the ability of enzymes found in washing powder to digest their substrates can be modified.

Timings	Activity
10 min	**Starter/Introduction** Ask learners to swap their completed Teacher Instructions 2 with a partner. Then hand out Worksheet E2 to all learners. Ask learners how their tables compare with the actual table that they will be using today. It is likely that learners have put the independent and dependent variables in inappropriate columns and/ or did not include reference to a control or to repeated data.
45 min	**Main lesson** Provide learners with Worksheet G1, which lists the method, and instruct them to collect their equipment and begin the practical task. Inform them that plates containing the various types of agar-substrate mixture have been provided. Ask learners to identify any aspects of the method they find problematic and to make a note of these as they go. They should write down possible solutions that they could employ to these problems, if they were to perform the practical again. This will develop their evaluative skills and prepare them for the task in the *Debriefing Lesson*. **Safety** Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate.
5 min	**Plenary** Ask learners to record the mean diameter for each zone of digestion on a common class spreadsheet (e.g. a shared *Google sheet*) or on the whiteboard. Class discussion might highlight the fact that the colours of the zones of digestion aren't what might be expected of the indicators used (iodine solution should become red/brown as starch is digested etc.). It is worth having to hand the ingredients list for each washing powder sample (normally available from the manufacturers website) to hand, which will likely explain why zones of digestion have not just changed colour, but decolourised (apart from enzymes, biological washing powders often include additional ingredients such as acids and bleaches for example).

Teacher notes

Watch the video (teacher version) and read these notes.

Each group will require:

- 5 g of two different biological washing powders
- 3 agar plates, containing either milk powder, vegetable oil, or starch solution
- 2 boiling tubes
- 2 beakers, each containing 50 cm^3 distilled water
- cork borer, 5mm diameter
- stopclock
- white tile
- tongs
- kettle
- Bunsen burner
- large beaker with a thermometer
- graph paper with 1mm squares
- 1 100 cm^3 beaker
- 1 permanent marker pen
- Ruler (30 cm)
- paper towels
- gloves

Safety

The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners.

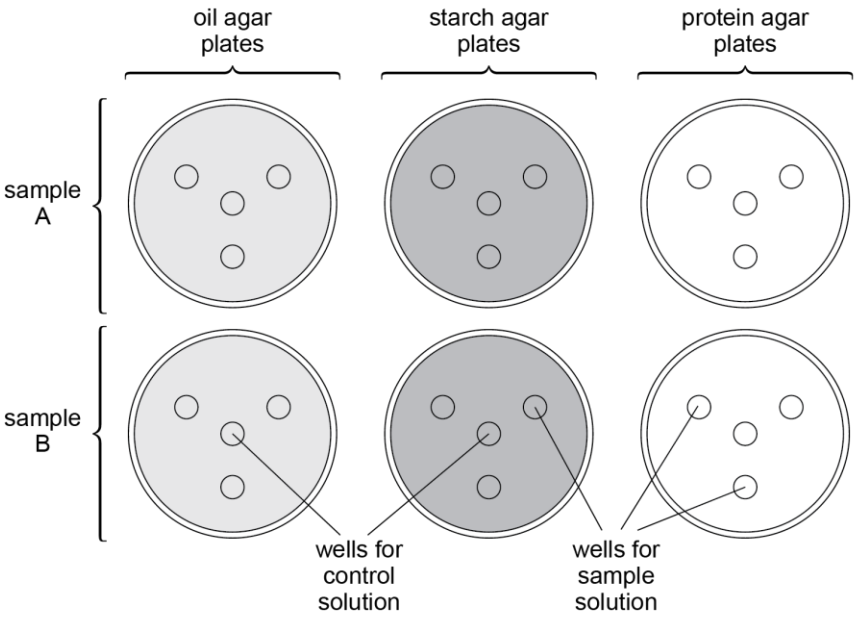
It is your responsibility to carry out an appropriate risk assessment for this experiment.

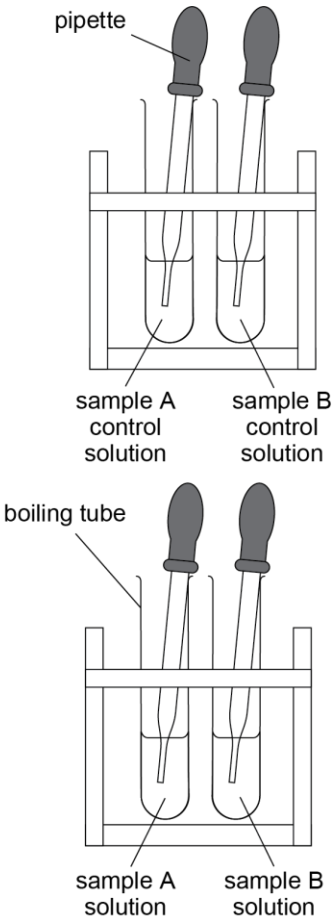
Substance	Hazard	First aid
Agar powder	Allergen	If inhaled, move person into fresh air. In case of skin contact, wash off with soap and plenty of water. In case of eye contact, flush eyes with water as a precaution. If swallowed, rinse mouth with water.
Agar cultures	Biohazard	**Spills of cultures:** Place paper towels over the spill, pour disinfectant (e.g., *Virkon*) on top and leave for at least 15 minutes.
Iodine solution [0.1 mol/dm^3]	 GHS09 (*hazardous to the aquatic environment* **N**)	**In the eye:** Flood the eye with gently-running tap water for 15 min. See a doctor.

Substance	Hazard	First aid
	Eyes: causes eye irritation. **Inhalation:** may be harmful if inhaled; causes respiratory tract irritation. **Skin:** may be harmful if absorbed through skin; causes skin irritation **Ingestion:** may be harmful if swallowed	**Vapour breathed in:** Take casualty to fresh air. Call a doctor if breathing is even slightly affected. **Swallowed:** Do no more than wash out the mouth with water. Do not induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor. **Spilt on the skin or clothing:** Remove contaminated clothing. Drench the skin with plenty of water. If a large area is affected or blistering occurs, see a doctor. **Spilt on the floor, bench, etc.,:** Ventilate the room. For small amounts, use a damp cloth. Rinse well. For larger amounts, cover with mineral absorbent (e.g. cat litter) and scoop into a bucket.
Universal indicator	 GHS02 (*flammable* **F**)	**In the eye:** Flood the eye with gently-running tap water for 10 min. See a doctor if pain persists. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor. **Spilt on the skin or clothing:** Brush compound off contaminated clothing. Rinse clothing or the skin as necessary **Spilt on the floor, bench, etc.:** Brush up compound spills, trying to avoid raising dust, then wipe with a damp cloth.
Glassware	Risk of cuts due to sharps, e.g. broken glass or scalpels. Wounds can lead to infection, especially if the blade or point is contaminated.	**Minor cuts:** Rinse the wound with water. Get the casualty to apply a small, sterile dressing. **Severe cuts**: Lower the casualty to the floor. Raise the wound as high as possible. If feasible, ask the casualty to apply pressure on or as close to the cut as possible, using fingers, a pad of cloth or, better, a sterile dressing (adding further layers as necessary). If the casualty is unable to do so, apply

Substance	Hazard	First aid
		pressure yourself, protecting your skin and clothes from contamination by blood if possible. Leave any embedded large bodies and press around them. Send for a first aider.
Bunsen burner	Burns	Flood burnt area with water for at least 10 minutes. For serious injuries see a doctor.

Experiment set-up





How to make the milk-agar plate

1. Dissolve 2 g of dried milk powder in 20 cm^3 of distilled water.
2. Add 2 g of agar powder in 80 cm^3 of distilled water. Heat the mixture and stir frequently to dissolve the agar completely.
3. Transfer the milk solution to the hot agar solution. Stir to mix.
4. When the milk-agar solution is cooled down to 45-50°C, pour it into clean petri dishes. Replace the lids and allow the plates to cool and set.

How to make the starch-agar plate

1. Add 2 g of agar powder in 50 cm^3 of distilled water. Heat the mixture and stir frequently to dissolve the agar completely.
2. Heat 1 g of soluble starch in 50 cm^3 of distilled water to form a colloidal solution.
3. Allow to cool and then mix with the agar solution and a few drops of iodine solution. Stir thoroughly.

4. When the starch-agar solution is cooled down to 45-50°C, pour it into clean petri dishes. Replace the lids and allow the plates to cool and set.

How to prepare the oil-agar plate

1. Add 2 g of agar powder in 85 cm^3 of distilled water. Heat the mixture and stir frequently to dissolve the agar completely.
2. Add 10 cm^3 vegetable oil and 5 cm^3 sodium hydrogen carbonate solution after the mixture has cooled for 5 minutes. Stir thoroughly.
3. When the oil-agar solution is cooled down for another 5 minutes to 45-50°C, add a few drops of universal indicator, stir thoroughly and pour it into clean petri dishes. Replace the lids and allow the plates to cool and set.

How to prepare the washing powder solutions

Add 5 g of each washing powder to 50 cm^3 of warm distilled water in a conical flask. Mix thoroughly by swirling and allow to settle, before decanting into the beakers used in the investigation. If the suspension is difficult to separate, pass through a filter paper in a filter funnel.

Teacher method

This is your version of the method for this experiment that accompanies the *Teacher walkthrough* video.

Do not share this method with learners. Give them Worksheet G1 (Lab lesson) or Worksheet G2 (Virtual Lab lesson).

Before you begin

Because of the variability in performance of washing powders worldwide, you should **run the experiment yourself** before the lab lesson to see how the agar plates react, and adjust incubation timings accordingly. A measurable result might take an hour, or substantially longer, possibly requiring plates to be kept overnight to incubate.

Plan how you will group your learners during the experiment session.

Think about:

- the number of groups you will need (group size 2–4 learners)
- the amount of equipment/chemicals required
- whether you are testing more than one carbonated drink.

Experiment

Walk around the learners during the experiment in case they encounter any difficulties.

Step	Notes
Prepare three pairs of agar plates, one pair for each of the three types of molecule: starch, protein and lipid.	One pair of plates contains agar with milk powder, which contains a white powder called casein. Another pair contains agar mixed with starch powder and iodine solution, to show the presence of starch. It is blue-black in colour. The final pair contains agar and vegetable oil with a small volume of alkali called sodium hydrogen carbonate. These also contain universal indicator solution.
Place the end of the cork borer into a Bunsen burner flame for a few seconds.	This ensures that the cork borer is aseptic before coming into contact with the agar. Keeping the plate closed minimises the opportunity for microorganisms to enter the plate, which could grow and interfere with the measurement of the results later.
Cut three small wells for each washing powder by gently forcing the cork borer into the agar. Use a dropping pipette to add the washing powder solutions to three of the four wells on each side of the plates.	Three wells containing the same washing powder solution will allow for the collection of repeats. Take care to ensure that the volume of washing powder added to the wells is just enough to fill them without spilling over the sides.

In the same way, cut another small well for each washing powder in the middle of the plate. Label this 'C.' Place a sample of each of the boiled washing powder solutions into these control wells of each plate.	The well in the centre will act as the control. The high temperature denatures the enzymes in the washing powder solutions, which removes the effect of the independent variable in this investigation.
Repeat this twice more for each plate, using the same washing powder solution.	It is important to ensure that a different pipette is used to transfer each washing powder to avoid cross-contamination.
Place the lid onto the plate unless being used.	The site of incubation must be free of disturbance so that the solutions remain within the wells and do not spill out.
Place the plates into a tray and on a shelf for incubation.	The site of incubation must be free of disturbance so that the solutions remain within the wells and do not spill out.
Allow around 1 hour for the plates to incubate.	This allows for the diffusion of the washing powder solution into the agar, and the enzyme-catalysed breakdown of substrate molecules.
After some time, 'halos' or rings of colour change appear around some of the wells containing the washing powder solutions.	The 'zone of digestion' represents the area of the agar that contains digested molecules: • As the starch is broken down by the amylase in the washing powder into simple sugars, the blue-black colour becomes colourless. • For the protein-agar plate, the white milk protein casein is digested by the proteases in the washing powder into amino acids, which decolourises the milk. • As the oil is broken down into fatty acids and glycerol by the lipases in washing powder, the fatty acids neutralise the sodium hydrogen carbonate. This causes the indicator to decolourise. Note that these results aren't exactly as would be expected with the indicators used on each plate – consider the other constituents of washing powder (acids, bleaches etc.)
Using a ruler with millimetre measurements, measure the diameter of each of the zones of digestion and record the results.	The zone of digestion is proportional to the distance from the centre of the well to the outer limit of the circle that has changed colour or decolourised.

Clean-up

After the experiment learners should:

- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up
- return all equipment and any unused chemicals to you.

The Petri dishes should be disposed of and sealed in bin bags.

Lab lesson: Option 2 – virtual experiment

Resources	• Teacher Instructions 3 • Worksheets E1 (from last lesson), E2 and G2
Learning objectives	By the end of the lesson: • **all learners** should be able to investigate the ability of enzymes found in washing powder to digest their substrates. • **most learners** should be able to evaluate the effectiveness of a method used to investigate the ability of enzymes found in washing powder to digest their substrates. • **some learners** will be able to suggest how an investigation into the ability of enzymes found in washing powder to digest their substrates can be modified.

Timings	Activity
10 min	**Starter/Introduction** Ask learners to swap their completed Worksheet E1 with a partner. Then hand out Worksheet E2 to all learners. Ask learners how their tables compare with the actual table that they will be using today. It is likely that learners have put the independent and dependent variables in inappropriate columns and/ or did not include reference to a control or to repeated data.
40 min	**Main lesson** Provide learners with Worksheet G2, which lists the method but which has some key terms missing. Play the video and challenge learners to determine the missing words as they watch. Ask learners to suggest which aspects of the method would be problematic to carry out and to make a note of these as they go. They should write down possible solutions that they could employ to these problems, if they were to perform the practical again. This will develop their evaluative skills and prepare them for the task in the *Debriefing Lesson*. You may wish to play the video twice in order to enable learners to check their work. **Safety** Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate.
10 min	**Plenary** Provide pairs of learners with sample data tables and ask them to record the mean diameter for each zone of digestion on a common class spreadsheet (e.g. a shared *Google sheet*) or on the whiteboard. Teacher Instructions 3 provides sample data tables that can be cut out and distributed.

Debriefing lesson: Improving investigations

Resources	• Teacher Instructions 4 • Worksheet H – K
Learning objectives	By the end of the lesson: • **all learners** should be able to understand the importance of some considerations in an experiment to the quality of the data. • **most learners** should be able to explain why some considerations in an experiment enhance the quality of the data. • **some learners** will be able suggest new considerations in an experiment to enhance the quality of the data.

Timings	Activity
10 min	**Starter/Introduction** Give pairs of learners Worksheet H, which shows a set of scores from a competition between five archers. Give learners a couple of minutes to consider the five scores, then ask 'Who was the best archer and why?' Have a class discussion and make sure you bring in the following five key terms that are important when considering the quality of data collected in a scientific investigation: validity, reliability, accuracy, random errors and systematic errors; make sure learners understand what each term means. To emphasise how this ties in with scientific experiments, explain that 'hitting the centre target' is equivalent to obtaining the true value through an investigation that involves repeats, precise use of calibrated equipment and controlling other factors that would otherwise invalidate the results. Give each learner a copy of Worksheet I and ask them to consider the answers to each question on their own, before sharing their answers in a whole-class discussion. Resolve any misconceptions.
40 min	**Main lesson** Divide learners into groups of three. Provide each learner Worksheet J, which lists an alternative method that could be used to investigate the effectiveness of washing powders. Outline how they need to choose one option from each pair of alternatives given, stating if the choice would enhance the validity (V), reliability (R) or accuracy (A) of the data they collect and how this would strengthen a conclusion. They must justify their choices. The questions posed at the end of the worksheet help learners understand what is meant by the independent variable, dependent variable, and standardised variables in an investigation. During the activity, provide learners with an opportunity to seek support if they encounter difficulty. This can be done by producing a series of 'clue cards,' available on request. These are provided on Teacher Instructions 4. If a learner feels like they need support, they can request a card from the teacher. Each card provides a 'hint' that is intended to give the learner just enough information to help them move on with their work.

Plenary

Divide the class into three, placing roughly one member from each group into the 'Accuracy' (error) group, one into the 'Reliability' group and the final member into the 'Validity' group. Issue Worksheet K to all learners. This provides prompts to encourage learners to consider the problems in the method that limited their given quality, and alternatives to… if they have any time, they should be asked to consider extensions… etc.

Ask the learners to get back into their original groups and discuss each of the other plans that they've learned about. Summarise the lesson by highlighting the most important points, before asking learners to complete the final task on Worksheet H (evaluating their own plan).

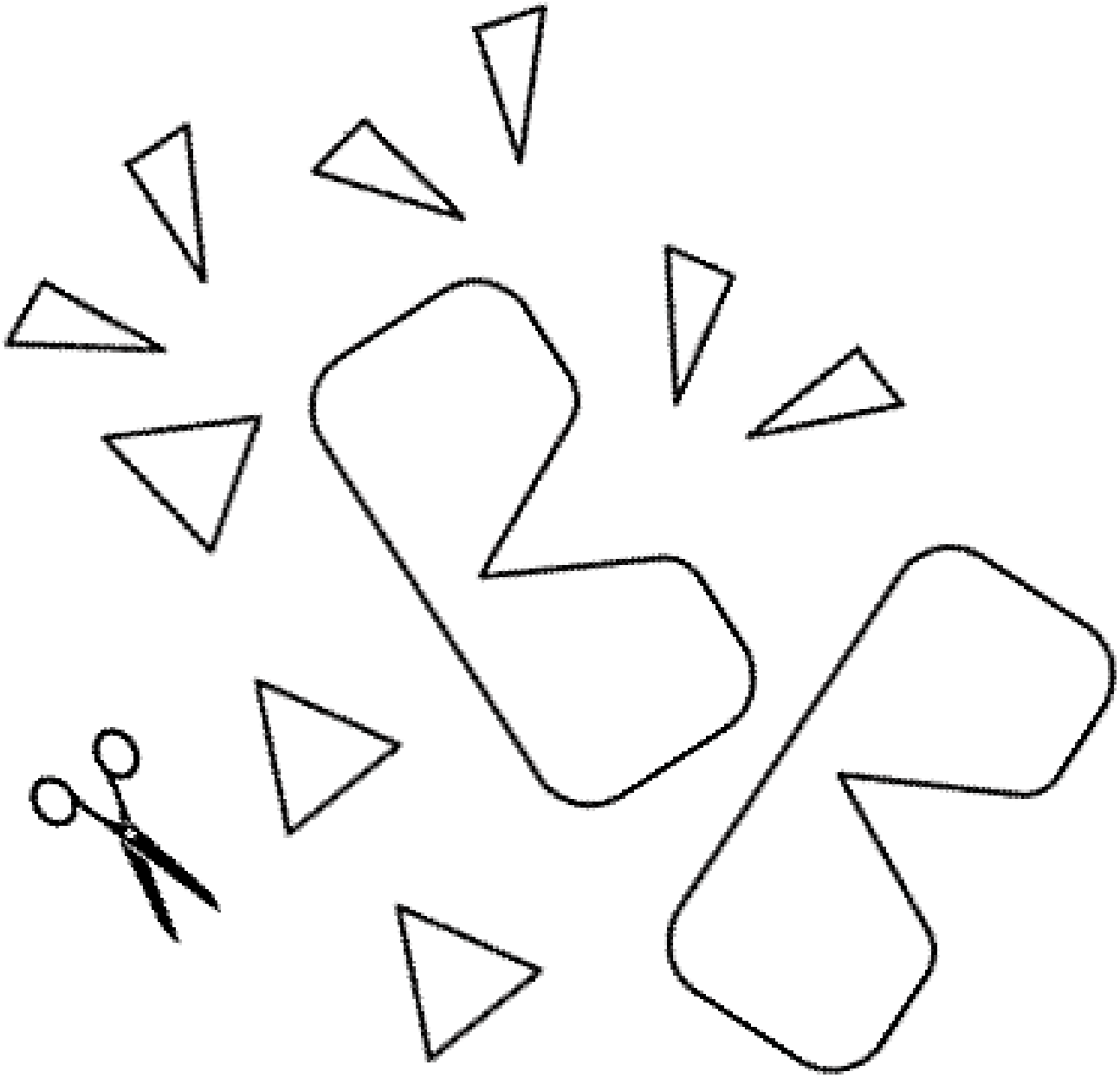
Worksheets and answers

	Worksheets	Answers
For use in the *Briefing lesson:*		
Teacher Instructions 1: Molecular puppet show	**20**	**N/A**
Teacher Instructions 2: Glossary for 'bingo' activity	**x**	**N/A**
A: Qualitative and quantitative data	**x**	**x**
B: Choosing equipment	**x**	**x**
C: Equipment set-up	**x**	**x**
D: Steps and rationales	**x**	**x**
E1: Designing a data table	**x**	**x**
F: Bingo	**x**	**x**
For use in *Lab lesson: Option 1:*		
E2: Data table	**x**	**x**
G1: Method	**x**	**x**
For use in *Lab lesson: Option 2:*		
Teacher Instructions 3: Sample data tables	**x**	**N/A**
E2: Data table	**x**	**x**
G2: Missing method statements	**x**	**x**
For use in the *Debriefing lesson:*		
Teacher Instructions 4: Clue cards	**x**	**N/A**
H: Aiming for accurate data 1	**x**	**x**
I: Aiming for accurate data 2	**x**	**x**
J: Group collaborations	**x**	**x**

Teacher Instructions 1: Molecular puppet show

This activity serves to remind learners of the mechanism of an enzyme-catalysed reaction.

Print this sheet on single-sided paper and cut out the shapes below. Project them onto the board using an overhead projector (OHP). Alternatively, photocopy enough of these sheets to provide each pair of learners. Follow the instructions overleaf.

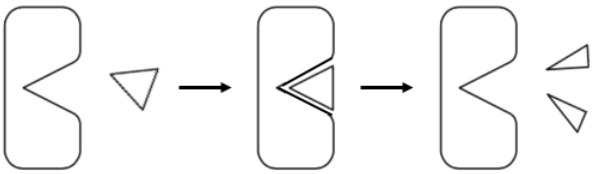


Teacher Instructions 1: Molecular puppet show *continued*

Guidance

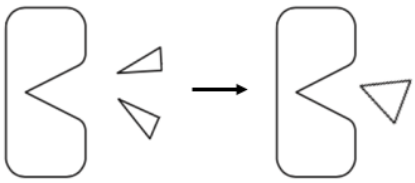
The cut-out shapes could be arranged in the following ways to demonstrate how an enzyme-catalysed reaction occurs and how it can be followed.

1. Formation of an enzyme-substrate complex relies on the complementary shape of the active site and the substrate:



2. The formation of an enzyme-substrate complex reduces the activation energy required to initiate a biological reaction:

3. The enzyme is not chemically changed by the biological reaction and another substrate can be catalysed:



4. The progress of an enzyme-catalysed reaction can be followed by assessing the colour of the reaction mixture:

when iodine is mixed with amylase, this mixture is blue/ black in colour 	when iodine is mixed with amylase, this mixture is red-brown in colour 
when milk powder is mixed with protease, this mixture is white in colour 	when milk powder is mixed with protease, this mixture is colourless 
when universal indicator is mixed with lipase in the presence of oil, this mixture is green in colour 	when universal indicator is mixed with lipase in the presence of oil, this mixture is yellow/orange in colour 

Teacher Instructions 2: Glossary for 'bingo' activity

Scientific terminology

Term	Definition (read this statement to the class)
enzyme	*proteins that function as biological catalysts*
substrate	*the substance on which an enzyme acts*
enzyme-substrate complex	*forms when a substrate enters the active site of an enzyme*
products	*the substance formed in an enzyme-controlled reaction*
activation energy	*required in order for a chemical reaction to begin*
amylase	*an enzyme which breaks down starch to maltose*
protease	*an enzyme that catalyses the breakdown of proteins*
lipase	*an enzyme that digests fats (lipids) to fatty acids and glycerol*
active site	*the part of an enzyme molecule into which its substrate fits*
denature	*an enzyme is said to be this when the molecule has changed shape so much that the substrate can no longer fit into its active site*

Investigative terminology

Term	Definition (read this statement to the class)
accurate	*data that is close to the actual value*
valid	*data that has been obtained in an investigation that has only one factor that was changed*
reliable	*data that is a mean value calculated from a number of consistent readings*
control	*an experiment in which the effect of the independent variable has been removed*
independent variable	*the factor that is changed during the investigation*
dependent variable	*the factor that is measured during the investigation*
standardised variable	*a factor that is kept constant during the investigation*
interval	*the 'gap' between the values of the independent variable*
range	*the difference between the highest and lowest values of the independent variable*
anomalous	*data that is very different from the expected or other values*

Teacher Instructions 3: Sample data

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	24	24	22	
Starch	B	23	26	25	
Protein	A	21	19	23	
Protein	B	20	21	21	
Lipid	A	13	14	12	
Lipid	B	13	14	10	

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	22	25	24	
Starch	B	27	29	30	
Protein	A	25	20	24	
Protein	B	23	23	22	
Lipid	A	13	11	12	
Lipid	B	10	10	8	

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	27	24	24	
Starch	B	28	24	25	
Protein	A	22	19	20	
Protein	B	16	18	20	
Lipid	A	17	14	15	
Lipid	B	16	14	16	

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	24	26	28	
Starch	B	23	24	27	
Protein	A	22	18	23	
Protein	B	19	23	22	
Lipid	A	12	11	12	
Lipid	B	10	8	11	

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	24	21	20	
Starch	B	26	24	25	
Protein	A	20	19	21	
Protein	B	16	20	20	
Lipid	A	14	11	12	
Lipid	B	15	16	10	

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A	29	25	27	
Starch	B	25	26	29	
Protein	A	24	19	24	
Protein	B	21	22	25	
Lipid	A	10	9	13	
Lipid	B	12	14	10	

Teacher Instructions 4: Clue cards

ACCURACY Consider why **aseptic** techniques were followed.	**ACCURACY** Consider why the wells were cut **far apart** from each other.	**ACCURACY** Consider why care was taken to **avoid disturbing** the plates during incubation.
RELIABILITY Consider **how many** wells were cut for each washing powder	**RELIABILITY** Consider why a **mean** was calculated.	**RELIABILITY** Consider whether the **repeated measurements** are consistent or are very different.
VALIDITY Consider the **control well**. What was the purpose of adding boiled washing powder solution?	**VALIDITY** Consider the features of the plates that were **kept the same**.	**VALIDITY** Consider whether there were any other factors that were **not standardised** during this experiment

Worksheet A: Qualitative & quantitative data

Read the five research questions provided in the table below.

experiment	research question: how does changing the…
1	light intensity affect the rate of photosynthesis in pond weed?
2	concentration of sodium chloride solution affect the mass of potato cylinders?
3	concentration of starch affect the intensity of the blue/ black colour of a solution?
4	mass of sodium chloride ingested affect the intensity of the feeling of thirst?
5	environmental pH affect the proportion of cress seeds that germinate?

List the numbers of the experiments that are likely to give **qualitative** data and which are likely to give **quantitative** data. Use the space below the table to reflect on whether more experiments in Biology give qualitative or quantitative data, and in which experiments the most accurate data can be obtained.

Qualitative	Quantitative
Experiment numbered: _____	Experiment numbered: _____

……

……

……

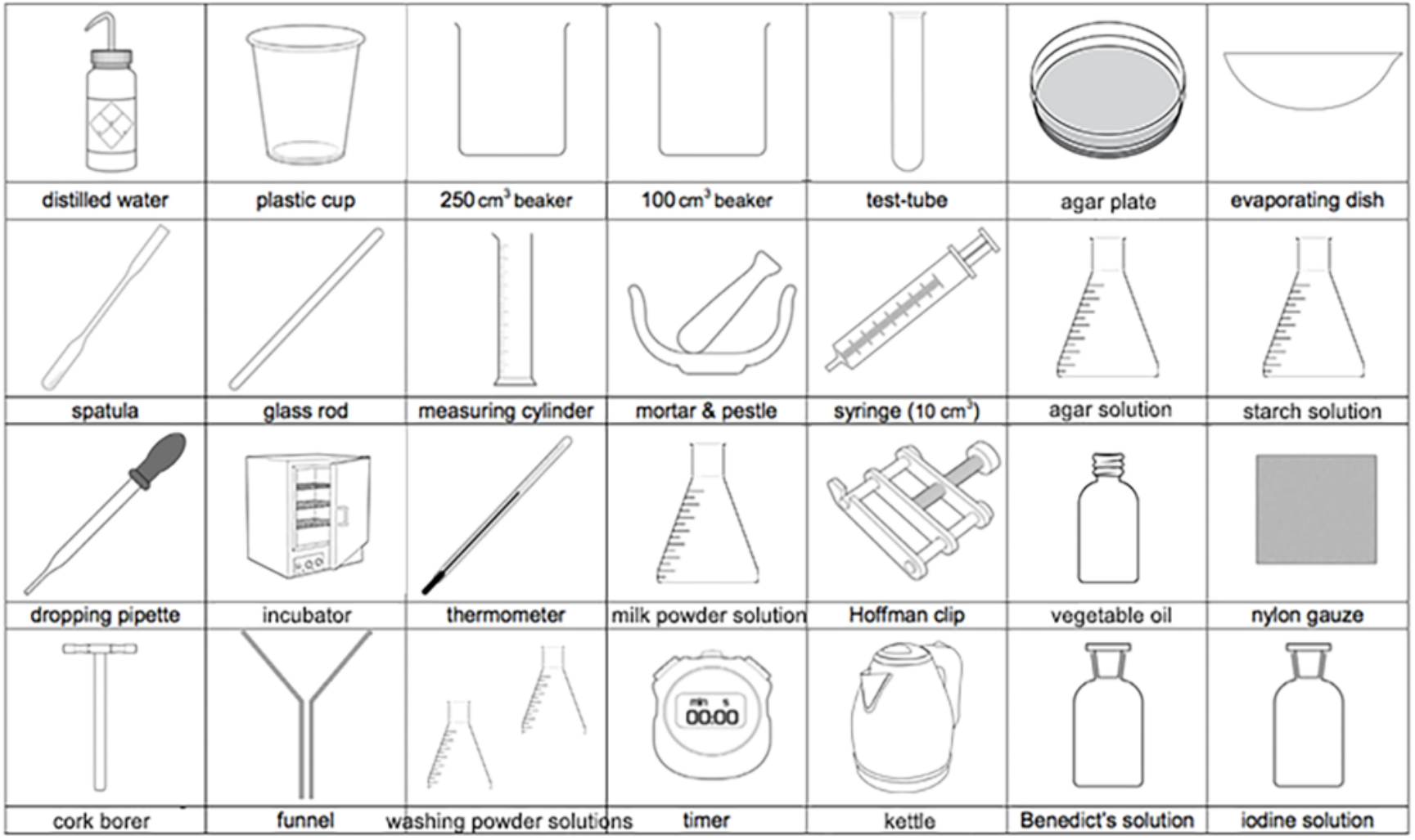
……

……

……

Worksheet B: Choosing equipment

Here is some typical laboratory apparatus available to you. Note that you will not need to use all items.



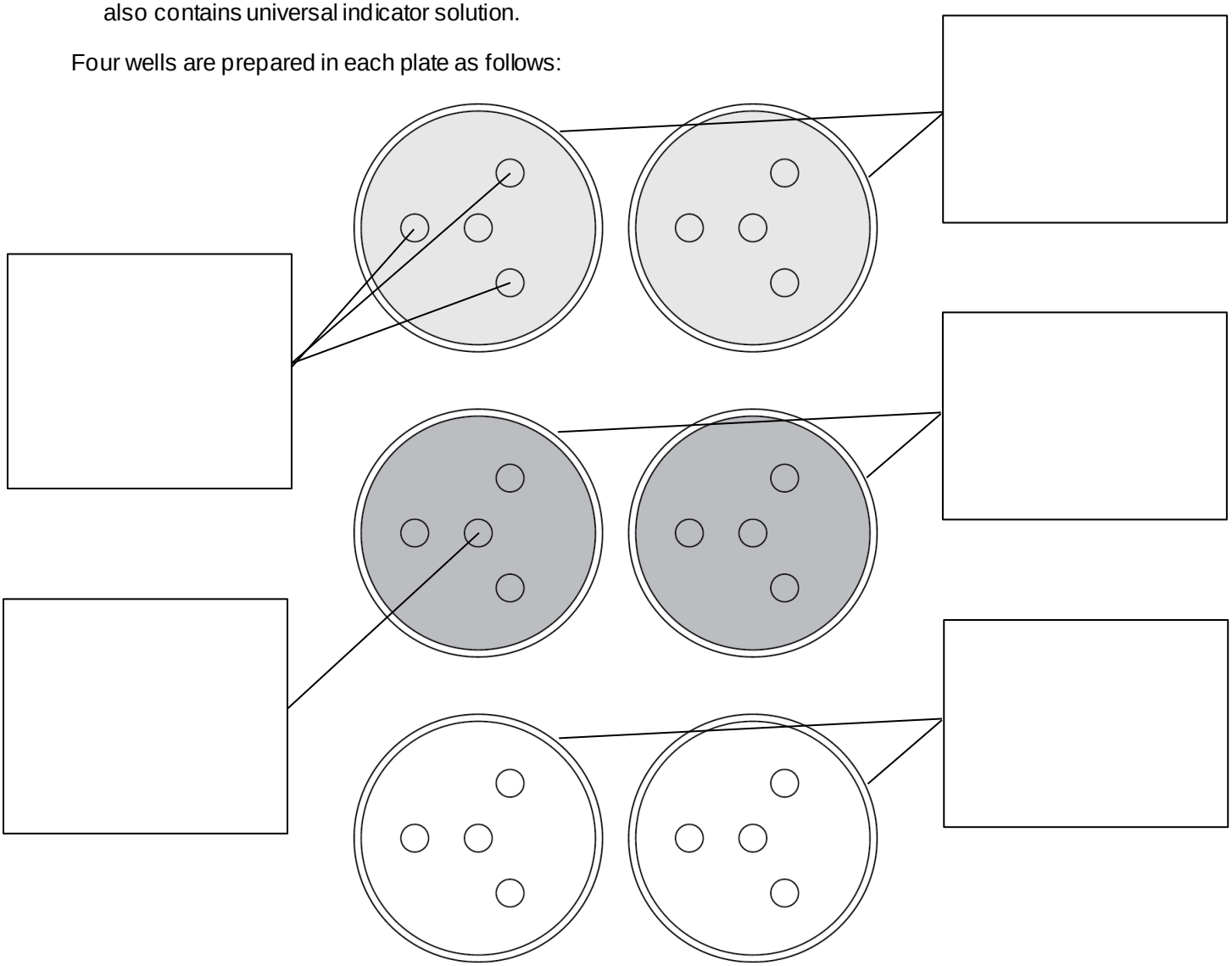
Worksheet C: Equipment set-up

This investigation uses agar plates of three types to investigate two different washing powder solutions, Brand A and Brand B. These can be produced by heating a solid agar powder in water and allowing the mixture to cool slightly before pouring into Petri dishes.

For the purposes of this investigation, three pairs of agar plates are prepared:

1. One pair contains milk powder, which contains a white powder called casein.
2. Another pair contains starch powder and iodine solution, to show the presence of starch. It is blue-black in colour.
3. The final pair contains vegetable oil and a small volume of alkali called sodium hydrogen carbonate. It also contains universal indicator solution.

Four wells are prepared in each plate as follows:



Consider your original plan. How is the arrangement of apparatus similar to your design? How is it different? How would arranging the apparatus in this way improve the quality of the investigation?

Worksheet D: Steps and rationales

Complete the table below by inferring the missing step or rationale from the method.

Number	Step	Rationale
1	Prepare three pairs of agar plates, one pair for each of the three types of molecule: starch, protein and lipid.	
2		This ensures that the cork borer is aseptic before coming into contact with the agar.
3	Cut three small wells for each washing powder by gently forcing the cork borer into the agar. Use a dropping pipette to add the washing powder solutions to three of the four wells on each side of the plates.	
4		The well in the centre will act as the control. The high temperature denatures the enzymes in the washing powder solutions, which removes the effect of the independent variable in this investigation.
5	Repeat this twice more for each plate, using the same washing powder solution.	
6		Keeping the plate closed minimises the opportunity for microorganisms to enter the plate, which could grow and interfere with the measurement of the results later.
7	Place the plates into a tray and on a shelf for incubation.	
8		This allows for the diffusion of the washing powder solution into the agar, and the enzyme-catalysed breakdown of substrate molecules.
9	After some time, ‘halos’ or rings of colour change appear around some of the wells containing the washing powder solutions.	
10		The zone of digestion is proportional to the distance from the centre of the well to the outer limit of the circle that has changed colour or decolourised.

Worksheet E1: Designing a data table

A good table:

- Contains an appropriate number of columns and rows.
- Is drawn with ruled lines and has a full border.
- Places the independent variable (the factor that is changed) into the left-most column.
- Includes units only in the headings of the table, and never in the table body.
- Contains numerical values that have the same number of decimal places.
- Has a column that records the mean values of repeated data.

For homework, use the space below to prepare a table in advance of the *Lab lesson*. Use the information above to help you.

Worksheet E2: Data table

Use the table below to record your data during this investigation:

Substance	Washing powder brand	Width of zone of digestion /mm			
		Well 1	Well 2	Well 3	Mean
Starch	A				
Starch	B				
Protein	A				
Protein	B				
Lipid	A				
Lipid	B				

If you finish collecting your data before other members of your class, consider the **graph** that you will plot:

- Bar chart or line graph? Why?
- Which labels (and units) should be on the x- and y-axes?
- What the most common mistakes regarding presentation that students make when drawing such a graph?

Worksheet F: Bingo

This sheet contains three grids, which is enough for three different learners.

Select three words from the first table and three words from the second table and copy them here:

If your words are described, cross them out. If you cross out all six, shout 'bingo!'

Select three words from the first table and three words from the second table and copy them here:

If your words are described, cross them out. If you cross out all six, shout 'bingo!'

Select three words from the first table and three words from the second table and copy them here:

If your words are described, cross them out. If you cross out all six, shout 'bingo!'

Worksheet G1: Method

Follow the method below during the *Virtual Lab lesson*.

1. Prepare three pairs of agar plates, one pair for each of the three types of molecule: starch, protein and lipid.
2. Place the end of the cork borer into a Bunsen burner flame for a few seconds.
3. Cut three small wells for each washing powder by gently forcing the cork borer into the agar.
4. In the same way, cut another small well for each washing powder in the middle of the plate. Label this 'C.'
5. Place the lid back onto the plate.
6. Prepare the control washing powder solutions by heating a small volume of each in separate test tube by placing into a water bath at 95 °C for at least five minutes.
7. Use a dropping pipette to add the washing powder solutions to three of the four wells on each side of the plates.
8. Repeat this twice more for each plate, using the same washing powder solution.
9. Place a sample of each of the boiled washing powder solutions into the control wells of each plate, which have been labelled 'C.'
10. After the washing powder solutions have been put into every well, place the plates into a tray and on a shelf for incubation.
11. Allow around 1 hour for the plates to incubate.
12. 'Halos' or rings of colour change appear around some of the wells containing the washing powder solutions. Using a ruler with millimetre measurements, measure the diameter of each of the zones of digestion and record the results.

Worksheet G2: Missing method statements

Watch the video during the *Virtual Lab lesson* and use this to help you complete the method below by finding the missing words and phrases.

1. Prepare three pairs of agar plates, one pair for each of the three types of molecule: _____, _____ and _____.

2. Place the end of the cork borer into a _____ _____ _____ for a few seconds.

3. Cut three small wells for each washing powder by gently forcing the _____ _____ into the agar.

4. In the same way, cut another small well for each washing powder in the middle of the plate. Label this _____.

5. Place the _____ back onto the plate.

6. Prepare the control washing powder solutions by _____ a small volume of each in separate test tube by placing into a _____ _____ at 95 °C for at least _____ minutes.

7. Use a dropping pipette to add the washing powder solutions to _____ of the four wells on each side of the plates.

8. Repeat this _____ more for each plate, using the same washing powder solution.

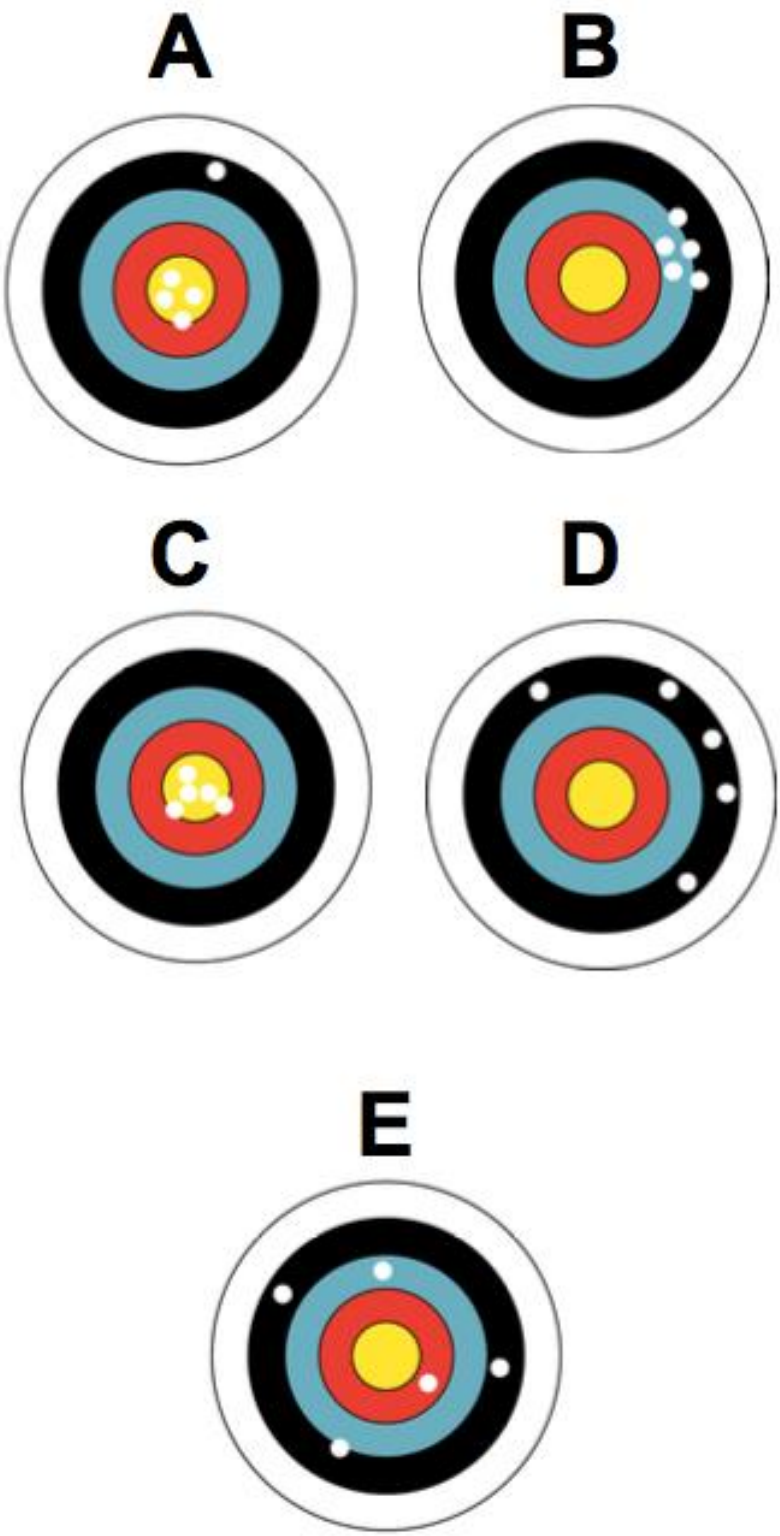
9. Place a sample of each of the _____ washing powder solutions into the control wells of each plate, which have been labelled 'C.'

10. After the washing powder solutions have been put into every well, place the plates into a tray and on a shelf for _____.

11. Allow around _____ for the plates to incubate.

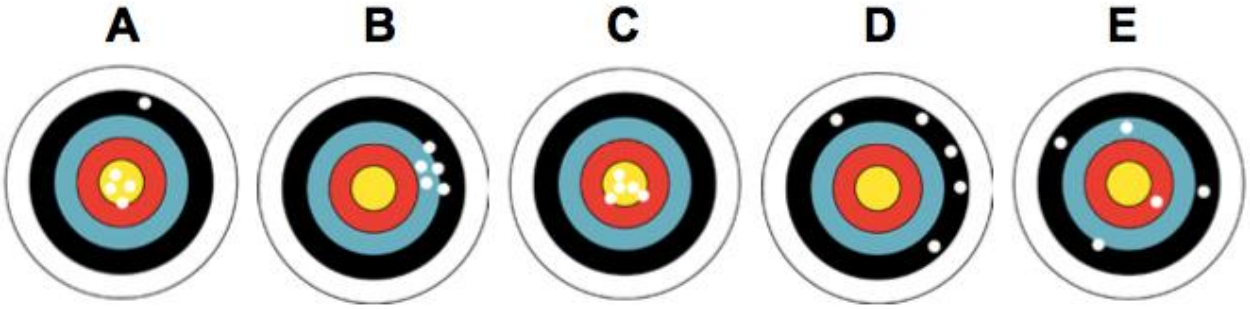
12. 'Halos' or rings of colour change appear around some of the wells containing the washing powder solutions. Using a ruler with millimetre measurements, measure the _____ of each of the _____ _____ _____ and record the results.

Worksheet H: Aiming for accurate data 1



Worksheet I: Aiming for accurate data 2

In a competition, five archers stand 20 metres from a target. Their aim is to hit the central 'bullseye' with five arrows. The results of the competition are shown below.



To answer the following questions, assume that hitting the centre of the target is equivalent to obtaining the actual (true) value for a piece of data in a scientific investigation.

1. Which archer had the most **precise** technique for shooting their arrows and fired the most **accurate** shots? Explain your choice.

2. Identify which two archers obtained the most **reliable** results with their technique. Explain your choice.

3. Archer A stood 1 metre closer to the target than the other four archers. Explain why this would **invalidate** any comparisons of the scores.

Worksheet J: Group collaborations

Use Worksheet I to help you distinguish between the terms **valid**, **accurate** and **reliable**. Your teacher will instruct you which team to join. Draw a box around the word that applies to your team.

The purpose of this exercise is to explore the quality of this investigation and whether the results can be accepted. Discuss the following questions in your team.

1. State a definition of this word. **Complete the following sentence: 'accurate/ reliable/ valid data is…'** (delete as appropriate)

……

2. Explain how the design of **this** investigation took into account this quality.

……

3. Suggest **two** extensions that could be undertaken to your investigation.

1……
2……

Extension questions for discussion
A student carries out a practical similar to yours but instead focuses only on the activity of protease. The student hopes to find the optimum temperature of the protease.

1. Outline a method that would enable the student to investigate this research question. Refer to safety precautions in your answer. [5 marks]

2. The student finds that the optimum temperature of the protease is 40 °C. Explain why it would be preferable for enzymes used in washing powders to have optimum temperature of less than this. [2 marks]

3. The student found that the control wells have a very small halo around them after the incubation period. Suggest what this suggests and how the student should deal with this. [3 marks]

4. The student carries out some research and finds that one brand of biological washing powder contains encapsulated form in which they are bound inside gel beads. Suggest **two** reasons why enzymes in biological washing powders are often added to the detergent in encapsulated form instead of powdered form. [2 marks]

Worksheet A: Answers

The experiments that are likely to give **qualitative** data are:

- Experiment 3: measuring the intensity of the blue/ black colour of a starch solution.
- Experiment 4: measuring the intensity of thirst.

The experiments that are likely to give **quantitative** data are:

- Experiment 1: counting the number of bubbles of oxygen / measuring the volume of oxygen using a gas syringe.
- Experiment 2: measuring the change in mass of the potato cylinder.
- Experiment 5: counting the number of cress seeds that germinate.

Learners should reflect on the fact that most experiments conducted in Biology give quantitative measurements. They may also point out that the accuracy of the measurement of the dependent variable is usually higher when a quantitative measurement is obtained. This is because subjectivity is required on the part of the experimenter to judge a colour or feeling.

Worksheet D: Answers

Number	Step	Rationale
1	Prepare three pairs of agar plates, one pair for each of the three types of molecule: starch, protein and lipid.	One pair of plates contains agar with milk powder, which contains a white powder called casein. Another pair contains agar mixed with starch powder and iodine solution, to show the presence of starch. It is blue-black in colour. The final pair contains agar and vegetable oil with a small volume of alkali called sodium hydrogen carbonate. These also contain universal indicator solution.
2	Place the end of the cork borer into a Bunsen burner flame for a few seconds.	This ensures that the cork borer is aseptic before coming into contact with the agar.
3	Cut three small wells for each washing powder by gently forcing the cork borer into the agar. Use a dropping pipette to add the washing powder solutions to three of the four wells on each side of the plates.	Three wells containing the same washing powder solution will allow for the collection of repeats. Take care to ensure that the volume of washing powder added to the wells is just enough to fill them without spilling over the sides.
4	In the same way, cut another small well for each washing powder in the middle of the plate. Label this 'C.' Place a sample of each of the boiled washing powder solutions into these control wells of each plate.	The well in the centre will act as the control. The high temperature denatures the enzymes in the washing powder solutions, which removes the effect of the independent variable in this investigation.
5	Repeat this twice more for each plate, using the same washing powder solution.	It is important to ensure that a different pipette is used to transfer each washing powder to avoid cross-contamination.
6	Place the lid onto the plate unless being used.	Keeping the plate closed minimises the opportunity for microorganisms to enter the plate, which could grow and interfere with the measurement of the results later.
7	Place the plates into a tray and on a shelf for incubation.	The site of incubation must be free of disturbance so that the solutions remain within the wells and do not spill out.
8	Allow around 1 hour for the plates to incubate.	This allows for the diffusion of the washing powder solution into the agar, and the enzyme-catalysed breakdown of substrate molecules.

Worksheet D: Answers *continued*

9	After some time, 'halos' or rings of colour change appear around some of the wells containing the washing powder solutions.	The 'zone of digestion' represents the area of the agar that contains digested molecules: • As the starch is broken down by the amylase in the washing powder into simple sugars, the blue-black colour becomes colourless. • For the protein-agar plate, the white milk protein casein is digested by the proteases in the washing powder into amino acids, which decolourises the milk. As the oil is broken down into fatty acids and glycerol by the lipases in washing powder, the fatty acids neutralise the sodium hydrogen carbonate. This causes the indicator to decolourise.
10	Using a ruler with millimetre measurements, measure the diameter of each of the zones of digestion and record the results.	The zone of digestion is proportional to the distance from the centre of the well to the outer limit of the circle that has changed colour or decolourised.

Worksheet G2: Answers

The missing words are underlined in the following passage.

Prepare three pairs of agar plates, one pair for each of the three types of molecule: starch, protein and lipid.

Place the end of the cork borer into a Bunsen burner flame for a few seconds.

Cut three small wells for each washing powder by gently forcing the cork borer into the agar.

In the same way, cut another small well for each washing powder in the middle of the plate. Label this 'C.'

Place the lid back onto the plate.

Prepare the control washing powder solutions by heating a small volume of each in separate test tube by placing into a water bath at 95 °C for at least five minutes.

Use a dropping pipette to add the washing powder solutions to three of the four wells on each side of the plates.

Repeat this twice more for each plate, using the same washing powder solution.

Place a sample of each of the boiled washing powder solutions into the control wells of each plate, which have been labelled 'C.'

After the washing powder solutions have been put into every well, place the plates into a tray and on a shelf for incubation.

Allow around 1 hour for the plates to incubate.

'Halos' or rings of colour change appear around some of the wells containing the washing powder solutions. Using a ruler with millimetre measurements, measure the diameter of each of the zones of digestion and record the results.

Worksheet I: Answers

1. Archer C.
The results were the most accurate as all five arrows hit the target (recorded a reading close to the true value), suggesting that their technique was the most precise.

2. Archers B and C.
For both, their arrows hit their target in roughly the same area each time (they got the same result each time) suggesting their technique was the most reliable.

3. Archer A.
The closer an archer is to the target, the more precise their aim can be as they can see the target more clearly and the arrow has less far to travel. As Archer A was closer to the target, they had an unfair advantage over the other archers; their technique and the distance from the target affect the shot here. Therefore, comparison of the scores obtained using their technique against those using the technique of the other archers would not be valid. (The distance from the target is a variable that needs to be standardised so that only the dependent variable (archer technique) is being investigated.)

Worksheet J: Answers

1. Either: Accurate data is close to the actual value of a measured factor.
Or: Reliable data is calculated from a mean value of several measurements of a factor.
Or: Valid data is obtained in an investigation in which all other factors are standardised.

2. Either: A ruler with millimetre measurements/ graph paper was used to estimate the diameter of the zone of digestion (accuracy).
Or: Each enzyme was placed into three wells on the same plate, and the mean diameter of the zone of digestion was calculated (reliability).
Or: The three wells were made in the same agar/ the plate was incubated at the same temperature/ the same volume of washing powder solution was placed into each well/ the three wells were incubated for the same time (validity).

3. Suggestions include:
- Repeat with a different type of amylase/ lipase/ protease
- Repeat with another type of enzyme (e.g. nuclease)
- Conduct at a lower temperature to assess whether the enzymes are as effective as the manufacturer claims at low washing temperatures.

Answers to extension questions:

1. The student could prepare a series (e.g. 5) of agar plates containing the same concentration of milk powder in agar. Four wells could be made in each plate, with the central well holding boiled washing powder solution to act as the control. The same volume of washing powder solution of equal concentration is then placed into each of the three wells on each plate. At the same time, the plates are placed into separate incubators set at a range of temperatures between 20 °C and 60 °C (chosen on the basis of the manufacturer's suggestions) at the same temperature for the same time. The diameter of the zone of digestion of each well is recorded and a mean is calculated, and these values are plotted on a graph. The student then draws a line of best fit to estimate the temperature at which the zone of digestion would be greatest. This is the optimum temperature for the enzyme.

2. The use of enzymes that have an optimum temperature of less than 40 °C would mean that washing machines could run at a lower temperature. This would save energy.

3. The presence of a very small halo around the control wells indicates that some of the white casein powder has been digested. This suggests that the enzyme in the boiled washing powder has not been completely denatured. It is possible to subtract the diameter of this zone of digestion from the mean zone of digestion of the wells containing washing powder on this plate.

4. Possible advantages include: they can be recycled/ will not be lost with each wash, and that they may be able to withstand and work effectively at a wider range of temperatures.

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