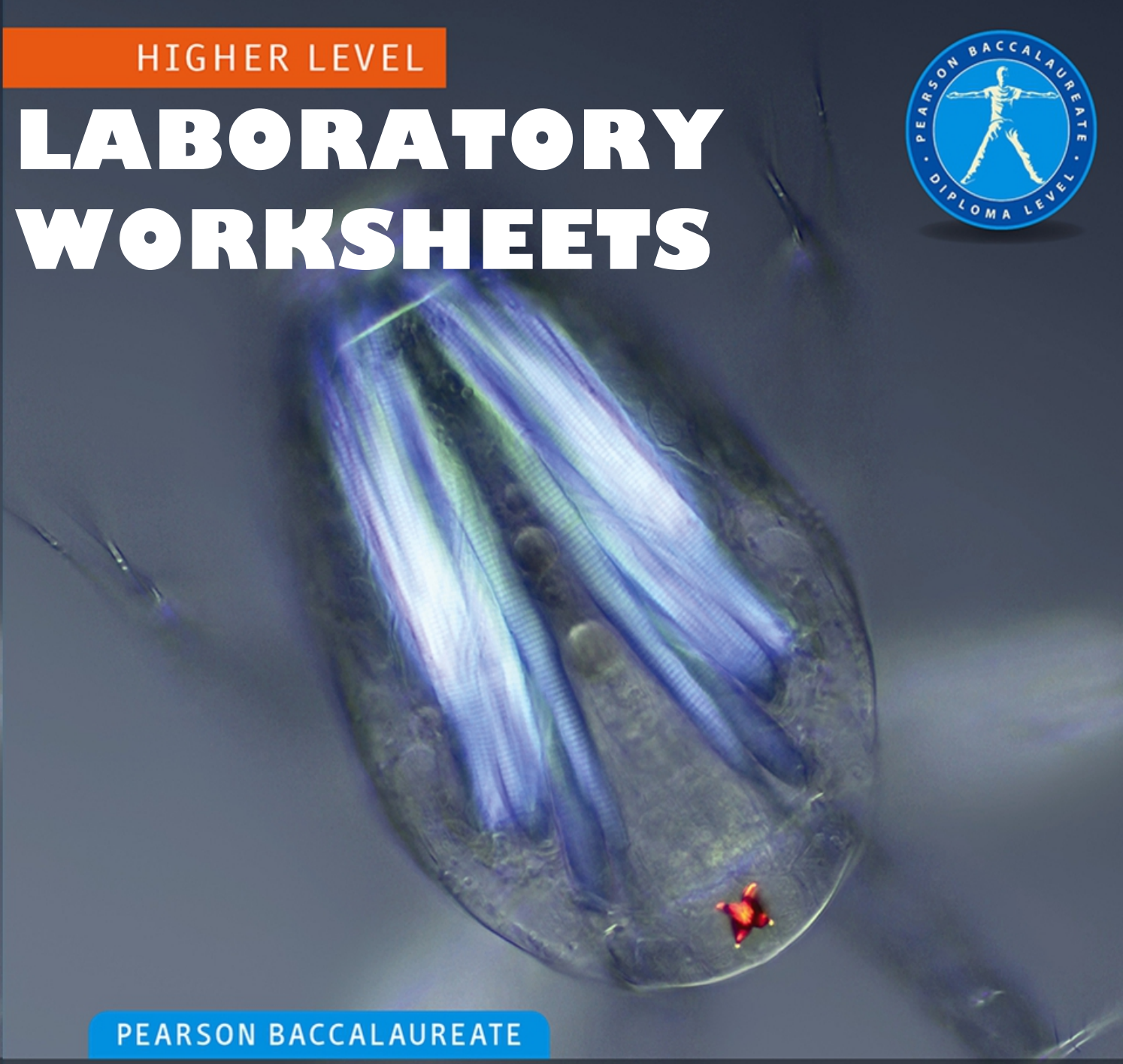
HIGHER LEVEL

LABORATORY WORKSHEETS



PEARSON BACCALAUREATE

HIGHER LEVEL

Biology

2nd Edition

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PEARSON

Chapter 2: The effect of increasing temperature on carbon dioxide output by germinating seeds

Brief summary

Looking at the influence of temperature on the rate of cell respiration as measured by the rate of CO_2 output. CO_2 sensors or respirometers could be used to monitor the level of CO_2 in a sealed chamber containing seeds. A large seed species works best, such as pea seeds.

Aim

To investigate the influence of temperature on a seed's rate of cell of respiration. In Part 1 of this investigation you will measure the basal CO_2 output of germinating seeds and learn the technique of measuring changes in CO_2 concentration using a sensor or respirometer within a closed chamber. In Part 2, you will alter the temperature of the environment of these seeds and measure the effect on the rate of CO_2 output.

Variables

- Independent variable: the air (or water) temperature.
- Dependent variable: the CO_2 output during a selected time period.
- Controlled variables: air pressure; light intensity; species of seed; germination stage of seeds.

Theory

Seeds (pea seeds or other) are embryonic plants. A typical seed undergoes a developmental period following pollination and subsequent fertilization, and develops into a very small embryonic plant surrounded by nutritious endosperm tissue and a seed coat (testa). The seed then goes into a dormancy period until the seed coat absorbs water, which initiates the events known as germination. During germination, the embryonic plant absorbs nutritive substances from the endosperm and begins to grow; the plant's metabolic rate is extremely high during this time period. Photosynthesis has not yet begun (and will not begin until the first true leaves of the plant are developed and above ground), and thus the only major cellular process involving carbon dioxide that is occurring is aerobic cell respiration.

Apparatus required

- CO_2 sensors and associated hardware/software (an alternative would be respirometers and trays for underwater submersion).
- Germinating seeds (pea seeds 2–3 days into germination would be ideal).
- Respiration chambers (a sealed chamber designed for sensor insertion or the volumetric pipette of a respirometer).
- If CO_2 sensors are to be used, a way of increasing the temperature will be needed, such as electric heating pads.

- If respirometers are to be used, a hotplate or microwave is necessary to provide different increments of water temperature.
- Thermometers to measure either the air (sensor method) or water (respirometer method) temperature.
- A timing device.
- Optional: apparatus to measure selected control variables, such as a barometer for air pressure, light meter for light intensity, etc.

Step-by-step method and procedure

The procedures given below are most appropriate for the sensor method of measuring CO_2; if respirometers are used, make changes as needed to account for the different methodology.

Part 1 procedure: Determination of the basal metabolic rate as reflected by the CO_2 given off in a 2-minute time period

- Count out and place 20 germinating seeds (more if the seeds are smaller than peas) in a respiration chamber (do *not* insert the CO_2 sensor yet).
- Allow this chamber to remain with the peas for a minimum of 2 minutes to equilibrate with the surrounding air temperature.
- Insert the CO_2 sensor into the respiration chamber and ensure a good stopper fit, and link up with the hardware/software necessary to measure the CO_2.
- Wait 30 more seconds as a further equilibration period (use a timer or clock).
- Start collecting your CO_2 data from within the sealed chamber and continue for 2 minutes.
- Use any other sensors or meters necessary to collect any control variable data, such as air pressure and light intensity.

Make *four more* data collection 'runs' (making a total of five) by repeating the steps shown above. To begin a data run you should return your 20 germinating seeds to the original tray, mix them up, and then choose 20 more seeds at random. Also, you should invert the respiration chamber (i.e. with the opening down) for at least 30 seconds to allow the previously accumulated CO_2 to move out of the chamber (CO_2 is more dense than other gases within air and thus moves down, not up).

Part 2 procedure: Determination of the influence of an environmental factor on the metabolic rate of germinating pea seeds

Your instructor may want to assign groups for this lab, with each group being responsible for the data collection of a specific temperature. There will need to be some initial planning by trial and error to determine what the temperature increments are going to be.

You will now be increasing the temperature of the respiration chambers in order to measure the effects on the rate of cell respiration.

If you are using respirometers immersed in water, you will be adding and taking away volumes of water in order to create increasing temperatures to test. You should always have a ready supply of hot water and a way of scooping out the water that is to be replaced. Use a bulb pipette immersed in the tray to monitor the water temperature.

If you are using CO_2 sensors, you will probably be using a heating pad to control the increasing temperatures. Tape a bulb thermometer to the outside of the respiration chamber and use that to estimate the internal

temperature after allowing a suitable equilibration period. The temperature variations can be obtained by using settings on the heating pad coupled with how far away the respiration chamber is from the actual pad (you might want to use a ring stand and clamp to hold the chamber).

You will need to collect five sets of data for each of the temperature increments. The data you collected in part 1 can be one of your five increments (as the lowest temperature). Don't forget to collect the data for the controlled variables during each trial and replicate.

Results

CO_2 output per time at various temperatures.

Guidance to undertake data processing

The rate of CO_2 output will need to be calculated based on the average of the five replicates for each temperature.

Guidance for presentation of processed data

An appropriate graph would have temperature increments on the *x*-axis and the mean rate of CO_2 output on the *y*-axis. If a linear pattern is detected, a line of best fit should be drawn through the five data points plotted. Error bars could be included representing either the range of data leading to each mean point plotted or the standard deviation of each mean point.

Conclusion

Base your conclusion on the pattern shown on the graph. Your discussion should be centred on the expectation of how an increase in temperature will affect the rate of cell respiration and why.

Evaluation

Look for the strengths and weaknesses of this procedure and technique while the investigation is proceeding.

Suggested reading

Chapter 2: Section 2.8.

Chapter 3: The rooting of stem cuttings

Brief summary

From the stem that has been removed from a plant, roots can grow and make a new plant. You will design an investigation to see what factors influence this process.

Aim

To design an investigation to explore the factors that influence the rooting of stem cuttings.

Variables

In your design, you need to consider all three types of variables:

- independent
- dependent
- controlled.

Theory

Flowering plants can reproduce sexually but they can also reproduce asexually. Under the right conditions, a portion of the plant can be removed from the original plant and made to grow into a new plant. Stem cuttings are one such portion of a plant. The growth of new roots in plants can be influenced by abiotic factors such as temperature, availability of water or light, and the presence of certain organic molecules such as ethylene, auxins, or gibberellins. Remember: in a design, you must choose only *one* variable that you modify.

Apparatus required

In your design, be sure to consider all the things that you will need. The following list contains possibilities for your apparatus and list of materials.

- A plant species that easily forms roots in either water or a solid medium, e.g. impatiens, begonias, jade, or African violet.
- A tool for cutting the stem.
- A water or solid medium in which roots can form, e.g. sand, agar, soil, or water.
- Any plant hormones you might consider using.
- Containers to hold the plant stems.

Step-by-step method and procedure

When you write your method, be sure to consider the following.

- How to collect or find the species of plant you will use.
- The exact technique for preparing a stem cutting: some research may be necessary.
- An explanation of how the liquid or solid medium will be prepared and how the stem cutting will be placed (horizontally or vertically).
- What quantities of plant hormones you will use (if any) and where to apply them.

Results

There are several possibilities for what you could decide to measure in your design.

- The number of roots and/or the lengths of the roots could be measured.
- The time it takes for roots to appear first or reach a certain length first could help you decide which factors have the most positive influence.

Guidance to record raw data

Think about how you would set up a blank results table. How many columns and rows? What text will go under the headings?

Guidance to undertake data processing

Although this is not really necessary for a design, you could think about how to process the data. Here are a few possibilities:

- averaging the lengths of the roots
- calculating the percentage change of the lengths of the roots
- plotting the root lengths over time and adding a trend line complete with an R^2 value (see the maths and ICT chapter for help with this)
- plotting the time it took for the first roots to appear or to reach a certain length
- using statistical tests to see if there is a difference between two populations of stem cuttings.

Suggested reading

See the IA chapter for suggestions on how to design investigations.

See the maths and ICT chapter for help on processing data.

Chapter 4: Random sampling using quadrats in a field

Brief summary

When trying to estimate the size of a population of a particular plant species in an area, it is often unrealistic to count every single plant. Instead, random sampling is used, whereby quadrats are placed in arbitrary locations around the field and an average number of plants per sample is determined.

Aim

To estimate the size of a population of a specific species of plant in a designated area.

Variables

- Independent: the random position of the quadrat in the area to be sampled.
- Dependent: the number of plants found in each quadrat.
- Controlled: same-sized quadrat; same species of plant being counted; same technique of determining the position of the next random sample; same convention used for deciding which plants are 'in' and which are 'out' of the quadrat when they are on the edge.

Theory

The technique of random sampling with a quadrat. A quadrat is a square of a particular dimension that can be made of a rigid material such as metal, plastic, or wood. This investigation is done in an area such as a typical sports field, which might be 5000 m^2. By finding an average number of plants per square metre, it is possible to estimate the total number of plants.

Apparatus required

- An area to sample that is comparable in size to a sports field and that has relatively clear boundaries.
- A 1-m^2 quadrat.
- A table of random numbers from 1 to 99.
- A pencil.
- A 10-m tape measure or equivalent for measuring the size of the field: it might be possible to use GPS coordinates.
- Something on which to record your data (see the blank data table below for a suggestion).

Step-by-step method and procedure

This lab is best carried out in groups of two to four students.

Pick a well-defined area, such as a fenced-in pasture, public park, or a sports field with natural grass (be sure you have permission to work there first).

Choose a species of plant that grows there that is easy to identify and that is widespread throughout the area, but not so numerous that counting the number of individuals growing in a square metre would take more than a minute or two. Possible examples are dandelions, docks, and yarrow, but the choice will depend on where you live and when you carry out the lab.

Each group should start in a different part of the area and spin a pencil to determine a random direction. Then, with your group, look at the first number on the random number table and walk in the designated direction that number of steps. If the border of the area is reached before the designated number of steps has been taken, you should 'bounce' off the border like a ray of light off a mirror, and continue in the direction dictated by an angle of incidence that is the same as the angle of reflection.

- Place the quadrat down on the ground at the point determined by the number of steps, and decide which of the four sides will be the 'top' and 'right' of the quadrat.
- Identify and count the number of individuals of the chosen species found inside the borders of the quadrat at that position.
- Repeat this as many times as possible in, say, an hour: the more quadrats done, the better.
- Before leaving the area you are working in, measure its dimensions so that its total surface area can be determined. This might be challenging for an irregularly shaped pasture or park, in which case online aerial views of the area might be useful. In that case, note the scale of the image.

Results

Fill in a table with your data. An example of a blank data table is shown.

Quadrat number	Number of plants counted
1	
2	
3	
. . .	
Average number of plants per m^2	

Note: a variation of this investigation is to count two different species of plant in each quadrat. If you decide to do this, another column should be added to your data table.

Guidance to record raw data

If the number of plants in a given quadrat is zero, be sure to record the result as such, otherwise your calculations will not be reliable.

Any plants touching the top or right of the quadrat should be considered 'in' and should be counted. Any plants touching the bottom or left side should be considered 'out' and not counted.

Guidance to undertake data processing

In the last row of the data table, calculate the average (or mean) number of plants per square metre, then use the surface area calculation to estimate the total number of individuals of that plant that are living in that area: (plants per m^2) × (surface area in m^2) = (population estimation).

It would be interesting to calculate the standard deviation to see whether the values differ greatly from the mean or not.

Conclusion

The conclusion will be the estimated number of plants per square metre. Looking at your numbers and at the standard deviation, you could comment on the variability of the data. You could also comment on whether the distribution of the species you studied is even or clumped. It will probably be difficult to compare your results with quantitative results from other scientists because they will probably have studied a different field in a different part of the world with a different plant species. However, you might find some published data about the number of plants of your chosen species that occur per square metre.

Evaluation

There are many sources of error to explore in this lab. Here are some possibilities:

- misidentification of plants, because some species look similar
- accidentally counting the same plant twice within a quadrat
- sometimes two plants can grow so close together that they look like one
- tall grass or other vegetation might hide smaller individuals of the species being counted
- by bad luck or human error, the randomness of the sampling might not be so random and certain parts of the field might be sampled more often than other parts of the field.

Suggested reading

See the maths and ICT chapter for hints on processing data, notably the section on standard deviation.

Chapter 5: Designing a simulation of natural selection

Brief summary

Designing an investigation to simulate available food and various mouthparts in order to show that some organisms will be better adapted than others.

Aim

To design an investigation simulating the process of natural selection.

Variables

In your design, you need to consider all three types of variables:

- independent
- dependent
- controlled.

One aspect of this lab to consider as a controlled variable is the rules that everyone must follow, not only to make the activity fair but also to keep it safe. It should be against the rules to use the mouthparts to intimidate or injure competitors, even though this happens in nature.

Theory

Animals compete for food, and some animals have better adapted mouthparts for gathering certain types of food than others. Thin, fine mouthparts would be well adapted for small food, whereas bigger, wider mouthparts would be better adapted for larger food.

Apparatus required

Be sure to think of different examples of laboratory equipment that could serve as mouthparts, and a variety of office supplies or other small objects that are easy to find in school that could serve as food.

- Examples for mouthparts: tweezers, clothes pegs or pins, plastic forks, wooden tongs, toothpicks, kebab sticks, and chopsticks.
- Examples for food: dry chickpeas or kidney beans, dry grains of rice, marbles, paper clips, and coins.
- A tray or some kind of feeding zone from which participants can take food.
- Petri dishes or similar containers that could serve as ‘stomachs’. The food is not considered to have been eaten until it is in this container.
- A stopwatch, if you want the activity to be timed.

Step-by-step method and procedure

There are multiple ways of designing this, but you need to decide whether participants will do this one by one and try for a best time, or whether they will compete with one, two, or multiple adversaries to get the most food.

Decide whether participants can choose their mouthparts or, better still, think of a system of picking a random mouthpart. In nature, organisms do not have the luxury of choosing what kind of mouth they are born with.

To make it more challenging, calorie values could be given so that the most difficult food to pick up is worth the most calories. Or, participants who get big mouthparts need more food points to survive. It's up to you to be creative here but the objective is to mimic nature as best you can.

It could be interesting to vary the food according to habitat: one combination of food items could represent one habitat, such as a tropical rainforest, and another combination could represent a desert.

It could also be interesting to show differences in the availability of food and the diversity of food from one season to the next.

Results

Based on how you have designed the method, set up a blank data table for the data.

Guidance to record raw data

If the activity was timed, but sure to include that data. Be sure to note down what types of mouthparts were used so that there is a way of comparing the different types. If you created seasons, how will you indicate them in the columns and rows?

Guidance to undertake data processing

If enough data are collected, it would be interesting to see whether there is a correlation between the types of food collected and which mouthparts are used. Are there statistically significant differences between seasons when food is abundant and varied, compared with seasons when food is scarce and of only one type?

Chapter 6: Learning anatomy using the Visible Human Project

Introduction

The Visible Human Project is an attempt to create a detailed data set of cross-sectional photographs of the human body. A male and a female cadaver were suspended in a gelatine substance, frozen, and then photographed starting with the bottom of the feet. A layer of the frozen specimen was then 'sanded' away and the cadaver photographed again. This process was repeated until the top of the skull was reached. Once photographed, the sections were no longer available because each layer was destroyed in the sanding process. The resulting high-quality photographs were digitized and were made available to various groups for medical education. The project is run by the US National Library of Medicine (NLM).

The male cadaver is Joseph Paul Jernigan, a 38-year-old Texas murderer who was executed by lethal injection on 5 August 1993. At the prompting of a prison chaplain, he agreed to donate his body to scientific research or medical use, although he had no idea he would be used for the Visible Human Project. Some people have voiced ethical concerns over this.

The female cadaver, a 59-year-old, remains anonymous. In the press she has been described as a Maryland housewife who died from a heart attack and whose husband requested that she be part of the project. It appears that the love for a spouse can manifest itself in many forms.

In all the photographs you are looking up from the person's feet towards the head. Thus the right side of the image is the patient's left side.

Aim

To understand human anatomy better.

Apparatus required

A computer and internet connection.

Step-by-step method and procedure

Access **this website**, which shows a selection of the Visible Human Project photos. This site is setup so that clicking on a structure's name shows an arrow indicating the location on the given photograph.

Listed below is a selection of views and structures that allow you to make use of the Visible Human Project without burdening you with too much anatomy. Your instructor may want to evaluate your ability to identify these structures in the following photos.

Sections to view	Structures to identify
Head & Neck	
Mid-skull, orbits, male	Nasal bone, optic nerve, optic chiasm, posterior chamber (eye), crystalline lens (eye)
Mid-skull, male	Nasal cartilage, brainstem, cerebellum, occipital lobe (of cerebrum)
Lower neck, female (C6 level)	Chin, trachea, scapula (spine of), deltoid (muscle), vertebral body, spinal cord
Chest, Male & Female	
Chest, male (T8 level)	Body of sternum, left ventricle (heart), right ventricle (heart), oesophagus, descending aorta, various lobes (of lungs), vertebral body, spinal cord
Abdomen & Pelvis, Female	
Upper abdomen, female	Right lobe of liver, gallbladder, body of stomach, right kidney, left kidney, descending aorta, inferior vena cava, vertebral body, spinal cord
Abdomen & Pelvis, Male	
Lower abdomen, male	Ascending colon, descending colon, ileum, descending aorta, inferior vena cava, vertebral body

Chapter 7: DNA extraction

Safety alerts

Wear goggles and an apron. Be careful with all the chemicals used in this procedure. Wash your hands thoroughly at the end of the procedure.

Brief summary

The extraction of DNA from strawberries and making observations of the DNA with a microscope.

Aim

To see that DNA does exist in cells and to determine some of its characteristics.

Theory

DNA is present in all cells. To liberate DNA from cells, the membranes and/or cell walls it is within must be broken down. DNA occurs in abundant amounts but as individual molecules is quite small.

Apparatus required

- Heavy-duty plastic bag (1-litre in size)
- Cheesecloth/muslin
- Funnel
- Large test tube
- Ethanol that has been chilled for 2 hours in a freezer
- Test tube rack
- Inoculating loop
- 0.1% methylene blue solution
- Laboratory balance
- 10-ml graduated cylinder
- Microscope

Step-by-step procedure

- Place a very ripe fresh or thawed frozen strawberry in a 1-litre sealable plastic bag.
- Seal the bag and mash the strawberry using your fingers for 2 minutes.
- Unseal the bag and add 1 ml clear detergent, 1.5 g NaCl, and 9 ml water.
- Seal the bag and mush the strawberry in the extraction solution for 1 minute. Do not produce bubbles in this process.
- Place a section of cheesecloth/muslin over the top of a funnel. Set the funnel in a large test tube.
- Slowly add the contents of the bag into the filtration apparatus. Let it drip directly into the test tube until the test tube is about one-quarter full.
- Obtain ethanol that has been placed in a freezer for 2 hours.

- Add this ice-cold ethanol slowly down the side of the slightly slanted test tube until the test tube is approximately half full. Be certain the ethanol forms a layer on top of the strawberry-extraction solution layer.
- Place the test tube in a test tube rack for 1 minute.
- Dip the inoculating loop into the layer of ethanol immediately above the strawberry-extraction solution layer and slowly rotate.
- Place a small amount of the DNA that collects on the rotating loop onto a microscope slide. Add one drop of 0.1% methylene blue solution. Place a cover slip over the DNA–methylene blue area of the microscope slide.
- Observe under a microscope.

Results

Your observations should include:

- a qualitative description of the DNA that moves into the ethanol layer
- a qualitative description of the DNA that is collected on the inoculating loop
- a drawing and verbal description of the DNA microscope slide observation.

Guidance to record the raw data

Your drawing must conform to guidelines for a proper biological drawing. It must include the microscope magnification the drawing is based on. Qualitative descriptions should be as detailed and as objective as possible.

Guidance for presentation of processed data

Consult your class guidelines for a proper drawing.

Conclusion

Explain the actions of the NaCl and detergent in the extraction solution in allowing the release of DNA from the cells of the strawberry. Discuss whether this procedure would work with other foods. Explain what you were observing with your naked eye when it is common knowledge that the individual strands of DNA are quite small.

Suggested reading

When it is ripe the strawberry produces pectinases and cellulases. Read about these enzymes. Also, the strawberry is octoploid. Find this term on the internet and be able to discuss the benefits of this condition to this lab.

Chapter 8: Experiment to demonstrate electron transfer in chloroplasts

Safety alerts

Wear goggles and an apron. Be cautious with the glassware. Wash your hands thoroughly at the end of the procedure. If an artificial light source is used, be careful of high temperatures produced by the bulb.

Brief summary

This lab demonstrates electron transfer in chloroplasts during photosynthesis using a chemical called 2,6-dichlorophenolindophenol (DCPIP).

Aim

To allow a visual demonstration of one of the reactions of photosynthesis.

Variables

- Independent variables: availability of light; temperature used to treat chloroplast extract.
- Dependent variable: absorbance measured by colorimeter or spectrophotometer.
- Controlled variables: light source; colorimeter or spectrophotometer for measuring absorbance; size and type of cuvettes; source of chlorophyll extract.

Theory

When the light-dependent reaction of photosynthesis occurs, electrons are transferred from the reaction centre, chlorophyll *a*, to an electron transport chain.

Apparatus required

- A blender
- Lab glassware, including graduated cylinders, beakers, and test tubes
- Cheesecloth/muslin
- A colorimeter or spectrophotometer
- Cuvettes
- Sucrose
- Distilled water
- DCPIP solution
- Spinach leaves

Step-by-step procedure

- Produce or obtain the following solutions:

- 171 g sucrose added to distilled water to produce a 0.5 M sucrose solution
- phosphate buffer with a pH of 6.5
- DCPIP solution produced as directed on the container (this varies depending on the supplier).

- Some spinach leaves should be placed in some light for a few hours. Keep the leaves cool and hydrated.
- Cover the blades of a blender with ice-cold 0.5 M sucrose.
- Loosely pack the blender with spinach leaves.
- Blend the spinach with 3–5 10-second bursts.
- Filter the resulting mixture (chloroplast suspension) through cheesecloth/muslin into a cold beaker.
- Place one-third of the chloroplast suspension into a boiling water bath for 5 minutes.
- Keep both the boiled and unboiled chloroplast suspensions under ice.
- Produce the following three test tubes.

	Dark and unboiled	Light and unboiled	Light and boiled
Buffer	1 ml	1 ml	1 ml
Water	3 ml	3 ml	3 ml
DCPIP	1 ml	1 ml	1 ml
Chloroplasts	5 drops	5 drops	5 drops

- Place aluminium foil around the dark test tube.
- Place all the test tubes an equal distance from a light source for 30 minutes.
- Maintain a constant temperature for all parts of this experiment.
- Use a colorimeter or spectrophotometer to obtain absorbance values. The colorimeter or spectrophotometer should be set to take readings at 605 nm.

Results

Quantitative observations should be taken for each test tube and recorded appropriately in the table provided below.

Guidance to record raw data

Experimental cuvette	Start colour/absorbance	Finish colour/absorbance
Dark/unboiled		
Light/unboiled		
Light/boiled		

Conclusion

The DCPIP out-competes the electron transfer chain for the electrons leaving the chlorophyll *a* when light is provided. This causes a decrease in the intensity of the blue colour of DCPIP. The result of this is a lesser absorbance value in the test tubes where light and unboiled chloroplasts are present. There will be no change in absorbance values when light is not provided or when the chloroplasts are boiled.

Evaluation

Answer all the questions provided in the written text on page 373for this lab.

Suggested reading

Read about the specific reactions involved in the Hill reaction of photosynthesis. Also, read about the specific occurrences of redox reactions.

Chapter 8: The optimum temperature for fermentation in yeast cells

Safety alerts

Wear safety goggles and an apron. Be very careful with the glassware and potentially hot temperatures. When applying the stopper to the flask, use doubled paper towelling to prevent potential cuts. Wash your hands thoroughly after cleaning up before leaving the lab area.

Brief summary

Determining the temperature at which yeast cells produce the most carbon dioxide through the process of fermentation. The carbon dioxide will be measured from the bubbles produced over a specified period of time.

Aim

To demonstrate that enzymes have different rates of activity at different temperatures.

Variables

- Independent variable: the temperature at which the yeast is maintained for 20 minutes.
- Dependent variable: the bubbles of CO_2 produced per minute.
- Controlled variables: type and amount of yeast; type and amount of sugar; water used to produce the yeast solution; same apparatus for all parts of the procedure.

Theory

Enzyme action is best at what is called the optimum temperature for that enzyme. If the temperature is too high, denaturation of the enzyme will occur, preventing enzyme action. On the other hand, at lower temperatures the rate of activity of the enzyme will be decreased.

Apparatus required

- Five 50-ml Erlenmeyer flasks, each with a one-hole stopper; each stopper should have a micropipette stem securely fitted into it with the tip pointing out the top.
- Five 1000- or 2000-ml beakers
- Five thermometers
- A hot plate
- Ice cubes
- A clock or stopwatch
- Molasses
- Dry yeast

Step-by-step procedure

- Produce a 10% mixture of molasses in water. The procedure requires approximately 150 ml of this mixture.
- Fifteen minutes before beginning the lab, activate the yeast by adding 10 g dry yeast to 100 ml water at 37°C.
- Keep this activated yeast solution at 37°C for the 15 minutes before the start of the procedure.
- Set up five water baths using the 1000- or 2000-ml beakers. Each beaker should have a constant temperature of water. The five temperatures should be: 20–25°C, 30–35°C, 40–45°C, 50–55°C, and 60–65°C. It is essential that these water baths are kept constant throughout the procedure.
- To each of the five Erlenmeyer flasks, add 25 ml fermentation mixture and 5 ml activated yeast mixture. Swirl the flasks so that the contents are mixed. Stopper the flask securely.
- Place one flask in each water bath, making certain the tip of the micropipette coming out of the stopper is well below the water line of the water bath.
- Once the flask is immersed, allow fermentation to proceed for 15 minutes. Be certain to maintain each water bath within its allocated temperature range.
- At the end of the 15 minutes, count the bubbles coming out of the top of the micropipette for 3 minutes. Divide by three to obtain the number of bubbles produced per minute.
- Disassemble and thoroughly wash all the components. Wash your hands thoroughly before leaving the lab area.

Results

Prepare a table recording the bubbles produced per minute for each of the water bath temperatures.

Guidance to record raw data

The independent variable should be shown on the far left of the table. The uncertainty should be stated for the independent variable based on the thermometer used. Uncertainty should be placed at the top of the dependent variable column in the table for the instrument used to measure time.

Guidance to undertake data processing

One example of data processing has already occurred when the bubbles were counted for 3 minutes and then divided by 3 to get the average number of bubbles produced per 1 minute. Another step in data processing would be to record data for this procedure from other groups in the class. This would allow the calculation of an average for the number of bubbles produced per minute that would have a greater degree of reliability.

Guidance for presentation of processed data

The average number of bubbles per minute for each temperature should be presented in an appropriate table. A graph can then be constructed with the independent variable on the *x*-axis and the dependent variable on the *y*-axis. These axes should be clearly labelled with the units included. The uncertainties should also be included with the axes labels.

Conclusion

Which temperature had the highest average number of bubbles per minute? Explain the reason for this finding using enzyme characteristics.

Evaluation

- What could be done to improve the reliability of the data obtained from this procedure?
- What were the independent, dependent, and controlled variables for this experiment?
- What could you do with this experiment to provide a control group or procedure?
- What was the gas produced in this procedure that was measured as bubbles per unit time?
- What is the other product that was produced in this fermentation process?

Suggested reading

Read about fermentation in cellular respiration. Be certain to understand the difference in products between fermentation in yeast/plants and animals. Find the equation that represents what happened in this procedure.

Chapter 10: Observing the stages of meiosis

Brief summary

Preparing and observing slides under the microscope of cells undergoing meiosis.

Aim

To see the different stages of meiosis in a living organism. In this case the reproductive cells of lily flowers.

Theory

Meiosis is a special kind of cell division reserved for making germ-line cells (cells that are not destined to build the internal or external body parts of the organism but instead are destined to make sexual reproduction possible). This investigation will allow you to see this preparation in progress.

Apparatus required

- Fresh lily flowers (one flower can be used for multiple groups).
- Acetocarmine in a dropper bottle.
- A microscope.
- A microscope slide and cover slip.
- A scalpel or dissecting needle.

Step-by-step method and procedure

- Place a drop of acetocarmine stain on an empty microscope slide.
- Collect one anther from a lily flower and place it in the drop.
- Using a scalpel or dissecting needle, slice the anther into sections that are as thin as possible.
- Keep two or three of the thinnest slices flat on the slide in the drop of stain and remove the rest of the anther.
- Place a cover slip gently on the slices without crushing them.
- Observe the cells and identify the stages of meiosis.

Optional: if your lab has some available, observe slides that have been prepared by professionals. Also, you could try other types of germ-line cells if your school's lab has some available.

Results

The results of this lab are mainly qualitative as you will be drawing the stages of meiosis that you see.

Guidance to record raw data

Be sure to follow the conventions for drawings in biology (see the presentation suggestions below). Sometimes a photo or video stream taken through the eyepiece can generate visual aids to assist you in the drawing process.

Guidance to undertake data processing

The only calculation you will need to do is to work out the magnification of the microscope. To do this, multiply the magnification of the eyepiece (e.g. 10×) by the magnification of the objective lens (e.g. 40×). 10 × 40 = 400 in the examples given, so you would write on your drawing 'observed at 400×'.

Guidance for presentation of processed data

Some rules to follow for drawings in biology:

- use a pencil
- make the drawings big enough to see the details
- label the parts that can be distinguished
- give the drawing a title
- state at which magnification the sample was observed.

Conclusion

- Were you able to draw all the stages of meiosis?
- Are some of the stages more difficult to observe and draw than others?
- Do the cells seem to be equally distributed in terms of the stages they are in or do some stages seem to occur more frequently than others?

Evaluation

Be sure to mention some of the difficulties you encountered when preparing and observing the samples.

Suggested reading

Reread Chapter 3: Section 3.3, on meiosis so that you know the various stages to look for.

A quick image search on the internet for the stages of meiosis could be helpful.

Chapter 11: How fast does a disease spread?

Safety alert

Notice that it is a very weak NaOH (0.2 M) that is to be used: do *not* use a concentrated form of sodium hydroxide!

Brief summary

Simulating the spread of a disease that is transmitted by person-to-person contact. A fluid that can be detected by an indicator represents the pathogen.

Aim

To understand the spread of a disease better.

Variables

The variables will depend on which variation of the lab is attempted. For the basic version of this investigation, the number of infection cycles will be the independent variable and the number of people infected will be the dependent variable.

Theory

Diseases often require person-to-person contact in order to transmit the pathogen. This contact may be in the form of exchanged fluids, skin-to-skin contact, or simply breathing in air that contains the pathogen after it has been expelled by someone else (e.g. by sneezing). This contact explains why quarantine is often effective in slowing or stopping an outbreak of disease.

Apparatus required

- 0.2 M sodium hydroxide solution (NaOH). The volume needed depends on the test tubes used.
- Deionized or distilled water.
- Phenolphthalein indicator.
- A test tube and stopper for each student.
- A marker pen for test tubes.
- Test tube racks as required.

Step-by-step method and procedure

Teacher preparation and directions

- Using a marker pen, number each test tube (from 1 to the highest number needed), and also place a mark on each test tube that shows approximately one-third of the volume of the test tube.
- Fill one (or more depending on the variation of this lab) random test tube to the one-third volume line with 0.2 M NaOH.
- Fill all of the other test tubes to the one-third volume line with either deionized or distilled water.

- Place a stopper in every tube.
- Provide a place on your board or a piece of paper for students to write down their name and test tube number. Because this lab works best when there is an even number, if the number of students in the class is odd the teacher can become one of the population subject to infection.
- Students need to be reminded to move randomly; if they constantly stay with or near a certain person the results will be skewed.
- Once a 'cycle of infection' has been completed, place a few drops of phenolphthalein indicator into each test tube to see who is 'infected', as indicated by the solution turning pink.

Variations of this lab

- The number of infection cycles can be varied, although it is best to start with about four.
- The number of patient zeros (the number of people who start with the dilute NaOH) can be varied (a ratio of about 1:10 is ideal).
- The effect of people vaccinated or otherwise having an immune status can be simulated by having certain tubes where the stopper is taped on and thus fluid cannot be shared.

Student directions

- Obtain a test tube and stopper that contains a clear liquid.
- Write your name and test tube number on the board or paper according to directions of your instructor.
- When your instructor directs you, wander aimlessly throughout your classroom.
- When your instructor says 'stop', find the person closest to you.
- Both of you should unstopper your test tubes and one of you should pour your contents into the other's test tube. Replace the stopper on this test tube and shake it to mix well. Unstopper this test tube and pour half of the volume into the empty test tube (the volume should be to the line marked on the test tube), so you both have mixed solutions.
- Put your stopper back on and, when the instructor directs you to, begin to wander aimlessly around the classroom once again.
- When you hear 'stop', repeat the last two steps, i.e. mixing the contents of your test tube with the person nearest you, and continue until your instructor changes the directions given.
- At some point in this process your instructor will ask you to unstopper your test tube so that an indicator can be added. If your solution turns pink, then you have become infected.

Results

The number and identity of each person 'infected' after each infection cycle (an infection cycle lasts from the beginning of the procedure until the indicator is added to the solutions).

Guidance to record raw data

A separate data table should be constructed for each variation of this lab. Each data table should include the number of patient zeros and the number of 'contacts' as well as the identity of each person, indicating whether they ended up being infected or not.

Guidance to undertake data processing

If multiple trials of this lab are attempted, each with a different number of infection cycles, calculate the percentage of people infected after each trial.

Guidance for presentation of processed data

If a percentage of people infected based on the number of infection cycles is calculated, plot a graph showing the number of infection cycles on the *x*-axis and the percentage of people infected on the *y*-axis.

Conclusion

In addition to noting the overall pattern of the graph, note the relationship to a logistic growth curve. Remember, you are 'tracking' the growth of a pathogen and you cannot infect an already infected individual.

Evaluation

Consider how this procedure is effective at simulating the spread of a pathogen, and how this procedure has limitations as a model.

Suggested reading

Chapter 11: Section 11.1.

Chapter 12, Option A: Olfaction

Brief summary

Chewing 10 jelly beans and trying to determine the flavour without the benefit of sight.

Aim

To determine whether there is a significant difference in the ability of males and females of a similar age to determine flavour. Only taste and olfaction are used to try and determine the flavour of 10 different jelly beans.

Variables

- Independent variable: the 10 flavours of jelly bean that are chosen by the student conducting the test.
- Dependent variable: the number of flavours correctly identified.
- Controlled variables: male and female students of a similar age who will be the test subjects.

Theory

Olfactory memory refers to remembering odours that have been experienced previously. You may guess the flavour of a jelly bean because you have eaten jelly beans many times previously. Memory plays an important part in your ability to identify odours.

The sense of smell greatly increases the flavours of the food that we eat, because the back of your throat connects your nose with your mouth. The flavours move from your mouth to the specialized neurones in your nose, which contain olfactory receptors. In this experiment, molecules are entering your nose from the back of your throat. The combination of several receptors is registered by the brain as a certain smell.

Apparatus required

- A timer.
- Twenty jelly beans of each of these flavours:
  - cherry
  - tangerine
  - bubble gum
  - peach
  - coconut
  - lemon
  - liquorice
  - pear
  - blueberry
  - lemon and lime
  - popcorn.
- A table to record the data from each participant.
- Students to do the testing.

- Students to help the tester.
- Ten females and 10 males of a similar age who will be tested for their ability to determine the correct flavour of the 11 jelly bean flavours listed above.
- Disposable spoons for each person tested.
- A small container or plastic bag to hold the jelly beans, with a label for the tester to identify the flavour of the jelly bean being given to the participant.
- Hand wipes for any one touching the jelly beans with their fingers.

Step-by-step method and procedure

- Select one or more students in the class to be the tester and another student to help the tester(s).
- Select five female students and five male students of approximately the same age to be tested.
- If enough students are available, five more males and five more females can be tested by a different tester, simultaneously.

Pretest

The cherry-flavoured jelly bean is used for the pretest. A 'nose-plugged' pretest shows that, without your sense of smell, you cannot taste the flavour of the jelly beans. Eyes are closed so that colour cannot be used as a clue to facilitate a correct guess. As the lab continues, the nose is unplugged for the testing, so that the sense of olfaction is operating.

The student to be tested must sit at a desk across from the tester and the helper and hold his or her nose closed with two fingers. Then the participant must be instructed to close his or her eyes. The participant is given a cherry jelly bean and asked to identify the flavour. Wait 30 seconds. If the participant does not know the flavour, he or she should be asked to open his or her nose and breathe. The participant can then put the jelly bean in his or her mouth and try to determine the flavour. If the participant guesses the flavour correctly, another jelly bean from the list should be tried.

Test

The student who is being tested:

- does not hold his or her nose, but must close his or her eyes so that he or she does not see the colour of the jelly bean
- has 30 seconds to look at the list of the 10 other possible flavours and decide which one he or she is chewing on
- states what he or she thinks the flavour is
- cannot mark off flavours on the sheet
- may repeat a flavour.

The tester records either yes or no in the table in response to a participant's guess.

During the test, the tester must select each jelly bean at random but must know the flavour before he or she gives it to a participant.

The student helper will hand the jelly bean on the spoon to the tester so that the participant takes the jelly bean off the spoon.

The helper will show a written label of the jelly bean to the tester so that he or she knows what flavour is being tested.

The tester can tell the participant his or her score at the end of the test.

Other questions that can to be explored

Does olfactory sensitivity change with age?

Test 10 people (equal numbers of male and female) in each of the following age ranges: 21–30, 31–40, 41–50, 51–60, 61–70. Look at the graph on page 528 in Chapter 12 on olfactory sensitivity and age. How do your data compare with the study shown on this graph?

Are any flavours guessed more accurately than others?

Results

A yes or no should be placed next to each jelly bean flavour for each participant. Participants are allowed to guess the same flavour more than once.

	M	M	M	M	M	F	F	F	F	F	
Tangerine											
Bubble gum											
Peach											
Coconut											
Lemon											
Liquorice											
Pear											
Blueberry											
Lemon and lime											
Popcorn											
Percentage correct											

Guidance to record raw data

- Record all the raw data.
- Give the results table labels and a title.
- Record quantitative data, e.g. if a participant has a cold so cannot smell very well, or has a sinus infection and cannot smell at all.

Guidance to undertake data processing

- Calculate the average of the percentage correct males and females.
- Determine the standard deviation of each group.
- Use the Student's *t*-test to determine whether the differences are significant.

Guidance for presentation of processed data

- A histogram with error bars might be appropriate for this discontinuous data set.
- For an alternative method, the data might be continuous and a trend line might be more appropriate.
- Make sure that labels are present on both the *x*- and *y*-axes of the graph. All graphs and data tables must have titles. Include uncertainties where appropriate.

Conclusion

Discuss the numerical data and how they support the conclusion. Use the data processing you have done to support the conclusion.

Error analysis

- What are the weaknesses and limitation of your methods?
- Did you use enough replicates?
- Variations that are uncontrollable should be mentioned.
- Variations that could have been controlled should be described.

Improving the investigation

- Improvements should be based on any weaknesses and limitations mentioned.
- Modifications to the technique should be relevant to any errors mentioned.

Alternative investigations

- Purchase a smell identification test.
- Percentage values for this test of males and females can be compared with normalized scores that are listed in a table as a function of age.
- Scores can be compared using sex, age, or smokers versus non-smokers, and the data set could be analysed statistically using the chi-square test.
- Perform an odour threshold test. Geometrically dilute an odorant by serial dilution. What is the range of threshold values for the odorant in a class of students? Is there a significant threshold value between males and females?

Suggested reading

Chapter 12: Section A.3.

Make sure to reference any information that you use in writing a lab report on this topic.

Chapter 12, Option A: Reaction time: measuring a reflex

Brief summary

This exercise will allow you to use some of the design skills that you have learned. You will collect and analyse quantifiable data appropriate to the question. New questions can then be generated that will be different to the question originally asked.

The initial question that you and your team of four other students will answer is: Is there a significant difference between reaction times when comparing the dominant and non-dominant hands of males and females in your class? This will be measured by determining the time it takes to catch a falling metre-long stick.

Each group will design their own procedure to measure this. At the end of the lab, a discussion of the different procedures that were used can be had. Results/conclusions and data analysis techniques can be compared between groups.

Aim

Formulate a hypothesis. Design an experimental procedure to measure the time it takes to catch a falling metre-long stick.

Working with a small group, collect data from the entire class (at least 10 students).

Variables

Because you are working with human subjects and working in a space that is not controlled, there are many variables that must be considered. One method that can be used to explain the variables and how they impact the data collection is to make a text box with two columns. One column is for the variable and the other column is for an explanation of how you attempted to control the variable.

Make sure you describe the independent and dependent variables.

Theory

Your reaction times play an important part in your everyday life. If you are old enough to drive a car, you should have realized by now how important reaction time is to this activity. You may know from playing sports that your reaction time improves the more you train and practise. Practice enhances the skills needed to get better at the sport.

Reaction time can also be slowed down or speeded up by outside factors. For example, it is known that alcohol slows reaction time, while caffeine usually improves it.

Apparatus required

- Metre-long stick.
- Timer/or mathematical formula to determine reaction time.
- Camera to record the procedure you have designed in labelled photographs.

Step-by-step method and procedure

You and your group will design a method to measure the time it takes for the reflex reaction of catching a falling metre-long stick.

You need to consider the following.

- Have you controlled the variables as much as possible?
- Do you have enough data so that standard deviation and/or a *t*-test can be used to analyse the data?
- Is the method completely repeatable so that another person could perform the procedure in the same manner and get the same results?
- Are all measurements in the metric system?
- Have you considered the uncertainties of your measuring tool when collecting data?

Results

Design a table that will include raw data, a title, headings, units, and uncertainties.

Guidance to record raw data

Record all the raw data and any qualitative observations that are applicable.

Guidance to undertake data processing

Data processing could include:

- averages
- standard deviation
- Student's *t*-test.

Guidance for presentation of processed data

A histogram with error bars might be appropriate for this simple question. Make sure to label the *x*- and *y*-axes using units and uncertainties. All graphs must have a title.

Conclusion

- Restate the hypothesis.
- Do your data support the hypothesis?
- Discuss the numerical data and how they support your conclusion.
- Use the data processing you have done to support your conclusion.

Evaluation

Error analysis

- What are the weaknesses and limitations of your methods?
- Did you use enough replicates?

- Were instrumental errors analysed?
- Have you mentioned random variations that were uncontrollable?

Improving the investigation

- Can you think of any improvements based on the weaknesses and limitations you have highlighted?
- Are there any modifications to the technique that you could make that are relevant to the errors you may have thought of?

Future questions that could be asked

- From the basic procedures that have been discussed, other questions could be asked to expand this investigation of reaction time.
- Can the non-dominant hand 'learn' to be as successful as the dominant hand?
- Does the stimulus (visual, touch, or audio) used for dropping the metre stick change the results?
- How do the reactions rates of video game 'addicts' compare with non-video game addicts.
- How does reaction time vary with age? (This would be a continuous variable rather than a discrete variable, resulting in a graph that is not a histogram.)

Suggested reading

Review Chapter 12: Section A.4 on reflexes.

Make sure to reference any information that you have used in writing a lab report on this topic.

Chapter 13, Option B: Testing bactericides using the disk diffusion method

Safety alerts

Although the bacterium you will be working with is non-pathogenic, certain protocols must be followed to avoid contamination of your plates and the surface area you are working on.

- Wipe down the lab bench with 95% ethanol.
- Wash your hands with anti-bacterial soap before and after the lab.
- If you have long hair, tie it back.
- Clear your workspace of any materials, books, and papers.
- No food or drink is allowed in the lab during this procedure.

Brief summary

In this activity you will choose commonly used spices to test their bactericidal properties on the growth of bacteria. The growth inhibitory property of the spice will be compared with the bactericidal effects of a known antibiotic.

Aim

To determine the bactericidal effect of a spice on the growth of bacteria by measuring the zone of inhibition in millimetres.

Variables

- Independent variable: spice.
- Dependent variable: zone of inhibition in millimetres.

Theory

Spices have been used for centuries to flavour food while also killing selective bacteria. Some spices contain molecules that are natural inhibitors to the growth of bacteria. A clear area around a disk containing a spice shows that the spice contains molecules that have diffused into the nutrient agar and inhibited the growth of bacteria in that area. The larger the clear area (the zone of inhibition), the more effective the spice.

Apparatus required

Materials per team

- Four Petri dishes filled with sterile nutrient agar
- One tube of nutrient broth that has been inoculated with *Escherichia coli* by the instructor and incubated overnight
- A metric ruler
- A biohazard bag
- Metal forceps
- An alcohol burner

- A pen
- Ethanol in a small beaker
- A vial of sterile paper disks
- A vial of antibiotic disks (the whole class will use the same antibiotic)
- A bent glass spreader
- A sterile plastic pipette (graduated)
- A mortar and pestle
- Sterile water

A choice of spices

- Fresh garlic
- Red pepper flakes
- Fresh hot pepper
- Powdered cinnamon
- Cloves
- Oregano oil
- Clove oil

Step-by-step method and procedure

Work as a team of two, three, or four students and choose one spice to use from the list above. Bring some of the chosen spice to class. Each team must use a different spice.

Do some research on your spice and bring the information to class to share with your teammates. Do you think your spice will work the best?

- Grind the spice in a mortar and pestle. Add 10 ml sterile water to the mortar and pestle. You will be dipping disks into this solution.
- Obtain four nutrient agar plates and one broth culture of *E. coli.*

Sterile plating technique

Work as a team and help each other. Do not put anything down on the lab table. Place all used pipettes in the hazard bag.

- Open one sterile pipette.
- Open the top of the nutrient broth culture with *E. coli*.
- Flame the top of the container to sterilize it.
- Insert the pipette and remove 1 ml broth.
- Open the lid of one Petri dish in a clamshell manner.
- Put the 1 ml of *E. coli* culture from the sterile pipette on top of the nutrient agar.
- Dip the glass spreader in alcohol and flame the spreader.
- When it cools, clamshell the Petri dish again and spread the 1 ml of *E. coli* around on the surface of the plate.
- Reflame the spreader before you put it down.

- After 2 minutes turn the plate over and label the back with a pen in the following way. Draw six circles about 5 mm in diameter around the edges of the plate. Label one with A for antibiotic. The rest will be for the spice. Put the date and team name in a small area on the bottom of each dish.
- Repeat the above procedure with three more Petri dishes.

Adding the spices

You will have a total of 5 disks of your spice and 1 disk with an antibiotic per plate. Have the disks handy.

- Dip the metal forceps in alcohol. Flame the forceps. Cool.
- Pick up one disk with the forceps and dip it in your spice.
- Clamshell the Petri dish and lay the disk on one of the circles you have drawn (but not the one labelled A).
- Tap it slightly to stick.
- Repeat with four more disks.
- Repeat the process with an antibiotic disk. Lay that one on A.
- Repeat this process with three more Petri dishes. This will give you a total of four dishes on which you have tested your one spice.
- Tape all four dishes together and turn them upside down.
- Place the taped unit upside down in an incubator for 24–48 hours at 37°C.
- Put all the used materials in a biohazard bag. Wash your hands and wipe the lab table down with 95% ethanol.

Results

Do not open the plates.

You will be measuring the zone of inhibition in millimetres. Most of the plate will be cloudy because of the *E. coli* covering the plate. Any area that is clear is where there is no *E. coli.* Around the antibiotic disk will be a definite zone of inhibition. Measure that first in this way: begin at the edge of the disk and measure to the edge of the clear area in millimetres.

Guidance to record raw data

Make a data table for all four dishes with all measurements and uncertainties. You will have 20 measurements for a spice and five measurements for the antibiotic. Share your data with your class. Hopefully, there will have been three or more teams.

Guidance to undertake data processing

- Work out the standard deviation for each spice and the antibiotic and include error bars in your histogram. Note on your histogram that the error bars represent standard deviation.
- Perform a Student's *t*-test for two groups that have averages that are very close. Are they significantly different? You can also use the Student's *t*-test to determine whether there is a significant difference between the antibiotic and one of the spices.

Guidance for presentation of processed data

Make a histogram of the averages for each spice and the antibiotic.

Conclusion

- Use data to support your conclusion.
- Use reference material to support your conclusion (remember to cite your sources).

Evaluation

Alternative ideas: instead of spices, you could use mouthwashes, cleaning products, antiseptics, or disinfectants. With these products, you could choose one and vary the concentration. Maybe do a test plate to see which mouthwash is the best. Next vary the concentration of that mouthwash to see what is the least concentration that will still be lethal to bacteria. Vary the bacteria. Try *Bacillus subtilis* or *Bacillus cereus*. Try one spice on all three bacteria. The variations for this project are endless! Just make sure to collect enough data to be able to carry out some data analysis.

Chapter 13, Option B: The action of enzyme-based dietary supplements

Brief summary

A class activity to collect data on the activity of an enzyme product of *Aspergillus niger*, a fungus, that has been found to be important in developing products for both the food and pharmaceutical industries.

Aim

To determine the effect of four temperatures on the enzyme activity of Beano® or a similar enzyme-based dietary supplement, measured by the amount of glucose produced from beans.

Variables

- Independent variable: temperature.
- Dependent variable: the amount of glucose as measured by Diastix in mg dL^{-1}.
- Negative control: a test tube with no Beano®.
- Positive control: honey (contains glucose).

Variables that must be controlled:

- type of bean
- test tube size
- timing
- Beano® concentration.

Theory

Industrial microbiology is now growing microorganisms on a large scale to produce commercially viable products. This process is referred to as fermentation. Currently, antibiotics like penicillin are the most important products of fermentation. However, food additives, such as citric acid, are another important product made from microorganisms. Citric acid can be made from a fungus, *Aspergillus niger.* This same fungus, *A. niger*, is used to make a pharmaceutical product called Beano®.

In 2000, Alan Kligemen of AKPharma Inc. found a mutant form of *A. niger* that produced an optimum amount of an enzyme called alpha-galactosidase. He used biotechnology methods to increase the production of this enzyme by *A. niger.* He filed for a patent for this discovery in 2000; this was subsequently turned into the product we will use in this investigation, called Beano®. Beano® was found to be effective in reducing flatus (gas) from undigested oligosaccharides (large complex carbohydrates) that pass into the large intestine and are broken down there by bacteria. After eating food, especially beans, uncomfortable gas is sometimes produced as a waste product.

Beano® actually contains two enzymes, alpha-galactosidase and invertase. These two enzymes break the oligosaccharides. Alpha-galactosidase breaks the oligosaccharides into galactose and sucrose. Invertase then breaks down the sucrose (disaccharide) into two monosaccharaides, glucose and fructose. Test strips, called Diastix strips, are used by diabetics to detect the concentration of glucose in the urine, but can also be used to detect the concentration of glucose in food. You can use these test strips to determine whether Beano® has done its job. If Beano® is consumed before eating beans, the complex carbohydrates will be broken down to simple

sugars before the bacteria in your gut get to work on them and produce uncomfortable gas. You will test this by putting Beano® in a container with some beans. A positive glucose test will tell you that Beano® has worked.

Apparatus required

- One can of white beans (garbanzo or cannellini)
- Beano® or a similar enzyme-based dietary supplement
- Distilled water
- Blender
- Balance
- Graduated cylinder
- Diastix glucose test strips
- 10% honey solution in distilled water
- Test tubes
- Disposable transfer pipette
- Test tube rack
- Thermometer
- Water bath
- Timer

Step-by-step method and procedure

Set up water baths at different temperatures (the recommended temperatures are 4°C, 25°C, 37°C, and 50°C). At least five tubes with Beano® and five tubes without Beano® should be placed in each water bath. Five sets of data should be collected for each temperature. This can be done as a class activity with four teams. Each team will maintain a water bath at a specific temperature.

For each temperature tested carry out the following.

- Dissolve 3 Beano® tablets in 25 ml of distilled water.
- Use a blender and puree 100 g canned beans with 200 ml distilled water.
- For the tube without Beano®, add 4 ml bean solution and 2 ml distilled water. Pipet up and down with a clean transfer pipette to mix them up.
- For the tube with Beano®, add 4 ml bean solution and 2 ml Beano® solution. Pipet up and down with a clean transfer pipette to mix them up.
- Take a baseline reading of the glucose by dipping a Diastix strip into the test tube mixture. Wait exactly 30 seconds and compare the colour chart on the bottle with the colour on the strip. Record the glucose concentrations in md dL^{-1}.
- Place the test tubes in the water bath and record the baseline reading as 0 minutes.
- Using a clean strip each time, take readings at 3 minutes, 6 minutes, 9 minutes, and 12 minutes.
- For a positive control add 4 ml bean solution and 2 ml 10% honey solution to a one test tube for each temperature.

Results

Record the class data in a data table.

Guidance to record raw data

Make sure you include:

- a title
- headings for each column
- units for each piece of raw data
- the degree of precision in the heading for each column (the precision for the Diastix is given next to the colour chart)
- the level of precision for each instrument (30 seconds ± 1 second)
- averages and standard deviation
- the number of decimal places for each piece of data in accordance with the precision of the measuring tool.

Guidance to undertake data processing

Processing might include:

- the averages of five test tubes for each temperature with and without Beano®
- standard deviations for each average
- a Student's *t*-test to compare the effect of temperature on the action of Beano®
- any other statistical test that might be appropriate (see the chapter on maths and ICT).

Guidance for presentation of processed data

- Do not graph raw data.
- Plot the averages of the five tubes.
- Use error bars to indicate the standard deviation.
- Make sure to indicate what the error bars mean.

Conclusion

Explain how the data collected answers the research question. Explain how the results either confirmed the hypothesis or refuted the hypothesis. Describe any unexpected results. Explain what can be learned from the results.

Evaluation

Describe the limitations and sources of possible error. Describe how these could be corrected in future lab work with Beano®.

Suggested reading

The activity of Beano® is that of an enzyme. Review the material in Chapter 2: Section 2.5, on the effect of temperature on enzyme activity. Compare the theory to the actual results that you obtained. Reference the section of the book that you have used in your discussion.

Refer to the chapter on maths and ICT to find appropriate statistical tests.

Chapter 13, Option B: Degradation of oil by ecofriendly bacteria

Safety alerts

Wear safety goggles and gloves during this lab, and during the clean up after the lab is finished.

Brief summary

Environmentally friendly drain cleaners containing bacteria will be added to vegetable oil. The length of time it takes for each cleaner to begin breaking down the oil will be measured. The measure of the breakdown of the oil is a colour change in an indicator molecule that will be added to the mixture of drain cleaner and oil. The indicator will change from colourless to pink as the bacteria begin to metabolize the carbon compounds in the oil.

Aim

To compare the effectiveness of four environmentally friendly drain cleaners that contain bacteria as they work to break down vegetable oil.

Variables

- Independent variable: environmentally friendly drain cleaners that contain bacteria (e.g. BioKleen, Dr Drain, Home Solv, Earth Enzymes, Enzyme Clear, Rid-X).
- Dependent variable: the breakdown of vegetable oil as measured by the time taken to reveal a colour change in tetrazolium chloride, an indicator of bacterial metabolism.
- Controlled variables: temperature; amount of drain cleaner; containers; time; use of a standardized procedure.

Theory

Over the last 3 billion years microorganisms have evolved the ability to digest many different carbon compounds. They use the energy trapped in the chemical bonds as a food source. Scientists have used *Pseudomonas* bacteria to clean up both oil spills and mercury in the marine environment. In this lab you will see the benefit of having bacteria in drain cleaners. A clogged drain actually contains many compounds that serve as energy sources for the bacteria. Mixtures of bacteria can digest the oil and other nutrients in the waste, allowing the remainder of the waste to dissolve.

Environmentally friendly drain cleaners contain mixtures of bacteria that break down grease. Chemical drain cleaners melt the grease by producing chemical reactions to generate heat. However, the chemical fumes are toxic. Bacterial drain cleaners are safer and more environmentally friendly. However, they may work more slowly to unclog a drain.

In this activity, an indicator molecule, tetrazolium chloride, is used to measure the breakdown of the oil. This indicator is colourless (when oxidized) but, as the bacterial action begins and the oil is broken down, the by-products created serve as electron donors to the tetrazolium chloride and it becomes reduced. When it is reduced, it turns pink. The pink compound you see is an insoluble compound. The reduction of tetrazolium from its colourless form to its reduced pink form is an indication that microbial action has begun to break down the oil.

How long will it take for the four drain cleaners to change the tetrazolium indicator to pink? Begin measuring the change in hours. Because bacterial drain cleaners are slower than chemical drain cleaners, it may take days! Be prepared to have your test tube rack in place for about a week. Make sure to record the room temperature every day in your data table. If the room temperature changes, that can affect the bacterial action occurring in our investigation.

Apparatus required

- Four brands of environmentally friendly drain cleaners that contain bacteria, such as BioKleen, Dr Drain, Home Solv, and Rid-X (drain cleaners that are ecofriendly can be ordered online from companies that sell ecofriendly products).
- Vegetable oil.
- Thirty test tubes with screw tops.
- Marker pens.
- Goggles.
- Gloves.
- Disposable 1-ml pipettes.
- Tetrazolium indicator 0.02% (this can be purchased from a biological supply company as 2,3,5-triphenyl-2H-tertazolium chloride; it comes in a powdered form and is water soluble).
- Three test tube racks with room for 30 test tubes.

Step-by-step method and procedure

- Label the tubes:
  - 5 tubes for one brand of drain cleaner
  - 5 tubes for the second brand of drain cleaner
  - 5 tubes for the third brand of drain cleaner
  - 5 tubes for the fourth brand of drain cleaner
  - 5 tubes for control water
  - 5 other tubes, one for each drain cleaner and one for water.
- To the first 25 test tubes mentioned above, add 2 ml of tetrazolium indicator.
- To all 30 tubes, add 2 ml of cooking oil.
- To the first 5 tubes, add 2 ml of one drain cleaner.
- To the next 5 tubes, add 2 ml of a second drain cleaner.
- To the next 5 tubes, add 2 ml of a third drain cleaner.
- To the next 5 tubes, add 2 ml of the fourth drain cleaner.
- To the next 5 tubes, add 2 ml of water.
- To the last 5 tubes, add 2 ml of one of the different drain cleaners into one each of the tubes and to the fifth tube add water. This group of 5 tubes will have no indicator added to them.
- Cap the test tubes and vortex them for 10 seconds each.
- Observe the tubes every hour for the first 7–8 hours and then as often as is practical for the next week. Record the time it takes for the tubes to first become what you are sure is a pink colour. Record the data in

hours. Some drain cleaners will not cause a change to a pink colour, indicating that oil metabolism is not occurring.

Results

Create a data table.

Guidance to record raw data

Record qualitative data in addition to the time it took for the pink colour to appear in each test tube. If no pink colour appears, 0 hours will eventually be recorded. This indicates that no bacterial breakdown of the oil has occurred. Made sure your data table and graphs contain complete titles**.**

Guidance to undertake data processing

- Find the average number of hours (± uncertainties) it took for bacterial action to begin.
- Find the standard deviation of this average.
- Make a graph of the data including standard deviation shown as error bars.
- Go online and find the website for calculating the Student's *t*-test. Using the Student's *t*-test, determine how significant the differences are in the number of hours it took for each drain cleaner to metabolize (break down) the oil compared with the others. Which drain cleaner was the most effective at breaking down the oil, based on your statistical analysis?

Guidance for presentation of processed data

The style of graph used for this would be a histogram, because the data are discontinuous.

Conclusion

Make sure you include the exact data that you are using to support your conclusion.

Evaluation

Evaluate the data you have collected and the procedures used to collect the data. What would you do to improve both the data and the procedures?

To take this lab further, consider the following.

- Would temperature affect the activity of these drain cleaners?
- Do different concentrations of the drain cleaners have measureable differences in oil breakdown?
- During an oil spill, fertilizer is spread over the oil spill to increase the action of the bacteria. Will the addition of nutrient fertilizer affect the activity of the drain cleaner?

Suggested reading

The bacteria in these cleaning products are industrially and environmentally important. Review the role of *Pseudomonas* in cleaning up oil spills and detoxifying mercury in our marine environments in Chapter 13: Section C:3.

Chapter 14, Option C: The *Allium* test for environmental toxicity

Brief summary

Allium cepa (green onion/scallion/spring onion) roots are grown in containers in order to test the ability of environmental pollutants to interfere with mitosis occurring at the root tip. Mitosis at the tip of the root (meristem) results in elongation of the root. The length of the roots grown in distilled water can be compared with the length of the roots grown in various concentrations of chemicals that might be polluting our environment.

Aim

To determine the effect of a specific environmental pollutant on the root length of *A. cepa*.

Variables

- Independent: various concentrations of a household cleaning product of your choice.
- Dependent: length of *A. cepa* roots after 2 weeks.
- Controlled: temperature; hours of light to which *A. cepa* is exposed; container size; size of *A. cepa.*

Theory

Allium cepa has been used in many scientific studies to assess environmental pollution. The cells of the root tip can be stained and the chromosomes observed and counted to determine the mitotic index (the ratio of cells undergoing mitosis to those not undergoing mitosis). Measuring the root length is an indirect measure of mitosis.

Some chemicals interfere with mitosis occurring at the *Allium* root tip. In one study, an increase in the concentration of waste water chemicals in the growth medium of the *Allium* roots resulted in a significantly decreased mitotic index and shorter roots. These results show that *Allium* is a reliable and useful tool for assessing toxicity of chemicals in the environment.

Apparatus required

- Thirty test tubes of the same size.
- A box of toothpicks.
- Six test tube racks holding five test tubes each.
- One household cleaning product.
- Thirty *A. cepa* with approximately the same size bulb and length (height).
- A graduated cylinder.
- A millimetre ruler.

Step-by-step method and procedure

- Make five serial dilutions of the household product you are testing using distilled water.
- Determine the amount of diluted household product to add to each test tube, so that the liquid will touch the bottom of the *Allium*. The roots will grow from the bottom. If the liquid evaporates over the 2 weeks, add more liquid so that the growing roots are always in solution.
- Insert three toothpicks into each *Allium* bulb and suspend it on the top of each test tube with the bottom of the bulb touching the liquid in the test tube.
- Set up your six test tube racks with *Allium* bulbs. One rack will have five tubes with only distilled water as a negative control. Each of the other racks will have five test tubes, each with one type of serial dilution of the household cleaner you are testing.
- Grow all the *Allium* bulbs at room temperature. Make sure to record the temperature of the room daily.
- Make qualitative observations daily.
- Grow all the *Allium* bulbs in the same amount of light. If it is daylight, then record the hours of light and dark for the 2 weeks.
- Measure the root length of all 30 of the *Allium* bulbs after 2 weeks of growth.
- Design and describe your method of measuring the roots. Will you measure the longest root or the general length of all the roots?

Results

Make a data table and record the results.

Guidance to record raw data

- Be sure to write a title for your table.
- Include any uncertainties of measurement.

Guidance to undertake data processing

Because you will have five data points for each concentration of household cleaner, you can calculate the average for each concentration and also calculate the standard deviation for each concentration.

Guidance for presentation of processed data

The concentration of household cleaner is the independent variable, while root length is the dependent variable. Because the concentration is a continuous variable, a line graph is appropriate. Standard deviation can be shown with an error bar at each data point.

- Make sure you give your graphs a title.
- Include information about what the error bars indicate.
- Add uncertainties to the labels on the *x*- and *y*-axes.
- Perform a Student's *t*-test to determine whether the different concentrations are statistically significant.

Conclusion

- Did any of the concentrations of the household chemical inhibit the root growth of *Allium*?
- Support your conclusion with specific numerical data from your data processing.
- Does any reference support this conclusion? Make sure to include a citation to the reference.

Evaluation

Specifically criticize your methods, errors, and the amount of data that you collected. Refer to the criticisms and make suggestions about how you could improve your methodology. You could use a table for this activity.

Further studies could include the following.

- Is root inhibition reversible? After 2 weeks, if the roots are placed in water, will they regain their ability to grow normally?
- Can you calculate the mitotic index of some of the cells? Go to a website that describes how to observe the cells by doing an aceto-orcein squash.

Suggested reading

Research online how the *Allium* test has been used to prevent environmental pollution by harmful chemicals.

Chapter 15, Option D: How long does it take to return to a resting heart rate after mild exercise?

Brief summary

Test subjects will be asked to carry out mild exercise in order to increase their heart rate 30% above their resting rate. The time taken for the test subjects to return to their resting heart rate will be measured and recorded.

Aim

To understand better heart rate regulation and the role of cardiovascular fitness on heart rate.

Theory

Cardiovascular fitness includes a multitude of physiological and anatomical factors. One of the better indicators of good cardiovascular fitness is the ability to return relatively quickly to a resting heart rate after exercise.

Apparatus required

- Stethoscopes
- Timers

Step-by-step method and procedure

Practise how to use a stethoscope to listen to the sounds of the heart, and to use a timer to count heart beats. Remember that you will hear two sounds with a stethoscope placed over the heart, a 'lub' and then a 'dub', followed by a short silence. These three events represent one heart beat. It is a good idea to work in groups for this lab and pool your data, as there is likely to be a great deal of variation in the data set and a larger data set will help minimize some counting irregularities. Prior to the lab, each participating test subject should be asked how many hours a week he or she spends in some sort of aerobic activity. Divide the total test subject pool into about five intervals based on the results of this information. These groupings will then be the independent variable of this investigation.

- Each test subject should have his or her resting heart rate measured by at least three different people. Count the number of heart beats heard for 20 seconds and then multiply that by 3 to get the number of beats per minute (the raw data will be beats 20 s^{-1}).
- Use the mean of the three measurements as the heart rate (mean heart rate).
- For each test subject, calculate what his or her heart rate will be if it is increased by 30%.
- Determine a common, safe, low-impact exercise that all test subjects can do to increase their heart rate. Walking stairs or jumping jacks are good possibilities. Each test subject will begin the exercise and his or her heart rate should be counted frequently (20 second counts) so that they reach a rate that is 30% higher than their resting rate but do not go much higher than that.

- Once a subject does increase his or her heart rate by 30%, he or she should stop the exercise immediately and be allowed to rest. The 20-second heart rate count should be taken every 30 seconds until the heart rate is back at the resting rate. (This means a 20-second count is followed by a 10-second rest, then another 10-second count, and so on.)
- Each test subject should be tested as many times as possible after having been given appropriate rest intervals between data collection events.

Results

The raw data will be the 20-second heart rate counts for each test subject. Each test subject will be assigned to one of five groups based on their aerobic activity.

Guidance to undertake data processing

All 20-second counts should be converted to true heart rates of beats min^{-1}. Each individual should have his or her data converted to a mean time of recovery to resting heart rate because he or she will have been measured multiple times.

Guidance for presentation of processed data

A graph should be drawn showing the aerobic ratings on the *x*-axis and mean recovery times on the *y*-axis.

Conclusion

Your graph should indicate whether there is a correlation between the aerobic rating scale generated and recovery time back to a resting heart rate.

Evaluation

Evaluate the strengths and weaknesses of the procedure and techniques used, and make suggestions for improvement.

Suggested reading

HL Chapter 15: Section D.4.