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INTRODUCTION

ExperimentFest is an experiment program designed to provide enriching educational experiences for senior high school students who are studying Physics, Chemistry and Biology. ExperimentFest is supported by the University of Newcastle’s Faculty of Science and Information Technology and takes place at both the Callaghan and Ourimbah (Central Coast) campuses of the University of Newcastle.

Callaghan Campus:
Physics - Friday 12 – Thursday 18 June, 2015

Chemistry - Monday 15 – Friday 19 June, 2015

Biology - Friday 12 - Friday 19 June, 2015

Ourimbah Campus:
Biology, Physics & Chemistry Monday 22 - Thursday 25 June, 2015

Tuncurry:
Physics - Saturday 20 June, 2015

All experiments are complemented by notes, follow-up discussions and questions to enhance your learning experience.

The activities allow students to engage in a range of hands-on experiments that are difficult to organise within a school setting, all under the supervision of University Staff and Postgraduate students. Each experiment is chosen to complement the NSW HSC syllabus for Biology, cementing classroom theory and providing a good basis for examination preparation.

Experiments include:
• Factors affecting the rate of enzyme activity
• Identifying and estimating the size of red and white blood cells using a light microscope
• Measuring oxygen saturation in the blood using a pulse oximeter

All experiments are complemented by notes, follow-up discussions and questions to enhance your learning experience.

For booking information contact:
Larry Milton on 0404 460 470; or
David Rushton on 4333 6965 or 0414 238 464
Welcome to the Faculty of Science and Information Technology at the University of Newcastle. ExperimentFest is a wonderful chance to give you practical experience which complements your classroom learning while giving you a first hand look at University life and facilities. Science is an exciting field of study, allowing you to move with the times and contribute actively and responsibly to society. There are many education opportunities in science after high school. Here in the Faculty we provide study and research programs in fastmoving modern fields that make our world work.

The Faculty staff and students who will be taking you through the experiments today are involved in contemporary science research. Please ask questions and utilise your time with them.

Take this day to enjoy being out of the class room, exploring science with fellow students and participating in valuable experiments and discussions which will help you in your HSC and beyond.

I wish you well in your studies. I hope you apply yourselves to the learning process with enthusiasm and you enjoy your time at the University. We hope to see you studying with us in the future!

Best wishes,

Prof. Eileen McLaughlin
Pro Vice-Chancellor – Faculty of Science and Information Technology
University of Newcastle
BIOLOGY
STUDYING BIOLOGY

Why study biology?

Biological Sciences is the science of life. Biologists study the world of living organisms; taking life and putting it into perspective, from microbes to human beings. Biology is a research-intensive science that can lead to a wide range of areas in which to study from environmental work to microbiology to agriculture. The discipline of Biological Sciences provides you with a great deal of hands-on learning and engaging research, moving you beyond text books and lectures. Course work provides you with the fundamental framework of knowledge, while labs provide you with practical hands-on experience.

Opportunities for further studies in Biological Sciences:

The Bachelor of Science degree program at the University of Newcastle provides a foundation of knowledge, skills and attributes that allows graduates to be employable not just today but into the future and to contribute actively and responsibly to society. Majoring in Chemistry, you have the opportunity to sample and/or specialise in any one of the following:

- Animal Biology
- Animal Research Science
- Biochemistry
- Biophysics (double major)
- Biostatistics (double major)
- Biotechnology
- Cell and Molecular Biology
- Environmental Biology
- Environmental Plant Biology
- Genetics
- Molecular Microbiology
- Park and Wildlife Biology
- Patent Law
- Physiology
- Plant Bioinformatics
- Plant Biology
- Plant Ecology
- Reproductive Biology

Research in Biological Sciences at the University of Newcastle

We conduct research on a wide range of contemporary biological problems. Staff are grouped in four major research themes but there are extensive collaborative links between these themes. By this approach we can develop areas of expertise and also generate multi-disciplinary and innovative research connections. The major research groupings are:

- Drug Design and Discovery
- Environmental Biology and Biotechnology
- Plant Science Group
- Reproductive Science Group

The discipline hosts two Australian Research Council Centres of Excellence, one University Priority Research Centre and one Research Centre:

- ARC Centre of Excellence in Biotechnology and Development
- ARC Centre of Excellence in Integrative Legume Research
- University PRC in Reproductive Sciences (joint with medicine)
- Centre for Sustainable Ecosystem Restoration (CSER)
Careers in Biology:

The Faculty of Science and IT care about our students and are interested in giving as much direction as possible to those making career choices and beyond. The possible career paths listed below include a range of opportunities for graduates at degree, honours, and post graduate study levels.

- Animal Biologist
- Animal Research Scientist
- Biochemist
- Biologist
- Biotechnologist
- Botanist / Plant Scientist
- Bushland Assessment Officer
- Clinical Research Coordinator
- Conservationist / Ecologist
- Environmental / Ecological Biologist
- Field Assistant
- Food Technologist
- Forensic Biologist
- Geneticist
- Graduate Trainee (Graduate Program)
- Laboratory / Research Assistant
- Marine Biologist
- Medical Scientist
- Microbiologist
- Molecular Biologist
- National Parks Ranger / Field Officer
- Pathology Assistant
- Pharmaceutical Sales
- Physiologist
- Reproductive Medicine / IVF Chemist
- Research Scientist
- Science Information / Education Officer
- Science / Biology Teacher
- Sciences Technician

For more information please visit the University’s website: www.newcastle.edu.au

For more information on the Faculty of Science and IT check out our website: www.newcastle.edu.au/science-it
Extract from HSC Biology Syllabus:

Factors effecting the rate of a named enzyme
Maintaining the balance
9.2.1
• Identify data sources, plan, choose equipment or resources and perform a first-hand investigation to test the effect of:
  – increased temperature
  – change in pH
  – change in substrate concentrations on the activity of named enzyme(s)

Identifying and estimating the size of red and white blood cells using a light microscope
Maintaining the balance
9.2.2
• Perform a first-hand investigation using the light microscope and prepared slides to gather information to estimate the size of red and white blood cells and draw scaled diagrams of each

Measuring oxygen saturation in the blood using a pulse oximeter.
Maintaining the balance
9.2.3
• Identify current technologies that allow measurement of oxygen saturation and carbon dioxide concentrations in blood and describe and explain the conditions under which these technologies are used

NAME: __________________________
Enzymes in Action

HSC Biology Syllabus 9.2.1 Maintaining a Balance
- Identify data sources, plan, choose equipment or resources and perform a first-hand investigation to test the effect of:
  - increased temperature
  - change in pH
  - change in substrate concentrations on the activity of named enzyme(s)

INTRODUCTION
Enzymes are proteins that catalyse (speed up) biochemical reactions. There are thousands of known enzymes and each cell contains hundreds of different ones.

In an enzyme-catalysed reaction the reactant is called the substrate. When enzymes and substrates combine they bind together at an area of the enzyme called the active site.

Each enzyme is highly specific to the reaction that it catalyses. One enzyme will catalyse only one particular substrate because of the exact match required between the shape of the substrate and the shape of the active site of the enzyme.

The lock and key model is used to explain this specificity of enzyme action.

![Figure 1.0 The lock and key model for enzyme action](image-url)
Factors affecting the rate of a named enzyme

The activity and efficiency of an enzyme can be significantly affected by temperature, pH and substrate concentration. Enzyme activity is measured by the rate of the enzyme-catalysed reaction.

TEMPERATURE
Enzymes have an optimum temperature at which they are most active. At low temperatures the molecules of the enzyme and substrate have low energy and the activity of the enzyme is low. As temperature increases the activation energy is reached by more molecules and the rate of reaction increases. Different enzymes have different optimum temperatures depending on their environments.

pH
The pH is a measure of the acidity of a solution and ranges from 1 to 14 on the pH scale. The presence of hydrogen ions (H+) in solution causes acidity and the higher the hydrogen ion concentration, the lower the pH. An acidic solution has a pH below 7. An alkaline or basic solution has few hydrogen ions and a pH above 7. A neutral solution has a pH of 7.

Different enzymes have different optimum pH values depending on their environments. Amylase is an enzyme found in the mouth and active within a pH range of 5 to 9 with an optimum pH of 6.8. Pepsin is an enzyme found in the stomach and is active within a pH range of 1 to 4 with an optimum pH of 2.

Enzymes are most active at the optimum pH. They lose activity if the pH varies too much from the optimum pH as this interrupts the bonds that hold the enzyme in its shape.

At high temperatures and extremes of pH, enzymes become denatured. This is when the protein molecules of enzymes become unravelled and the active site is destroyed.
Substrate Concentration

In most enzyme-catalysed reactions of biological systems, the enzyme concentration is much lower than the substrate concentration. An increase in the enzyme concentration results in a linear increase in the rate of reaction (Figure 2).

When the substrate concentration is increased, and the enzyme concentration remains the same, the rate of reaction eventually reaches a constant rate even with further increases in substrate concentration. This point is called the maximum velocity (Vmax) and is reached when all the active sites of the enzyme are occupied (or saturated) with substrate and the reaction proceeds at its maximum rate (see Figure 3 above).

The Experiment

PURPOSE

In this experiment you will investigate the effect of change in temperature, pH, and substrate concentration on the activity of the catalase enzyme.

Background

Hydrogen peroxide (H2O2) is a toxic by-product of metabolism that can damage and destroy cells if not quickly removed or decomposed. Catalase is an enzyme that rapidly catalyses the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules.

Catalase is one of the most efficient enzymes known. It is present in peroxisomes of nearly all aerobic cells. Peroxisomes are specialised microstructures in the cell which contain oxidation reactions that produce toxic peroxide by-products. All animals use catalase in every organ, with the liver containing particularly large concentrations. Catalase is also found in many plants.
EQUIPMENT USED

Each bench will have:

- Working solutions of catalase in graduated 10mL sterile tubes stored on ice
- 3% hydrogen peroxide with detergent added (this causes the bubbles of oxygen from the reaction to produce foam)
- Range of different concentrations of Hydrogen peroxide (0%, 0.5%, 1%, 2%, 3%)
- Range of different pH solutions of hydrogen peroxide (pH 3, 5, 7, 11)

The enzyme reactions will be enabled using 10mL sterile tubes and 5mL vials as pictured below.

The photograph below illustrates the typical reaction you will observe between the catalase enzyme and hydrogen peroxide solution. The foam component is indicative of the oxygen by-product.
METHODOLOGY

Procedure A: Effect of temperature on catalase activity

- Remove the five temperature labeled 10mL sterile tubes (4°C, 25°C, 60°C, and 90°C) containing 100µL of catalase enzyme from the esky.
- Place each of the tubes in the corresponding water baths and leave for 5 minutes. This allows the catalase to equilibrate to the temperature being tested.
- Collect five of the small 5mL yellow top vials and label them 4°C, 25°C (room temperature), 37°C, 60°C and 90°C.
- Using a pipette set at 1000µL (1mL), add 2mL of 3% hydrogen peroxide (pH7) to each of the labelled 5mL vials. Replace the lids and position vials in the appropriate water baths next to corresponding 10mL tubes. Allow to equilibrate for several minutes.

**NOTE** – the next step involves timing the reaction! Read carefully.

- Carefully pour the 2mLs of 3% hydrogen peroxide from the small vial into the corresponding 10mL tube containing catalase, and at the same time start the stop watch (keep the centrifuge tube in the water bath during the reaction).
- After 30 seconds (reaction time) record the volume of the foam (height of the bubbles in the tube) in mL.
- Repeat the above method for other tubes at different temperatures to be tested, with the following exception; **EXCEPTION:** perform the reaction for 90°C temperature point on the bench top using the test tube rack provided (i.e. do not undertake reaction in 90°C water bath).
- Record your results in the table and on the white board at the front of the lab.
- Graph the average of all groups’ results at each temperature

RESULTS

<table>
<thead>
<tr>
<th>Tube</th>
<th>Temperature (°C)</th>
<th>Volume of foam produced in reaction time (mL)</th>
<th>Rate of Reaction Volume/ reaction time (mL)/ second</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Explain the shape of your graph. Account for what happens to the enzyme activity at low and high temperatures, and identify the optimum temperature for catalase activity.

NOTE: Variables are quantities that change during an experiment, either deliberately by the experimenter or due to external influences. The independent variable is the quantity that is deliberately changed by the experimenter. The dependent variable is the quantity that responds to the change made to the independent variable.

2. Identify the independent variable and dependent variable for this experiment.
Procedure B: Effect of pH on catalase activity

- Remove four of the 10mL tubes containing the 100µL of catalase enzyme from the esky and label as pH3, pH5, pH7, pH11.
- Place the tubes in a test tube rack on the bench and leave for a few minutes to allow the enzyme to equilibrate to room temperature.
- Collect four of the small 5mL yellow top vials and label them pH3, pH5, pH7, and pH11.
- Using a pipette set at 1000µL (1mL), add 2mL of the appropriate pH solution into relevant (previously labelled) 5mL yellow top vials.
  **NOTE: use a new pipette tip for each pH solution.**
- Replace the lids and position vials next to the corresponding 10mL tubes containing enzyme in the test tube rack.

  **NOTE – the next step involves timing the reaction! Read carefully.**

- Carefully pour the 2mL of pH solution from the 5mL vial into the corresponding 10mL tube containing 100µL of catalase enzyme and simultaneously start the stopwatch.
- After 30 seconds (reaction time) record the volume of foam in mL (height of the bubbles in the tube).
- Repeat for other pH solutions in the test tube rack.
- Record your results (and other groups) in the table.
- Graph your results to show the effect of pH on the enzyme activity (rate of reaction).

### RESULTS

<table>
<thead>
<tr>
<th>Tube</th>
<th>pH</th>
<th>Volume of foam produced in reaction time (mL)</th>
<th>Rate of Reaction Volume/ reaction time (mL)/ second</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Explain the shape of your graph and account for what happens to the enzyme activity in conditions of low pH and high pH. Identify the optimum pH for catalase activity.

2. List the variables from this experiment that were kept constant.

3. What is pH?
**Procedure C: Effect of substrate concentration on catalase activity**

- Remove five of the 10mL tubes containing 100µL of catalase enzyme from the esky and label 0%, 0.5%, 1%, 2%, 3%.
- Place the labelled tubes in a test tube rack on the bench and leave for a few minutes to allow the catalase enzyme to equilibrate to room temperature.
- Collect five of the small 5mL yellow top vials and label them 0%, 0.5%, 1%, 2% and 3%.
- Using a pipette set at 1000µL (1mL), add 2ml of each of the different concentrations of hydrogen peroxide solution (substrate), into the previously labelled 5mL yellow top vials. [NOTE – use a new pipette tip for each concentration of hydrogen peroxide]. Replace the lid and position vials next to the matching 10mL tubes in the test tube rack.

  NOTE – the next step involves timing the reaction! Read carefully.

- Starting with the 0% substrate concentration, carefully pour the 2mL of hydrogen peroxide solution from the 5mL vial, into the corresponding 10mL tube containing 100µL of catalase enzyme and simultaneously start the stopwatch.
- After 30 seconds (reaction time) record the volume of foam in mL (height of bubbles in the tube).
- Repeat for other substrate concentrations in the test tube rack.
- Record your results (and other groups) in the table.
- Graph your results to show the effect of substrate concentration on enzyme activity (rate of reaction)

**RESULTS**

<table>
<thead>
<tr>
<th>Tube</th>
<th>Substrate Concentration (%)</th>
<th>Volume of foam produced in reaction time (mL)</th>
<th>Rate of Reaction Volume/ reaction time (mL)/ second</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Explain the shape of your graph and account for what happens to the enzyme activity as substrate concentration increases. What is the maximum rate?

2. Use your graph to predict what would happen to the enzyme activity with a substrate concentration of 5% hydrogen peroxide.
DISCUSSION AND CONCLUSIONS

1. Outline the relationship between reaction rate and substrate concentration.

2. How do extremes of pH cause denaturation (breakdown of structure of a protein) and loss of enzyme activity?

3. Outline possible causes for the variation in the class data other than experimental error.
The microscope is a major tool of biologists. Through the use of the compound light microscope, knowledge of cell structure was gained and one of the fundamental theories of biology, the Cell Theory, was developed. Without the microscope, we would have very little knowledge of living things too small to be seen by the unaided human eye (e.g. cells and their contents).

Pre-lab Questions:
Read and complete the following before entering the laboratory.
As you read the descriptions provided, label the parts on the diagram on the following page.

### Ocular Lens
- Also known as the eyepiece
- May be one only or a set of two
- Lens closest to your eye, usually the highest part of the microscope
- Includes a magnification factor engraved on the barrel, e.g. 10x indicates the image is magnified ten times
- Should be cleaned only with lens cleaner and lens paper

### Nosepiece
- Revolving circular mechanism that holds the different objective lenses
- Rotation of this changes the objective lenses

### Objective Lenses
- Individual lenses attached to the nosepiece
- Includes a magnification factor engraved on the barrel
- Changed by rotating the nosepiece

### Arm
- Provides the safest way to hold a microscope. Use two hands.

### Stage
- Also called the mechanical stage
- A surface that supports the slide, with the help of stage clips

### Stage controls
- Usually located on the top or side of the stage
- Moves the slide around the stage.

### Condenser
- Located under the stage
- Focuses the light through the hole in the stage and onto the specimen
- Adjusts the quality and the amount of light passing through the specimen
- May be raised or lowered with the condenser-adjustment knob

### Iris Diaphragm
- Located under the condenser
- Adjusts the intensity of light passing through the specimen
- Use the iris diaphragm lever to open or close

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**USING A LIGHT MICROSCOPE**
Coarse Adjustment knob
[ ] Large knob located on the arm
[ ] Adjusts the distance between the stage and the objective lens, in large increments
[ ] Used initially to bring the specimen into focus. It is dangerous to use this knob when the objective lens is already near the slide
[ ] Should be turned very slowly to avoid breaking the slide

Fine adjustment knob.
[ ] Small knob located inside the course adjustment knob
[ ] Adjusts in small increments
[ ] Typically used after the objective lens is already near the slide and the specimen is almost in focus

Lamp
[ ] Small light source located under the condenser
[ ] May be turned on / off with switch on the base

Base
[ ] Square or horseshoe–shaped support for the whole microscope
[ ] Usually quite heavy to prevent tilting

Identify and label the parts of the microscope.
IDENTIFYING AND ESTIMATING THE SIZE OF RED AND WHITE BLOOD CELLS USING A LIGHT MICROSCOPE.

HSC syllabus reference: Maintaining the balance 9.2.2
- perform a first-hand investigation using the light microscope and prepared slides to gather information to estimate the size of red and white blood cells and draw scaled diagrams of each

During the identification and estimation of size of red and white blood cells experiment you will be using a compound light microscope.

The binocular compound microscope is used for detailed examination of thin sections of plant and animal tissues. It has a total magnification range of 32X to 1000X. Magnification is calculated as the power of the eye piece times the power of the objective lens e.g. eye piece 10X, objective lens 40X, magnification = 10 x 40 = 400X.

PRECAUTIONS FOR MICROSCOPE USE
- Microscopes are expensive and delicate instruments – please be gentle.
- If you need to move the microscope, always carry it in an upright position with one hand holding the arm of the microscope and the other supporting the base.
- Never place the microscope close to the edge of the lab bench.
- Never touch the lens of the microscope with your hands; only special lens paper should be used to clean the lenses in the eyepiece and objectives.
- Always turn on the microscope light first, before you look down the eyepiece. When you have finished using the microscope, turn off the light and rotate the low-power (shortest) objective into viewing position. Never leave the high-power objective in the viewing position.
- Always use the low-power objective to locate the specimen to be studied and bring it into focus using the large focus knob, then turn to the high-power objective for more detail.
- Use the fine focus knob when the high-power objective is in the viewing position.
- When changing to a higher objective lens always watch it from the side (not through the eye piece) to prevent damaging the objective lens by hitting the stage or slide.
- Ask a Demonstrator for help if you’re having difficulty using the microscope or your microscope is faulty – do not attempt to make any repairs yourself.
USING THE COMPOUND MICROSCOPE

Refer to Figure 2 below to help you identify parts of a compound microscope.

Setting up the compound microscope to view a slide
1. Ensure the microscope is securely on the bench and everything is in working order.
2. Rotate the nose piece so that the lowest power objective is in position.
3. Obtain a slide (hold it by its edges to avoid fingerprints) and, making sure it is the correct way up with the cover slip on top, put it on the stage and hold it in place with the stage clips.
4. Switch on the microscope light and adjust the light intensity.
5. Using the coarse focus knob and watching from the side of the microscope, wind the stage up until the tip of the low power objective lens is 5mm from the slide.
6. Look through the eyepiece and use the coarse focus knob to slowly move the stage downwards to bring the specimen into focus.
7. Use the fine focus knob to bring the specimen into sharp focus. If necessary move the slide around so that it is in the centre of the field.
8. Adjust the diaphragm until it just fits your field of view.

Using high power
1. First set up for low power examination as detailed above.
2. Find the part of the slide to be examined under low power first, centre it in the field of view, then carefully rotate the nose piece until the next highest power objective is in position.
3. Use the fine focus knob to bring the specimen into sharp focus and re-centre it in the field of view.
4. Rotate the highest objective lens into position and refocus and re-centre the slide.
5. If necessary adjust the diaphragm and condenser to give the sharpest and brightest image possible.
FIRST HAND INVESTIGATION TO ESTIMATE THE SIZE OF RED AND WHITE BLOOD CELLS AND DRAW SCALED DIAGRAMS OF EACH

CALCULATING THE MAGNIFICATION

The magnification is calculated by multiplying the ocular lens (eyepiece) and the objective lens.

For example:

* if the magnification of the ocular is 15X and the objective is 40X, then the total magnification is 15 x 40 = 600X.

CALCULATING THE FIELD OF VIEW

Using the microscope place the mini-grid on the microscope stage and move it so that the edge of the grid line is against the side of the field of view (circle). Count the number of divisions across the field of view.

* Remember 1mm = 1000µm (microns)
* If you increase the field of view (i.e. what you see) then the magnification will decrease by the same factor.

For example

* if you change the field of view (i.e. what you see) by doubling it, then the magnification will decrease by 1/2. That is, you see more (field of view) but you see it in LESS detail.
* if you change the field of view (i.e. what you see) by halving it, then the magnification will increase by 2. That is you see less (field of view) but you see it in MORE detail.

Increase FOV = Decrease MAGNIFICATION
Decrease FOV = Increase MAGNIFICATION

METHOD

- Place the micrometer grid on microscope
- Set the microscope to the lowest magnification- record this in the table.
- Adjust the micrometer grid so that it is in the field of view. Make sure that the line of the micrometer grid lines up with the diameter of the field of view.
- Count the grid lines – large are 1 mm, small 0.1mm
- Record in the table below the size of the field of view (FOV)
- Switch to each higher magnification and repeat

A closer look at the micrometer grid slide

<table>
<thead>
<tr>
<th>Microscope magnification</th>
<th>FOV diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Part B: ESTIMATING THE SIZE OF RED AND WHITE BLOOD CELLS.

METHOD

1. Place the prepared slide of a blood smear on the microscope stage under low magnification. Once it is in focus, rotate the high power objective into place. (Do not change the focus before doing this, as the microscopes are parfocal—they remain in focus while you rotate objectives and will not crush your slide.)

2. Distinguish between the numerous small red blood cells and the few, larger white blood cells (the nucleus in the white blood cells takes up a stain and appears dark blue or purple in colour). (See pictures provided to assist in identification)

3. Estimate the size of a red blood cell. Count or estimate the approximate number of red blood cells that would fit across the diameter (imaginary centre line) of the field of view (using HIGH magnification). Using this number and the known diameter for the field of view, calculate the size of each blood cell by dividing the diameter of the field of view by the number of cells which fit across it. For example, if about 50 red blood cells fit across the field of view and the field of view = 500 µm, then each cell is 500/50 = 10 µm.

4. Improve accuracy and validity. Repeat this process three times within different 'field of views' to determine an average size for red blood cells. Record your results on the Results page

5. Estimate the size of white blood cells
Since there are so few white blood cells, it is not possible to count the number of white cells across the diameter and therefore much more difficult to estimate how many would fit across the diameter. Another method of estimating their size is to compare their proportions with that of red blood cells. For example, is the white blood cell half the size or twice the size of a red blood cell? —i.e. how much bigger or smaller is the white blood cell compared with the red cell?

Use this estimate to calculate the size of white blood cells.

6. Improve accuracy and validity. To increase accuracy and validate of the conclusion based on the data gathered, repeat the process with three different white blood cells and obtain an average. Record your results on the Results page
Part C: DRAWING A SCALE DIAGRAM OF RED AND WHITE BLOOD CELLS.

METHOD

1. Draw a 1 cm line. This will be your scale bar that represents 10 µm.

2. Using this scale, draw a circle in the space provided below to represent the average size of each cell.

3. Now add the detail to the cells.

For example:
— red blood cell: represent the cytoplasm, cell membrane and use appropriate shading to show its biconcave shape
— white blood cell: draw the nucleus, cell membrane and cytoplasm.

4. Label all parts of each cell.
RESULTS

ESTIMATING THE SIZE OF RED & WHITE BLOOD CELLS

A) Red Blood Cells
Diameter of Field of View = ______________

Number of Red Blood Cells across diameter of the Field of View (Average) = ______

Estimated size of one Red Blood cell = ________

B) White Blood Cells
Are White Blood Cells smaller or larger than Red Blood Cells? ________________

Number of Red Blood Cells which fit across ONE White Blood Cell _____________

Estimated size of ONE White Blood Cell = ________________

RED BLOOD CELL DRAWING

WHITE BLOOD CELL DRAWING
MEASURING OXYGEN SATURATION IN THE BLOOD USING A PULSE OXIMETER

HSC Biology Syllabus 9.2.3 Maintaining a Balance
- Identify current technologies that allow measurement of oxygen saturation and carbon dioxide concentrations and explain the conditions under which these technologies are used

Introduction
Haemoglobin is a protein found in red blood cells and is responsible for carrying most of the oxygen in the blood. It can bind with oxygen and/or carbon dioxide. Oxygen saturation is the amount of oxygenated haemoglobin in the blood. Haemoglobin with oxygen attached to it is called Oxyhaemoglobin. The amount of oxygen bound to haemoglobin is determined by the oxygen concentration, carbon dioxide concentration and pH.
How haemoglobin works…
1. Blood entering the lungs has haemoglobin bound with carbon dioxide in it.
2. Through breathing, the lungs contain high concentrations of oxygen and low concentrations of carbon dioxide.
3. In the lungs, haemoglobin releases carbon dioxide and binds oxygen.
4. Haemoglobin gets transported through the heart and blood vessels to the muscle.
5. In muscle, the carbon dioxide concentration is high and the oxygen concentration is low due to metabolism.
7. Haemoglobin gets transported back to the lungs to repeat the cycle.

The pulse oximeter is a non-invasive device that measures the amount of oxygen in blood (oxygen saturation SpO2), and the pulse (heart) rate. It is used on adults and children in hospitals, medical clinics, and at home. It is a simple, quick, and effective instrument that consists of a probe attached to a person’s finger (some can attach to toes, nose or ear lobes), and a digital display showing both the percentage of haemoglobin saturated with oxygen and the pulse rate.
Pulse oximeters use two low powered LEDs of different wavelengths of light (red 660nm and infra-red 910nm), and a photodetector to record the amount of light absorbed as it passes through the finger. A processor in the device uses this information to calculate oxygen saturation (%SpO2) and pulse rate.

Figure 1 Pulse oximeter in use
Factors affecting pulse oximeter results:
- Poor circulation in the fingers, toes, or earlobe.
- Severe hypotension.
- Cardiac failure.
- Cold temperature.
- Bright external light.
- Smoking can affect blood oxygenation.

Typical 'normal' readings from a resting adult are 95 – 100 % oxygen saturation and 50 – 90 BPM pulse rate. It is considered abnormal if oxygen saturation declines by more than 5% during exercise or sleep.

NOTE: (BPM – Beats Per Minute).

FIRST HAND INVESTIGATION TO USE A PULSE OXIMETER TO MEASURE OXYGEN SATURATION IN BLOOD.

Purpose
In this experiment you will use a pulse oximeter to investigate the changes in blood oxygen saturation during aerobic exercise.

Background
During exercise your blood vessels dilate to increase the flow of oxygen-rich blood to your muscles. The metabolic activity is high, and the pH is lower than normal as more hydrogen ions and lactic acid are produced. The low pH weakens the attachment between oxygen and haemoglobin causing the haemoglobin to release more oxygen than usual. This increases the oxygen delivered to the muscle.

Method
1. Obtain a pulse oximeter and stop watch from a demonstrator.
2. Choose someone in your group to be the 'rider' of an exercise bike!
3. Turn the pulse oximeter on by pushing the button on the front, and attach it to the 'rider’s’ index finger, allow 8 seconds before recording the ‘at rest’ readings of oxygen saturation and pulse rate.
4. Start the stopwatch as the rider starts pedalling (at a moderate to fast rate).
5. After 2 minutes record the oxygen saturation and pulse rate readings.
6. Continue recording the oxygen saturation and pulse rate at 2 minute intervals.
7. Stop riding the bike after 10 minutes then walk slowly around the room to cool down and catch your breath, continue recording the oxygen saturation and pulse rate at 2 minute intervals for another 3 readings.
## RESULTS

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen saturation (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pulse rate (BPM)</td>
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</tbody>
</table>

Graph your results showing two curves, one for % oxygen saturation and one for pulse rate, against time.

![Graph](image)

1. Explain the shape of these curves considering what happens to blood oxygen saturation and pulse rate before, during, and after aerobic activity.

2. Describe some benefits and limitations of pulse oximetry.