Faculty of Science

Laboratory Manual

Genetics

Bachelor of Biotechnology (Hons.)

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Genetics

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')
- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful

- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

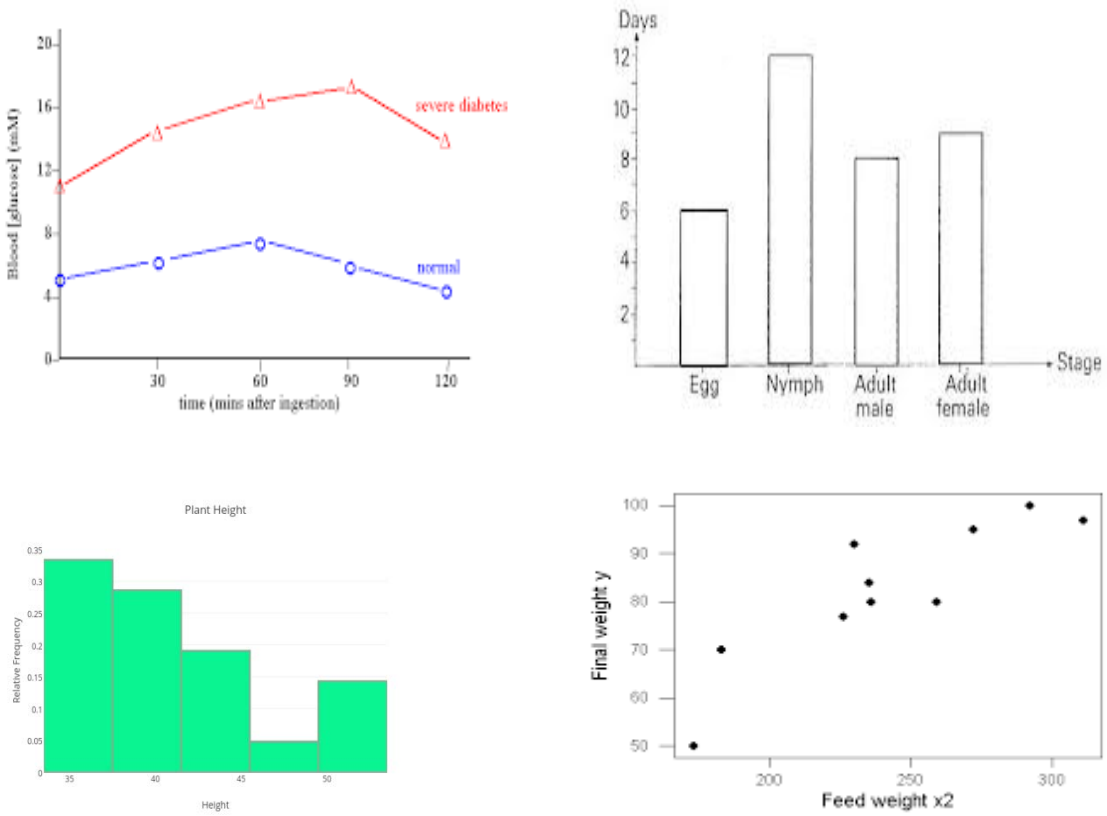

Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.
- Cite sources to substantiate background information.

- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1
Title: Mitosis in onion root tip

Objectives:
After completing the practical, you will be able:

1. To understand the process and different stages of mitosis
2. To visualize different phases of mitosis

Introduction

A process by which a parent cell divides into two or more daughter cells is called cell division. Cell division is a small part of the cell cycle. In normal eukaryotic cells, the type of cell division is known as mitosis.

Another type of cell division is present in reproductive cells of eukaryotes and is known as meiosis. Cell cycle is mainly classified into two segments: M-phase and Interphase. Interphase is the longer period of cell division. During this phase the cell prepares for its next stage.

This is a period of diverse activities and these activities are a prerequisite for the next mitotic phase. Interphase is mainly divided into three phases: G1 phase, S phase and G2 phase. S phase is the period of replication. G1 and G2 are the two gap phases during which the cell grows, producing proteins and preparing the cells. These phases also have certain check points and the whole cell cycle is strictly regulated.

M phase of the cell cycle stands for Mitosis or nuclear division. In eukaryotes, DNA replication is followed by a process called mitosis which separates the chromosomes in its cell nucleus into two identical sets, in two individual nuclei.. Mitosis is followed by cytokinesis. The process of Mitosis is divided into four stages: Prophase, Metaphase, Anaphase and Telophase (Figure 1).

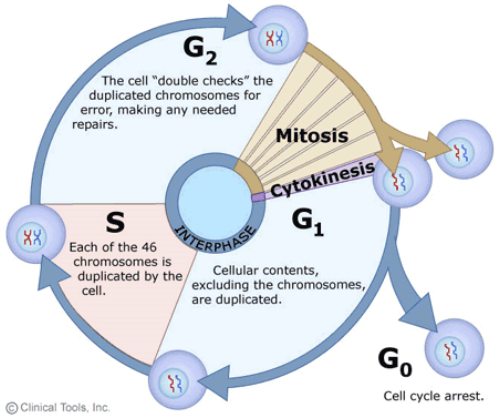

Figure 1: The process of mitosis

The genetic information of all organisms resides in the individual DNA molecules or chromosomes. An onion cell possesses eight chromosomes whereas human cells possess forty six chromosomes.

In 1842, C. Nageli first saw chromosomes and in 1888 W. Waldeyer named them. Walther Flemming studied and named the process of cell division as mitosis. Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs.

The most commonly used root tips in labs to study mitosis are onion, wheat, lentil, barley and alfalfa. An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, like Feulgen stain and Acetocarmine stain.

THE MITOTIC PHASE

The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved into two new, identical daughter cells. The first portion of the mitotic phase is called karyokinesis, or nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into the two daughter cells.

KARYOKINESIS (MITOSIS): NUCLEAR DIVISION

Karyokinesis, also known as mitosis, is divided into a series of phases that result in the division of the duplicated chromosomes:

1. Prophase
2. Prometaphase (sometimes considered part of prophase)
3. Metaphase
4. Anaphase
5. Telophase

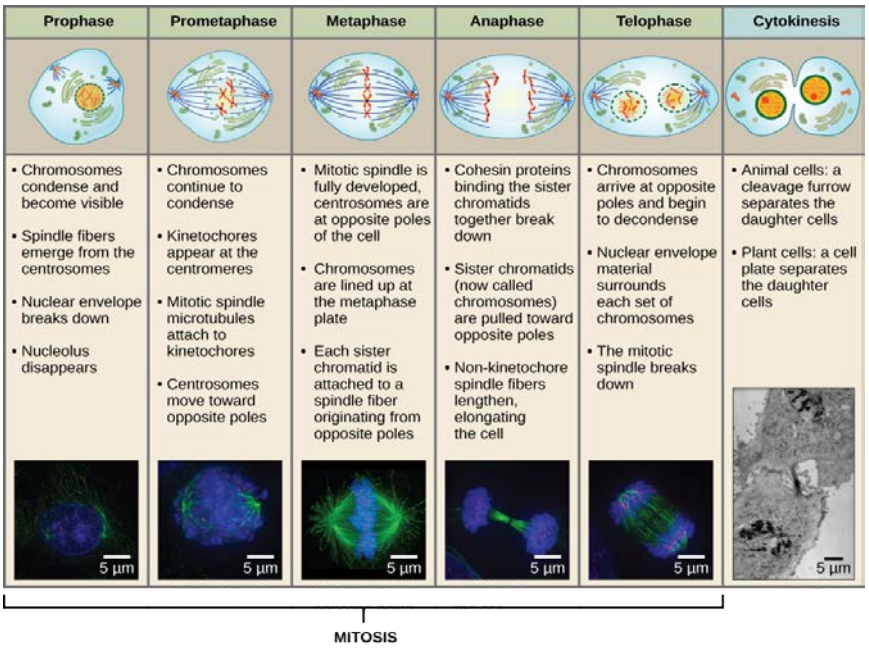

Figure 2: Karyokinesis

Materials required

1. Onion plant with root
2. Acetocarmine stain
3. 1 N HCl
4. Scissors
5. Forceps
6. Razor blade
7. Pasture pipette or dropper
8. Microscopic slides and cover slips
9. Heater
10. Light microscope

Procedure:

1. Take the onion plant with newly sprouted roots and cut two or three root tips using scissors and transfer them on a microscope slide.

2. Cover the roots with a drop of 1N HCl using a dropper.

3. Place the slide on a heater and heat the slides (~60˚C) for 10 minutes.

4. Discard the HCl by washing using drops of water (Rinse the roots at least three times).

5. Then remove the water from the surface of slide using a pipette.

6. After the washing step add 2-3 drops of stain on the slides with root tips and incubate the roots for 10 minutes at ~60˚C using a heater.

7. After the incubation remove the stain using a pipette.

8. Again rinse the root tips with distilled water. (Rinse the roots at least three times).

9. Transfer a root from the slide to the centre of a new microscopic slide and add a drop of water or stain over it.

10. Take a razor blade and cut most of the unstained part of the root.

11. Cover the root tip with a cover slip and then carefully push down on the cover slide with a clean tissue. (Push hard, but do not twist or push the cover slide sideways). The root tip should spread out to a diameter of about 0.5- 1cm.

12. Observe it under a compound microscope in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x for a better view.

Questions:

1. Why use onion roots for viewing mitosis?
2. What are the three cellular regions near the tip of an onion root?
3. Is it possible to view the chromosomes? Why?
4. What are the steps for softening the roots so that they later can be spread on a microscope slide?

Practical 2

Title: Blood grouping test

Objective:

After completing the practical, you will be able:

1. To understand the basic concept of blood grouping

Introduction

It was in 1901, that Austrian-American immunologist and pathologist Karl Landsteiner discovered human blood groups. Karl Landsteiner's work helps to determine blood groups and thus opened a way for blood transfusions which can be carried out safely. He was awarded the Nobel Prize in Physiology or Medicine in 1930 for this discovery.

Death of the patient was the result in most cases before 1900, when blood transfusion was attempted. Blood transfusion was made much safer by the discovery of blood groups, as blood of the same ABO group could be chosen for each patient. However, there were still many cases of unexplained blood transfusion reactions. Biologists still went in search of these unexplained questions.

In 1902, the fourth main type, AB was found by Decastrello and Sturli. It was the observations of Levine and Stetson in 1939, and Landsteiner and Weiner in 1940, which laid the foundations of our knowledge about the remaining major blood group- the Rhesus system. Once reliable tests for Rhesus grouping had been established, transfusion reactions became rare! For this discovery Landsteiner was awarded the Nobel Prize in Physiology or Medicine in 1930.

The Components of Blood

The circulatory system distributes about 4-6 liters of blood to the adult human body. The blood mainly has 2 portions: the Plasma and the Blood Cells. Plasma is mainly composed of water, but contains different types of proteins and other chemicals such as: enzymes, glucose, fat particles, salts, hormones, antibodies etc. It constitutes about 60% of the blood. Blood cells can be observed under a microscope on staining. The formation of blood cells occurs in the bone marrow by the 'Hematopoietic stem cells'. They can be divided into 3 basic cell types:

Erythrocytes- Red Blood Cells (RBC):

As the name suggests, these red coloured cells give blood its red colour. (The word erythrocyte is from erythro-Gk.meaning red and Latin-cytos meaning cell). One ml of blood contains approximately 5 million RBCs! The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%. Mature RBCs are biconcave in shape, lack a Nucleus and many other organelles. They circulate in the system for about 120 days, carrying out their job, i.e., to supply oxygen.

This function is carried out by them most efficiently. Since they are rich in an Iron-containing biomolecule called haemoglobin. Haemoglobin has high affinity for oxygen, thus binds to it and is transported from the alveoli (in lungs) to every part of the body. There is constant replenishment of RBCs in order to remove old cells that break down. (This process is carried out in organs like the liver, also producing by-products like bile pigments.) Millions of cells are released into the bloodstream from the bone marrow each day.

Leukocytes- White Blood Cells:

These cells are key players in our immune system. They are of different types such as neutrophils, lymphocytes, eosinophils, monocytes, basophils. Each of them has a variety of functions in our immunity. 1 ml of blood of an adult human contains about 4,000-11,000 leukocytes. Basically, they destroy and remove old or aberrant cells and clear cellular debris, as well as attack foreign substances and infectious agents (pathogenic entities).

Thrombocytes- Platelets:

The coagulation or blood clotting process is taken care of by them. They act on clotting proteins like Fibrinogen, converting it into Fibrin. They create a mesh onto which RBCs collect and form a clot. This prevents excessive blood loss and also checks the entry of pathogens into the body. 1 ml of blood of an adult human contains about 200,000-500,000 platelets.

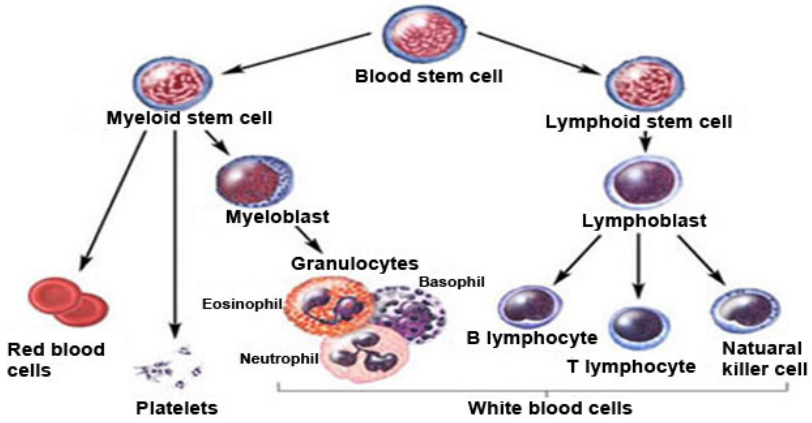

Figure 1: Platelets

The observations that led to the discovery of blood groups:

At times, it was observed that mixing blood from two individuals led to blood clumping or agglutination. Later it was understood that the agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The agglutinated red blood cells also crack and their contents leak out in the body.

The RBCs contain haemoglobin which becomes toxic when outside the cell. This must have been the phenomena that occurred in the blood transfusion cases that ended up with fatality of the patient at the receiving end. Karl Landsteiner discovered that blood clumping was an immunological reaction which occurs when the receiver of a blood transfusion has antibodies against the donor blood cells! People learned that, compatibility of blood groups needed to be checked before anything else was done. If they are not, the red blood cells from the donated blood will agglutinate. This can have fatal consequences for the patient.

ABO blood grouping system:

According to the AB0 blood group system there are four different kinds of blood groups: A, B, AB and O (null) (Figure 2).

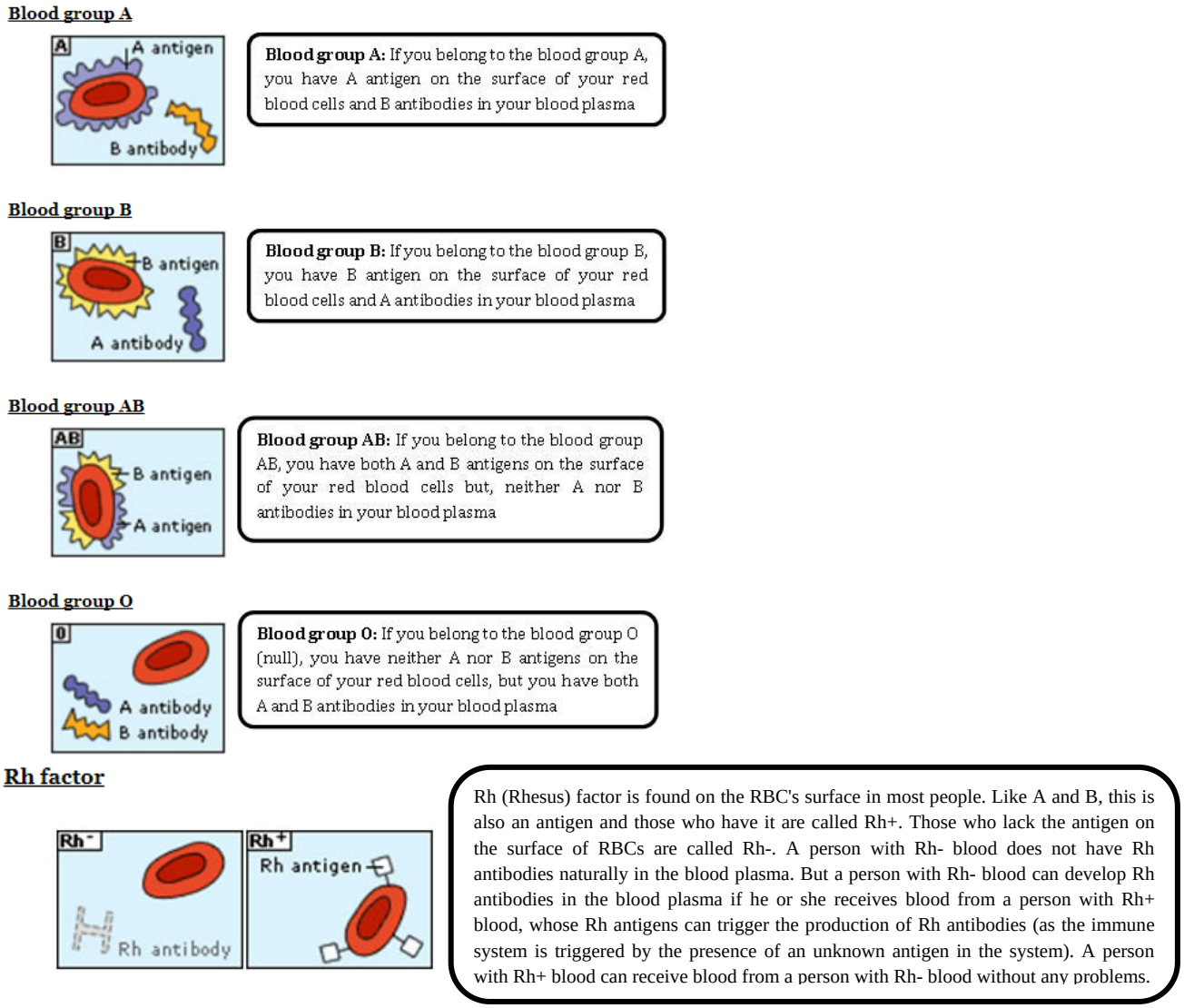

Figure 2: Blood grouping system

Inheritance of Blood Groups:

Blood groups for each individual are determined by genes or alleles (small packets of information in cells contained in the DNA) which are inherited from both parents. Genes for the Rh-ve and O groups from one parent are masked (i.e., they are recessive) by the presence of Rh+ve and A or B genes from the other parent. That is, O and Rh negative genes only produce an effect when there is a "double dose" of such genes, i.e., one from each parent (homozygous condition). Thus, people who are apparently A or B Rh+ve may also carry genes for the O and Rh-ve blood groups which can be inherited by their children (Figure 3).

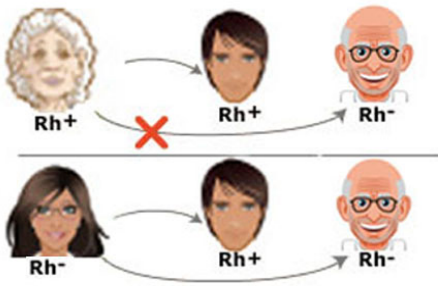

Figure 3: Inheritance of Blood Groups

Principle behind blood tests: Blood clumping or Agglutination observation

Compatibility between the blood groups of donor and recipient determines the success of a blood transfusion. The AB0 and Rh blood groups are looked at while conducting the test. In a diagnostic lab, Monoclonal antibodies are available for A, B and Rh antigen (Figure 4). Monoclonal antibody against Antigen A (also called Anti-A), comes in a small bottles with droppers; the monoclonal suspension being BLUE in colour. Anti-B comes in YELLOW colour. Anti-D (monoclonal antibody against Rh) is colourless. All the colour codes are universal standards. When the monoclonal antibodies are added one by one to wells that contain the test sample (blood from patient), if the RBCs in that particular sample carry the corresponding Antigen, clumps can be observed in the corresponding wells. A drop of blood is left without adding any of the antibodies; it is used as a control in the experiment. The monoclonal antibody bottles should be stored in a refrigerator. It is recommended to tilt the bottle a couple of times before use in order to resuspend the antibodies that have settled at the bottom of the bottle.

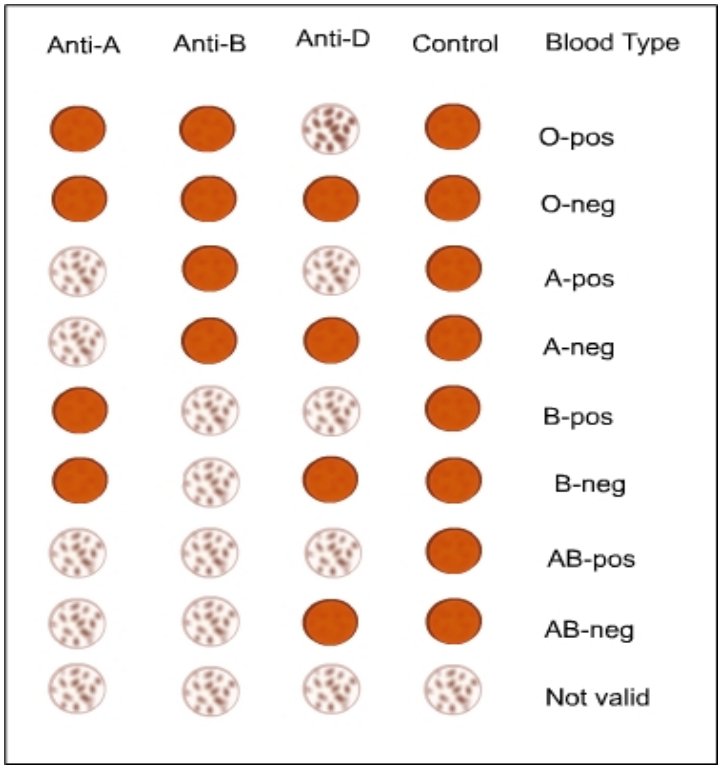

Figure 4: Blood test

Table 1: Blood group, antigens and antibodies

Blood Group	Antigens	Antibodies	Can Donate To	Can Receive from
A Rh+	A and Rh	B	A Rh+ AB Rh+	A Rh+ A Rh- O Rh+ O Rh-
A Rh-	A	B (Can develop Rh antibodies)	A Rh+ A Rh- AB Rh+ AB Rh-	A Rh- O Rh-
B Rh+	B and Rh	A	B Rh+ AB Rh+	B Rh+ B Rh- O Rh+ O Rh-
B Rh-	B	A (Can develop Rh antibodies)	B Rh+ B Rh- AB Rh+ AB Rh-	B Rh- O Rh-
O Rh+	Rh	A and B	O Rh+ A Rh+ B Rh+ AB Rh+	O Rh+ O Rh-
O Rh-	None	A and B (Can develop Rh antibodies)	AB Rh+ AB Rh- A Rh+ A Rh- B Rh+ B Rh- O Rh+ O Rh-	O Rh-
AB Rh+	A, B and Rh	None	AB Rh+	AB Rh+ AB Rh – A Rh+ A Rh- B Rh+ B Rh- O Rh+ O Rh-
AB Rh-	A and B	None (Can develop Rh antibodies)	AB Rh+ AB Rh-	AB Rh- A Rh- B Rh- O Rh-

Questions:

1. What are the consequences of Rh incompatibility and what does it lead to?

2. Hemolytic disease of new born due to maternal fetal ABO in compatibility occurs more frequently as a result of which specific mismatch?

3. A maternity case involves an infant with type AB blood. Four women claim to be the mother. You be the judge, as far as the blood type evidence allows. Why?
    - The woman with type A blood could not be the mother.
    - The woman with type AB blood could not be the mother.
    - The woman with type O blood could not be the mother.
    - The woman with type B blood could not be the mother.
    - All the four could be the mother.

4. Two parents with blood types A and O have a child who has type O blood. What is the probability that their next child will be type A?

5. A woman is married for a 2nd time. Her first husband was blood type A and her son by that marriage was type O. Her new husband is type B and their child is type AB. What is the woman's blood group?

Practical 3
Title: Chi-Square analysis

Objectives:

After completing the practical, you will be able:

1. To visualize different phases of mitosis Calculate χ^2 to determine whether a data set approximate a theorically expected ratio.
2. Interpret a calculated χ^2 value at an approximate number of degrees of freedom.

Introduction

The extent an observed data set fits or differs from the predicted or expected occurances can be evaluated by testing the goodness of fit of the data. When the data do not fit exactly, we will want to find out how much deviation can be allowed before we reject a null hypothesis. One of the simples statistical tests to assess the goodness of fit of the null hypothesis is Chi-square analysis (χ^2), which tests the difference between observed (O) and expected (E) values. This χ^2 value is then used to estimate how frequently the observed deviation can be expected to occur strictly as a result of chance, allowing us to determine whether progeny phenotype ratios fit our assumptions about their genotypes. The formula for calculating the Chi-square value is:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where O = the observed number of individuals in a particular phenotype,
E = the expected number in that phenotype, and
Σ = the summation of all possible values of $(O - E)^2 / E$ for the various phenotypic categories

The degree of freedom (*df*), which is equal to n – 1, where n is the number of categories (the number phenotypes considered), will be then be determined. Degrees of freedom must be taken into account because the greater number of categories, the more deviation is expected by chance alone. The calculated χ^2 value can now be interpreted at a corresponding probability value (*p*), which can be obtained from χ^2 table (Appendix) for (n -1) degree of freedom.

The *p* value can be thought as a percentage. For example, a *p* value of 0.26 indicates that were the same experiment repeated many times, 26% of the trials would be expected to exhibit chance deviation as great as or greater than that seen in the initial trial. Conversely, 74% of the repeats would show less deviation than initially observed as a result of chance. The interpretation of the *p* value reveals that a hypothesis is never proved or disproved absolutely. Instead, a relative standard must be set to serve as the basis for either rejecting or failing to reject the hypothesis. This standard is often a probability value of 0.05. If the calculated χ^2 value is smaller than the critical value given, in the table at (n – 1) *df*, then we accept the null hypothesis and conclude that the difference between observed and expected results is not significant, and vice versa.

Materials:

Non-biological materials: container of equal quantities of colored and white beads, Petri dishes, two equal-value coins and a calculator

Activity 1 (suggested time: 30 mins)
You will be given a beaker containing 150 coloured and 150 white beads that have been throughly mixed. Proceed with the following:

1. Remove one petri dish full random sample of beads from the beaker.
2. Segregate and count the beads of the different colours.
3. Record your data in Table 1 and then calculate the expected numbers based on the size of the sample and the known ratio of coloured to white beads in the entire population. Complete Table 1 and calculate χ^2.

Table 1: Calculation of χ^2 for a sample removed from a large population consisting of equal numbers of coloured and white beads.

Classes (Phenotypes)	Observed (O)	Expected (E)	Deviation (O-E)	$(O-E)^2$	$(O-E)^2 / E$
Coloured					
White					
Total				χ^2 =	

Degree of freedom:_____

p value: _____ Critical value:_____

I accept / reject the hypothesis that the data approximate the expected ratio.

Activity 2 (Suggested time: 30 mins)

1. Toss two equal-value coins together 30 times.
2. Record your data in Table 2 and then calculate the expected numbers. Note that the probability of two or more independent events occuring simultaneously is the product of their individual probabilities.
3. Complete Table 2 and calculate χ^2.

Table 2: Calculation of χ^2 on data from tossing coins.

Classes (Phenotypes)	Observed (O)	Expected (E)	Deviation (O-E)	$(O-E)^2$	$(O-E)^2 / E$
Heads on both coins					
Heads on one, tails on the other coin					
Tails on both coins					
Total				χ^2 =	

Degree of freedom:_____

p value: _____ Critical value:_____

I accept / reject the hypothesis that the data approximate the expected ratio.

Activity 3 (Suggested time: 30 mins)

You will be given a breaker containing 100 beads of two different colours in the ratio of 3:1. The beads have been through roughly mixed. Proceed with the following:

1. Remove one petri dish full random sample of beads from the beaker.
2. Segregate and count the beads of the different colours.
3. Record your data in Table 3 and then calculate the expected numbers based on the size of the sample and the known ratio of coloured to white beads in the entire population. Complete Table 3 and calculate χ^2.

Table 3: Calculation of χ^2 for a sample removed from a large population consisting of beads of two different colours.

Classes (Phenotypes)	Observed (O)	Expected (E)	Deviation (O-E)	$(O-E)^2$	$(O-E)^2 / E$
Coloured					
White					
Total				χ^2 =	

Degree of freedom:_____

p value: _____ Critical value:_____

I accept / reject the hypothesis that the data approximate the expected ratio.

Activity 4 (suggested time: 30 mins)

The following are the approximate frequencies of the various ABO blood groups in a hypothetical population: 41% A, 9% B, 3% AB, and 47% O. Determine if the data given in Table 4 represent a satisfactory sample of the hypothetical population. Calculate E and χ^2 values to two decimal places, complete the table, and answer the following questions.

Table 4: Calculation of χ^2 on blood group data from a hypothetical population.

Classes (Phenotypes)	Observed (O)	Expected (E)	Deviation (O-E)	$(O-E)^2$	$(O-E)^2 / E$
A	413				
B	94				
AB	77				
O	516				
Total				χ^2 =	

Degree of freedom:_____

p value: _____ Critical value:_____

I accept / reject the hypothesis that the data approximate the expected ratio.

Cautions When Using Chi-Square

Statistians suggest that calculating χ^2 is inappropriate when the sample size in any class is less than five. With certain exceptions, χ^2 calculations must be based on numerical frequencies and not on percentage or ratios.

Appendix: Table of χ^2 values at various degrees of freedom*

Percentage Points of the Chi-Square Distribution

Degrees of Freedom	Probability of a larger value of x^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72
12	3.571	5.226	6.304	8.438	11.340	14.85	18.55	21.03	26.22
13	4.107	5.892	7.042	9.299	12.340	15.98	19.81	22.36	27.69
14	4.660	6.571	7.790	10.165	13.339	17.12	21.06	23.68	29.14
15	5.229	7.261	8.547	11.037	14.339	18.25	22.31	25.00	30.58
16	5.812	7.962	9.312	11.912	15.338	19.37	23.54	26.30	32.00
17	6.408	8.672	10.085	12.792	16.338	20.49	24.77	27.59	33.41
18	7.015	9.390	10.865	13.675	17.338	21.60	25.99	28.87	34.80
19	7.633	10.117	11.651	14.562	18.338	22.72	27.20	30.14	36.19
20	8.260	10.851	12.443	15.452	19.337	23.83	28.41	31.41	37.57
22	9.542	12.338	14.041	17.240	21.337	26.04	30.81	33.92	40.29
24	10.856	13.848	15.659	19.037	23.337	28.24	33.20	36.42	42.98
26	12.198	15.379	17.292	20.843	25.336	30.43	35.56	38.89	45.64
28	13.565	16.928	18.939	22.657	27.336	32.62	37.92	41.34	48.28
30	14.953	18.493	20.599	24.478	29.336	34.80	40.26	43.77	50.89
40	22.164	26.509	29.051	33.660	39.335	45.62	51.80	55.76	63.69
50	27.707	34.764	37.689	42.942	49.335	56.33	63.17	67.50	76.15
60	37.485	43.188	46.459	52.294	59.335	66.98	74.40	79.08	88.38

*Selected data from R.A. Fisher and F. Yates, *Statistical tables for biological, agricultural and medical research* (London: Oliver and Boyd, 1943).

Practical 4
Title: Monster genetic lab

Objective:
After completing the practical, you will be able:

1. To observe how the results of different gene combinations produce certain traits

Introduction

Heredity is the passing on of traits, or characteristics, from parent to offspring. The units of heredity are called *genes*. Different versions of the same gene are called *alleles*. Genes are found on the *chromosomes* in a cell. *The combinations of genes for each trait occur by chance*.

When one allele in a pair is stronger than the other allele, the trait of the weaker allele is masked, or hidden. The stronger allele is the *dominant allele*, and the allele that is masked is the *recessive allele*. *Dominant alleles are written as capital letters and recessive alleles are written as lowercase letters*. If both alleles are different, the trait is said to be *heterozygous*, or *hybrid*. If both alleles are the same, the trait is said to be *homozygous*, or *purebred*. Sometimes alleles are neither dominant nor recessive. The result of such a situation is a *blending* of traits.

The genetic makeup of an individual is known as its *genotype*. The observable physical characteristics of an individual that are the result of the genotype are known as its *phenotype*.

Procedure:

1. Flip a coin twice to determine the genotype for each trait and record it in the data table.
   Heads = allele 1, Tails = allele 2 (*Example: if you flipped heads twice, your monster will have two copies of allele 1 for his genotype.*)

2. Determine the phenotype resulting from the allele pair for each trait.
   Repeat steps 1-2 for each trait and complete the female monster's Table 1.

Table 1: Genotypes & Phenotypes for Female Monster

Trait	Allele 1	Allele 2	Genotype	Phenotype
Eye	Two small eyes (E)	One large eye (e)		
Eye Color	Red (R)	White (r)		
Skin Color	Green (G)	Blue (g)		
Tail Shape	Curly (C)	Straight (c)		
Tail Color	Purple (P)	Orange (p)		
Tail	Have tail (T)	No tail (t)		
Teeth	Sharp (S)	Round (s)		
Feet	Four toes (F)	Three toes (f)		
Horn Color	Purple (W)	White (w)		
Ear shape	Pointy (Y)	Round (y)		
Ears	Four ears (N)	Two ears (n)		
Claws	Long (L)	Short (l)		

The female monster (described in Table 1) is married to a male monster (see Table 2 below) and they plan to have baby monsters. They are interested in finding out the probabilities of which traits their offspring will have. Fill in the missing genetic information in the table for the male.

Table 2: Genotypes & Phenotypes for Male Monster

Trait	Genotype	Phenotype
Eyes	ee	
Eye Color		White
Skin Color		Green
Tail Shape		Straight
Tail Color	Pp	
Tail		No tail
Teeth		Round
Feet	Ff	
Horn Color	ww	
Ear shape	yy	

Ears		Have 2 ears
Claws		Short

Analysis and Conclusion:

1. Create Punnett squares (attach your work to this handout) to predict what traits would result from a cross between the two monsters for each trait, and write out the genotype & phenotype ratios for each:

a. Eye
b. Eye Color
c. Skin Color
d. Tail
e. Feet
f. Horn Color
g. Ears
h. Claws

2. Write out three possible parental crosses that could produce a child with a heterozygous genotype for tail shape (Cc).

3. In humans, the sex of an individual is determined by the particular combination of the two sex chromosomes. Individuals that have two X chromosomes (XX) are females, whereas those with an X and a Y chromosome (XY) are males. Monster genetics works the same way. What percent chance do the male and female monsters have of producing a male offspring? A female offspring? Explain your answer. (Yes use COMPLETE SENTENCES!)

Critical Thinking and Application: ANSWER IN COMPLETE SENTENCES!!!

1. How might it be possible for the baby monster to show traits that neither of the parents exhibit?
2. Do you think the baby monster could have some traits of the grandparents?
3. A small monster colony has a large amount of monsters that have an abnormal amount of horns. Why might this trait be so prevalent in this colony?

Draw a picture of either your female monster or one of your possible baby monsters.

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