Faculty of Science

Laboratory Manual

Biophysics

Bachelor of Biotechnology (Hons.)

Edited By: Dr. Tapash Rudra

Published by:

LINCOLN UNIVERSITY COLLEGE
Wisma Lincoln, No. 12,14,16 & 18,
Jalan SS 6/12, Off Jalan Perbandaran
47301 Petaling, Jaya,
Selangor Darul Ehsan, Malaysia
Tel.: +603-7806 3478
Fax: +603-7806 3479
Toll Free: 1-300-880-111
E-mail: lucp@lincoln.edu.my
info@lincoln.edu.my
Web: www.lucp.net
www.lincoln.edu.my

ISBN: 978-967-2257-16-5

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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).

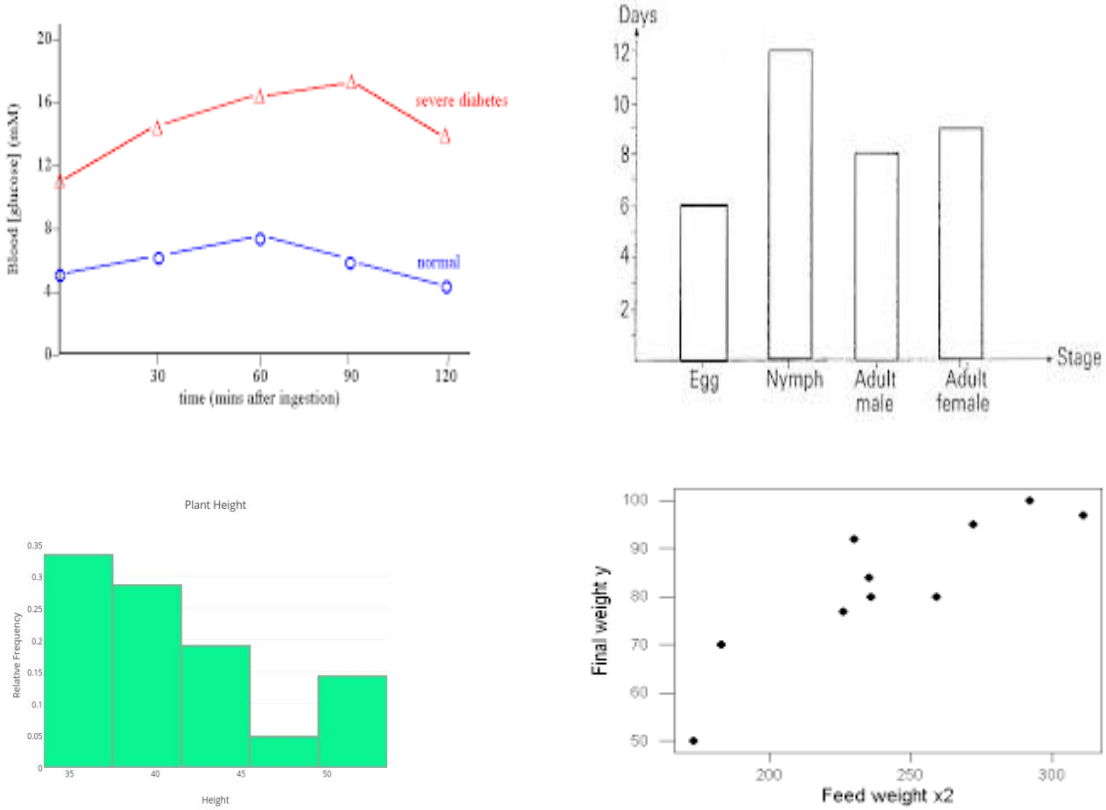

Figure 1: Line graphs, bar charts, histograms and scatter graphs

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: Determination of the optical density (absorbance) of Bromophenol blue dye through spectrophotometer

Objective:

After completing the practical, you will be able:

1. Estimate the absorbance and transmittance of an analyte by spectrophotometry

Introduction:

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications. A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

Materials

1. Beaker
2. Distilled water
3. Bromophenol blue dye
4. Cuvette
5. Micropipette and tips
6. Test tubes
7. Spectrophotometer

Procedures:

1. Make sure no sample in cuvette or sample compartments and the test tubes are closed.
2. Raise the power button to switch ON the spectrophotometer.
3. Warm up the instrument.
4. Enter the decided wavelength (visible 480-530 nm).
5. Clean the cuvette properly containing the blank solution and place in the cuvette at the respective position in the spectrophotometer.
6. Close the sample compartment door.
7. Press the Auto 0 button of spectrophotometer.
8. Remove the blank. Place the cuvette again containing the analytic (Bromophenol blue dye) in to the sample compartment and close the door.
9. Record the digital display readings.
10. Rinse the cuvette properly to wipe-out any analyte is present.

Observation/Results:

Analytic Bromophenol blue dye	Transmittance	Absorbance
Sample A		
1		
2		
3		
Sample B		
1		
2		
3		

Questions:

1. Explain the Law that holds spectrophotometry.
2. Explain absorbance and transmittance in spectrophotometry.

Practical 2

Title: Determination of pressure at the bottom most position of a cylinder using the concept of thermodynamic principle

Objective:

After completing the practical, you will be able:

1. To determine and measure the parameters of a system with the aids of thermodynamic principle

Introduction:

Thermodynamics can be defined as the study of energy, energy transformations and its relation to matter. The analysis of thermal systems is achieved through the application of the governing conservation equations, namely *Conservation of Mass*, *Conservation of Energy* (1st law of thermodynamics), the 2nd law of thermodynamics and the property relations. Energy can be viewed as the ability to cause changes.

1. First law of thermodynamics: one of the most fundamental laws of nature is the conservation of energy principle. It simply states that during an interaction, energy can change from one form to another but the total amount of energy remains constant.

2. Second law of thermodynamics: energy has quality as well as quantity, and actual processes occur in the direction of decreasing quality of energy. Whenever there is an interaction between energy and matter, thermodynamics is involved. Some examples include heating and air-conditioning systems, refrigerators, water heaters, and more.

Closed and Open Systems

A system is defined as a quantity of matter or a region in space chosen for study. The mass or region outside the system is called the surroundings.

- Boundary: the real or imaginary surface that separates the system from its surroundings. The boundaries of a system can be fixed or movable. Mathematically, the boundary has zero thickness, no mass, and no volume.
- Closed system or control mass: consists of a fixed amount of mass, and no mass can cross its boundary. But, energy in the form of heat or work, can cross the boundary, and the volume of a closed system does not have to be fixed.
- Open system or control volume: is a properly selected region in space. It usually encloses a device that involves mass flow such as a compressor. Both mass and energy can cross the boundary of a control volume.

Problem:

The piston of a cylinder-piston device has a mass of 60 kg and a cross-sectional area of 0.04 m^2, the depth of the liquid in the cylinder is 1.8 m and has a density of 1558 kg/m^3. The local atmospheric pressure is 0.97 bar, and the gravitational acceleration is 9.8 m/s^2. Determine the pressure at the bottom of the cylinder.

Solution:

Practical 3
Title: Derivation of Beer-Lambert Law

Objective:
After completing the practical, you will be able:
1. To learn and derive the Beer- Lambert Law and its implications in biophysics

Introduction:

Beer-Lambert law helps to correlate the intensity of absorption of UV-visible radiation to the amount of substance presence in a sample. Beer-Lambert law has been widely used in many fields of pharmaceutical sciences, chemistry and quantification testing. It allows UV-visible spectroscopy to be useful as not just a qualitative but also a quantitative tool.

Derivation

As it has been seen in the process of light absorption of ultraviolet and visible light that occurs due to absorption of energy by electrons which can be excited (such as π-electrons). Practically when a beam of light hits a substance, the machine can measure the intensity or power of light before it strikes the substance (I_0), and then after it goes through the substance (I_T). The incident light may undergo absorption, reflection, interference, and scattering before it is transmitted. Thus the intensity of light through the substance (I_T) will be reduced. The change in intensity of light due to absorption, reflection, interference and scattering, can therefore be equated as:
$\Delta I = I_0 - I_T$
Where I_0 is the intensity of incident light, I_T is the intensity of light transmitted and ΔI is the change in intensity of light due to absorption, reflection, interference and scattering by the solution. Example: $\Delta I = I_{absorbed} + I_{reflected} + I_{interference} + I_{scattering}$
Normally, in order to measure just the amount of light that is absorbed by the sample, we keep an appropriate control sample with just the solvent without any dissolved solute. In such a case

$\Delta I = I_{absorbed}$

The ratio of the light passing through a substance is measured as transmittance which can be calculated as I_T/I_0. The % transmittance is therefore calculated as:

$$\%T = \frac{100 I_T}{I_0}$$

Percent Transmittance

Absorbance is defined as the amount of light which is absorbed by the substance and is calculated as the negative logarithm of transmittance:

$$A = \log_{10}\frac{I_0}{I_T} = \log_{10}\left(\frac{1}{T}\right) = -\log T = 2 - \log(\%T)$$

Absorbance (A)

History of the Beer-Lambert Law and Classical Derivations

Lambert Derivation

In 1760, Lambert stated that the rate of decrease in the intensity of light with thickness (*b*) of the medium is proportional to the intensity of the incident light. When expressed mathematically:

$$-\frac{dI}{db} \propto I \text{ or } -\frac{dI}{db} = k' I$$

Where *k'* is the proportionality constant, therefore when we take the reciprocal of the entire equation:

$$-\frac{db}{dI} = \frac{1}{k'I}$$

Integrating the above equation we get:

$$-b = \frac{1}{k'} \ln I_T + C$$

Where I_T is the intensity transmitted at thickness *b* and *C* is the constant of integration.

Now in order to solve for the constant of integration C, consider the case where *b*=0 (i.e. the thickness of the substance is 0). In such a case I_T would be = *I0*. In such a case the equation would breakdown to give us:

$$C = -\frac{1}{k'} \ln I_0$$

Solving for C in the lambert derivation when b=0

Therefore if this value of *C* may be substituted in the above equation as:

$$-b = \frac{1}{k'} \ln I_T - \frac{1}{k'} \ln I_0$$

$$\ln \frac{I_0}{I_T} = k'b$$

Since it is normal to convert to common log_{10} we get:

$$\log \frac{I_0}{I_T} = \frac{k'b}{2.303}$$

By definition, the left side of this equation (i.e. $log\ I_0 / I_T$) is nothing but absorbance (*A*). Therefore the equation then becomes:

$$A = \frac{k'b}{2.303}$$

The Lambert Derivation

Thus Lambert's Law was defined as: The intensity of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogenous thickness. Simply put, the absorbance is proportional to the thickness (path length *b*) of the solution.

Beer Derivation

In 1852, in a similar derivation to Lambert's, Beer demonstrated the following relationship between absorbance and concentration of a solution:

$$\log \frac{I_0}{I_T} = \frac{k''c}{2.303} \quad \text{OR} \quad A = \frac{k''c}{2.303}$$

The Beer Derivation

Where, k^n is a proportionality constant, c is the concentration and A is the absorbance.

Thus, Beer's law can be defined as: the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. More simply, it stated that absorbance is proportional to the concentration (c) of the solution.

Questions:

1. Explain the relationship between absorbance and transmittance.
2. How can we calculate the transmittance with respect to absorbance?

Practical 4

Title: Separation of components of two different coloured liquids using thin layer chromatography

Objective:

After completing the practical, you will be able:

1. To learn and acquire knowledge about thin layer chromatography and its implications in biophysics

Introduction:

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures.[1] Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptane are used. The mobile phase may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase.

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area. Chemical processes can also be used to visualize spots; anisaldehyde, for example, forms colored adducts with many compounds, and sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

To quantify the results, the distance travelled by the substance being considered is divided by the total distance travelled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.) This ratio is called the retardation factor (Rf). In general, a substance whose structure resembles the stationary phase will have low Rf, while one that has a similar structure to the mobile phase will have high retardation factor. Retardation factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound to the sheet before running the experiment.

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents.

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis. This method is referred to as HPTLC, or "high-performance TLC". HPTLC typically uses thinner layers of stationary phase and smaller sample volumes, thus reducing the loss of resolution due to diffusion.

Materials

1. Chromatography chamber
2. Jar
3. Filter paper
4. Ethyl alcohol
5. Distilled water
6. Mixture of blue and red colour.
7. Pencil
8. Scale
9. Capillary tube
10. Glass rod

Procedure:

1. Take a filter paper and using a pencil draw a 4cm horizontal line from one end to the other. Then draw another line vertically on the filter paper at the centre. Name the point at which different point intersect as P.
2. Using a fine capillary tube put a drop of mixture of red and blue inks at the point P. Let it be dry.
3. Put another drop at the same point and dry again, so that the spot is rich in the mixture.
4. Pour equal amount of ethyl alcohol and distilled water into the chromatographic chamber and mix it well using the glass rod. This acting as the solvent.
5. Suspend the filter paper vertically in the chromatographic chamber containing the solvent. In such way, so that the pencil mark remain 2cm above solvent level.
6. Close the jar with the lid and let it undisturbed.
7. Notice the raising solvent along the red and blue inks.
8. After the solvent raising about 15cm, you'll notice 2 different spots of red and blue colors respectively on the filter paper.
9. Take the filter out and using a pencil, mark the distance that the solvent raisin on the paper. This is call the solvent flaunt.
10. Dry the filter paper and put pencil marks at the center of the red and blue spots.
11. Measure the distance of two spots from the original line and also measure the distance of the solvent from the original line.
12. Calculate the (Retardation Factor) RF values of the red and blue inks using the formula.

$$RF = \frac{\text{Distance travelled by components from the origin line}}{\text{Distance travelled by the solvent from the origin line}}$$

Observations/Result:

Questions:

1. Explain RF with respect to chromatography.
2. Explain stationary phase, mobile phase and supportive medium.
3. Describe a chromatogram.

Notes

LINCOLN UNIVERSITY COLLEGE

Wisma Lincoln, No. 12, 14, 16 & 18,
Jalan SS 6/12,47301 Petaling Jaya,
Selangor Darul Ehsan, Malaysia.
Tel.: +603-7806 3478 Fax: +603-7806 3479
Toll Free: 1-300-880-111
E-mail: lucp@lincoln.edu.my
Web.: www.lucp.net

ISBN 978-967-2257-16-5