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Practical Skills Handbook

Contents

Introduction

It is important to remember that the practical assessment is an examination. The results of this examination will directly count towards your overall final grade, so it is essential that you are as prepared beforehand as you can possibly be.

Important Practical Skills

1. Carrying out basic laboratory techniques and understanding the principles that underlie them
2. Working safely, responsibly and legally in the laboratory, with due attention to ethical aspects
3. Designing, planning and conducting biological investigations
4. Obtaining, recording, collating and analysing biological data
5. Using data in several forms e.g. numerical, textual, verbal and graphical
6. Evaluating your experimental technique

Approach to Practical Work

- Read handouts in advance, where possible, so you understand <u>why</u> you are doing a particular practical and the principles behind it
- Be aware of the time in which you have to work
- Consider safety hazards before you begin
- Organise your working bench space
- Write up work as soon as possible after the practical

Equipment

Why revise for months for your written papers to not bring in the correct equipment and lose marks in your assessed practical examination?

You must have;
- Sharp pencil
- Clear 30 cm ruler
- Rubber
- Black biro pen
- Calculator
- Sharpener

Recording data

In many of your assessed practical examinations you are tested on your ability to present data in a clear and logical manner. It is important that you follow the correct conventions for presenting data in tables;

Use a table, which should have:-
- All raw data should be presented in a single table
- The table should be drawn in pencil with straight tidy ruled lines and a complete border (not an open ended box).
- The Independent variable (IV) is always in the first column starting with the smallest value;
- The dependent variable (DV) in columns to the right (for quantitative observations)
  OR Descriptive comments in columns to the right (for qualitative observations).
- Observations of results (not your interpretation of them!) Write these in as much detail as possible. i.e colour observed not what this colour means.
- Repeated readings; use a separate column for each
- Processed data (e.g. means, rates, standard deviations) in columns to the far right.
- No calculations in the table, only calculated values.
- Each column headed with physical quantity and correct SI units (for quantitative data);
  OR informative description (for qualitative data)
- Units separated from physical quantity using either brackets or a solidus (slash). The method must be consistent throughout.
- No units in the body of the table, only in the column headings.
- Raw data recorded to a number of decimal places and significant figures appropriate to the least accurate piece of equipment used to measure it. (2 d.p – Except when recording time in sec, which is to the nearest second).
- All raw data recorded to the same number of decimal places and significant figures.
- Processed data recorded to up to one decimal place more than the raw data.

- Design your table to make the recording of data as straightforward as possible, to minimise the possibility of mistakes
- Explain any unusual data values in a footnote; don't rely on memory! E.g. forgot to start stopwatch
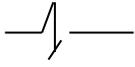
Graphs

The following general guidelines should be followed when presenting data in graphs.

- The type of graph used (e.g. bar chart, histogram, line graph, pie chart or scattergram) should be appropriate to the data collected.
- The graph should be of an appropriate size to make good use of the paper (at least 50%).
- There should be an informative title.

Drawing Graphs

- Graphs give a visual impression of the content and meaning of your results. Tables provide an accurate numerical record of data values.

- Graphs should:-
    - include all material necessary to convey the appropriate message without reference to the text

- When drawing graphs:-
    - Collect all data values to be plotted
    - Consider whether a graph is the best way to present the data
    - Choose a concise explanatory title to establish the content
    - Consider the layout and scale of the axes carefully
    - Use the x axis for the independent variable
    - Use the y axis for the dependent variable
    - When neither variable is determined by the other, or where variables are interdependent, the axes may be plotted either way round
    - Each axis should have a descriptive label showing what is represented and including units of measurement, separated by / or written in brackets.

- Each axis must have an appropriate marked scale, showing the location of all numbers used. Fill the available space on the paper
- If scale breaks are used, show them clearly
- Choose the symbols for each set of data points, if plotting more than one set of data
- Include a key to symbols of different data sets if necessary
- To plot very large or small numbers, the plotted values may be measured numbers multiplied by a power of ten e.g. 10^{-3} x population of bacteria / mm^3
- Draw a trend line for each set of points:
    - Curve?
    - Straight line?
    - Points joined with a ruler? (This is really only valid if there is zero error. i.e. all the plotted points are precise and accurate)

(a)

(b)
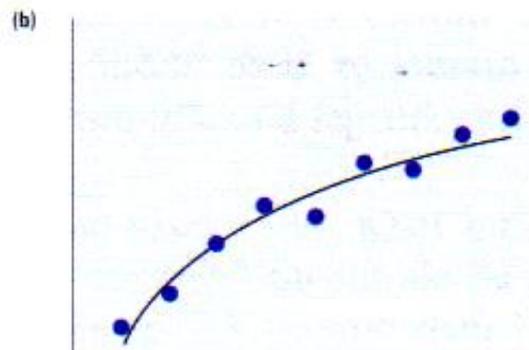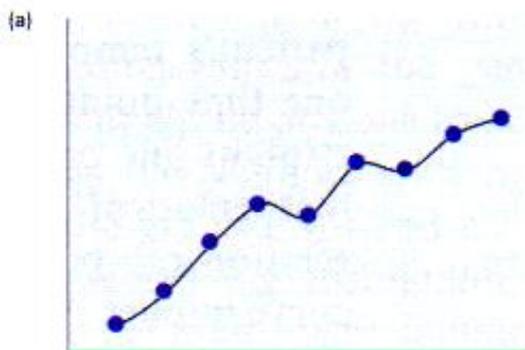
- Always draw the simplest line that fits the data reasonably well and is biologically reasonable.
- Try to avoid the need to extrapolate plotted curves by better experimental design. *(This could be a point for evaluation of an experiment).*
- Never allow a computer programme to dictate what a graph looks like; make sure you can alter scales, labels, axes etc and make appropriate selections. Draw curves freehand if necessary.

Guidelines for specific types of graphs follow.

1   Bar charts and histograms

These are used when the dependent variable on the y-axis is discrete, i.e. whole numbers, fractions are impossible and the data under consideration deal with frequencies.

1A   Bar charts

Bar charts are used when the independent variable is non-numerical, e.g. the number of different insect species found on trees. These data are discontinuous.

• They can be made up of lines, or blocks of equal width, which do not touch.

• The lines or blocks can be arranged in any order, but it can aid comparison if they are arranged in descending order of size.

• Each axis should be labelled clearly with an appropriate scale.

1B   Histograms

These are used when the independent variable is numerical and the data are continuous. They are sometimes referred to as frequency diagrams.

• One axis, usually the x-axis, represents the independent variable and is continuous.
It should be labelled clearly with an appropriate scale.

• The number of classes needs to be established. This will largely depend on the type and nature of the data. However, five times the log of the number of observations is one approach.

• The blocks should be drawn touching.

• The edges of the blocks should be labelled, so a block might be labelled '7' at the left and '8' at the right; this is expressed as a class range 7 - 8 units but it is implied that 7.0 is included in this range but 8.0 is not. 8.0 will be included in the next class range, 8 - 9.

• The other axis, conventionally the y-axis, represents the number or frequency, and should be labelled with an appropriate scale.

2   Pie charts

These can be used when displaying data that are proportions or percentages.

• Sector angles are calculated by dividing their percentage by 100 and multiplying the answer by 360o (if figures are proportions then just multiply by 360o).

• When comparing two or more pie charts, the sequence of segments should be the same.

• The size of the pie circle can be made proportional to the size of the sample.

• Pie charts should not contain more than 6 to 7 sectors, otherwise they become confusing.

• There should be segment labels or a key.

3   Line graphs

• Straight lines should join points. A smooth curve is only drawn if there is reason to believe that intermediate values fall on the curve.

• The independent variable should be plotted on the horizontal axis (x) and the dependent variable plotted on the vertical axis (y).

• Axis labels should be stated horizontally and in lower case, using units or in full.

• Axes should have an arrow end when there is no scale. If the origin (0,0) is not included in a printed graph, the axis should be broken.

• Points should be plotted with encircled dots (⊙) or saltire crosses (x). When multiple curves are being plotted, vertical crosses (+) can be employed.

• If a graph shows more than one curve, then each curve should be labelled to show what it represents.

4   Scattergrams

These are used when investigating the relationship between two variables of a sample or replicate and observations are in pairs. The data can then be used to establish if there is a relationship between the variables. The relationship can be a positive correlation, a negative correlation or no correlation at all.
• The two axes of the graph are marked out with appropriate scales.
• The two variables are plotted for each sample as a point so that each point on the graph represents an individual.

Interpreting Graphs – Analysis & Evaluation

1. Describe the relationship between the variables, quoting data.
2. Explain what the relationship means with reference to the biological principles involved.
3. Consider the content:
   a. What was the aim / hypothesis of the investigation?
   b. Why were the observations made?
4. Consider what kind of graph is presented; is it an appropriate choice for the data?
5. Look carefully at the scale of each axis.
   a. What is the starting point?
   b. What is the highest value?
   c. Do the values start at zero? A non-zero axis emphasizes the differences in measurements by reducing the range of values covered by the axis.
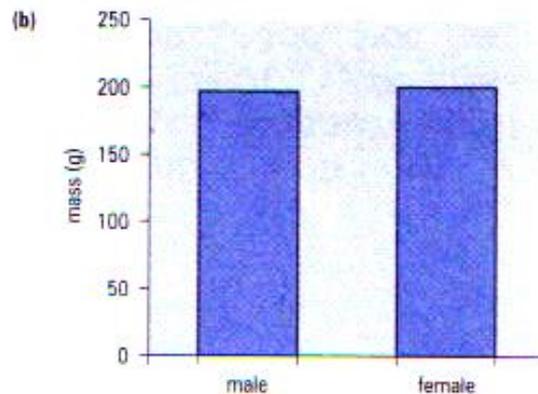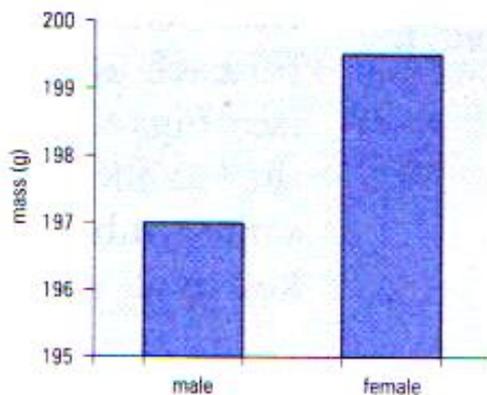

6. Examine symbols and trend lines; e.g. if 2 conditions have been observed while a variable is altered, when do they differ from each other; by how much and for how long?
7. Evaluate errors:-
   a. Look for variability in the data; have anomalies been recognized? Have they been included in the trend line? Is it
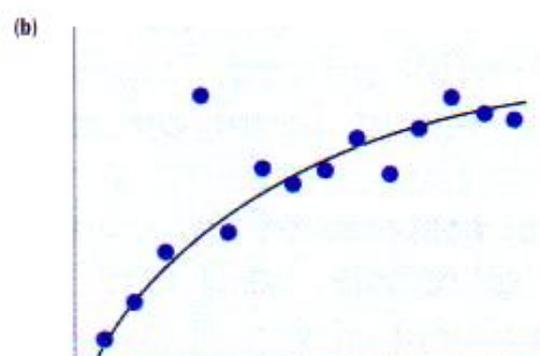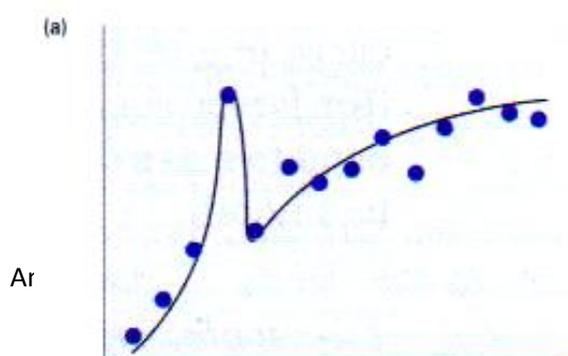
reasonable to ignore such points?

b. If mean values are presented, the underlying errors could be large
c. Has a graph been extrapolated correctly? There can be no guarantee that relationships will hold under new conditions, so the extrapolation may be inaccurate.
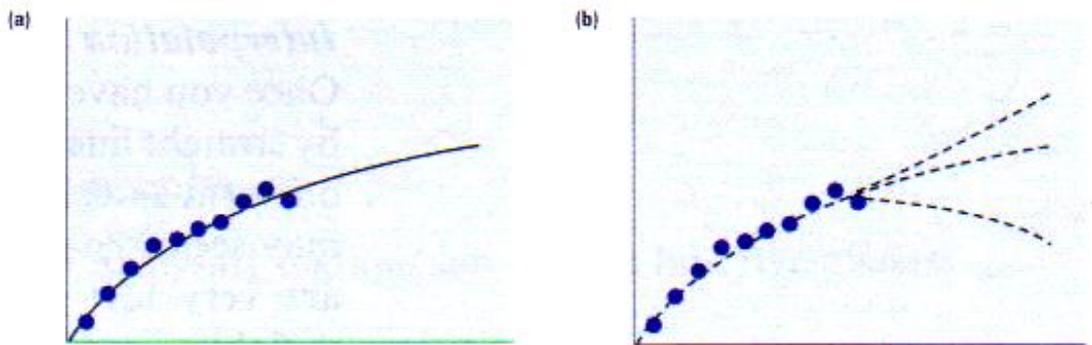
d. Is the trend line appropriate? Would a curve / straight line be more appropriate? Consider the theoretical validity of the line. A straight line implies one factor varies in direct relationship to another; the true situation may be more complex e.g. an exponential relationship could be more correct.
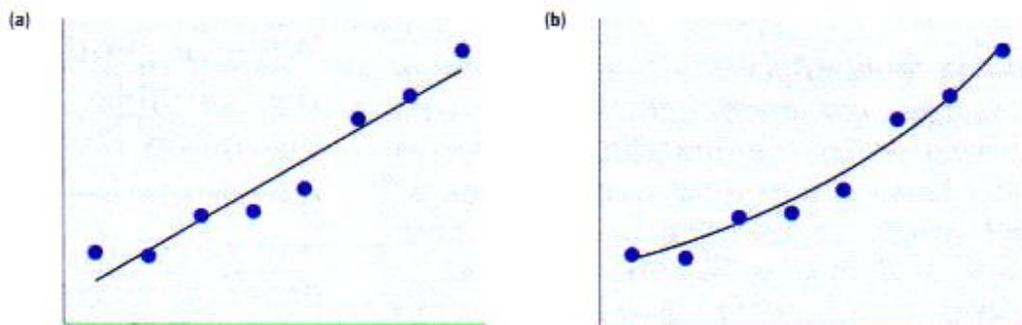
Accuracy & Precision in techniques

Accuracy = the closeness of a measured data value to its true value

Reliability = the closeness of repeated measurements to each other
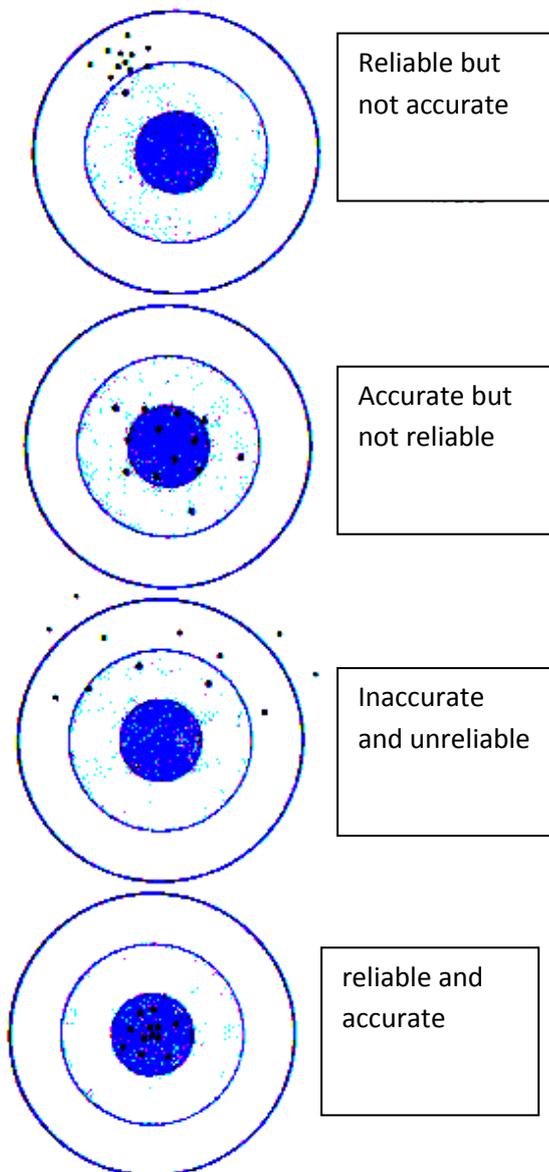
So... a balance with a fault in it could give reliable (i.e. very repeatable) results but inaccurate (untrue) results.

Bias can be due to:-

- Incorrectly calibrated instruments e.g. faulty water bath
- Experimental manipulations e.g. using a thermometer to measure temperature can itself decrease the temperature
- Subjective ideas by the experimenter e.g. judging when an end-point is reached, or fixing results to fit those expected

Minimising Errors

When designing an experiment:-

- Ensure that the independent variable is the only major factor that changes
- Incorporate a control experiment to show it is only the independent variable which causes the measured effect
- Where appropriate, select experimental subjects randomly to cancel out variation arising from biased selection (this is important in ecological investigations)

Reliable but not accurate

Accurate but not reliable

Inaccurate and unreliable

reliable and accurate

- Keep the number of replicates as high as possible
- Ensure the same number of replicates is done for each value of the independent variable
- Identify other factors which could affect the dependent variable and keep them constant (control variables) e.g. temperature, volumes of solutions, light intensity, time for reaction

Minimising Measurement Error
- Common source is carelessness e.g. reading a scale in the wrong direction; reduce this by more careful recording (think how you can do this) and by repeating the measurement

Accuracy depends on using *suitable equipment* with *care*.

Accurate measurement of liquids

- High viscosity liquids are difficult to transfer; allow time for all the liquid to transfer
- Organic solvents or hot liquids may evaporate quickly, making measurements inaccurate; transfer these liquids quickly and cover containers
- Liquids likely to froth e.g. yeast or protein solutions are difficult to measure; transfer slowly
- Suspensions e.g. yeast or cell cultures may sediment; mix well before transferring
- Use measuring cylinders on a level surface so the scale is horizontal; fill to below the desired mark, then add liquid slowly e.g. by pipette to reach desired level
- Make sure there are no air bubbles in syringes when measuring volumes. Expel liquids slowly and touch the end of the syringe on the vessel to remove any liquid stuck to the end
- Potential errors: inaccurate measurements for reasons given above. What effects would this have on the results?

Using balances

- Never weigh anything directly onto a balance's pan; this will contaminate it for other users. Use a weighing boat or slip of aluminium foil, or paper
- Note reading to 2 decimal places. N.B. When calculating a mean average of readings, the average should also be to 2 decimal places
- Potential errors: samples spilt onto the pan will be measured but not used; as will samples left if the weighing boat is not scraped clean. How would the results be affected?

Measuring & Controlling temperature

- Heating samples:
  - Wear safety glasses
  - Use a *thermostatically-controlled* water bath if possible and suitable; check the temperature using a thermometer; do not rely on the temperature shown on the dial
  - *Note that if you are not using a thermostatically controlled water bath, this would be a good improvement you could mention in an evaluation*
  - State the temperature used and the time for which heating is carried out e.g. Benedict's test – heat for **5-10 minutes** in a **water bath at 80ºC**
  - Use insulation if necessary or possible

- Potential errors: has the temperature varied from the stated value? How would this affect the results?

Measuring Time

- Use a stop watch rather than a clock
- Make sure you know which buttons to press *before* the experiment starts!
- Note time readings to a suitable number of decimal places. N.B. When calculating a mean average of readings, the average should also be to the same number of decimal places. Could you actually measure the time this accurately?
- Potential errors: how easy is it to know when to start or stop the timer? What difference would this make to the results?

Preparing Dilutions

Solutions are usually prepared with respect to their

- molar concentrations (mol/l or mol/dm^3) (a mole is a given number of molecules of a compound; 1 mole has a mass in grams equal to the relative molecular mass of that compound) or
- mass concentrations (g/l or g/dm^3)

Both these are the amount of a substance per unit volume of solution.

$$\text{i.e.} \qquad \text{Concentration} \quad = \quad \underline{\text{Amount}}$$
$$\text{Volume}$$

Concentration must always be given units.

In practical assessments, you will often be given stock solutions to use. These are solutions of known concentration and are valuable when making up a range of solutions of differing concentrations. They also save work if the same solution is used over a long time e.g. a nutrient solution. Stock solutions are more concentrated than the final requirement and are diluted as appropriate.
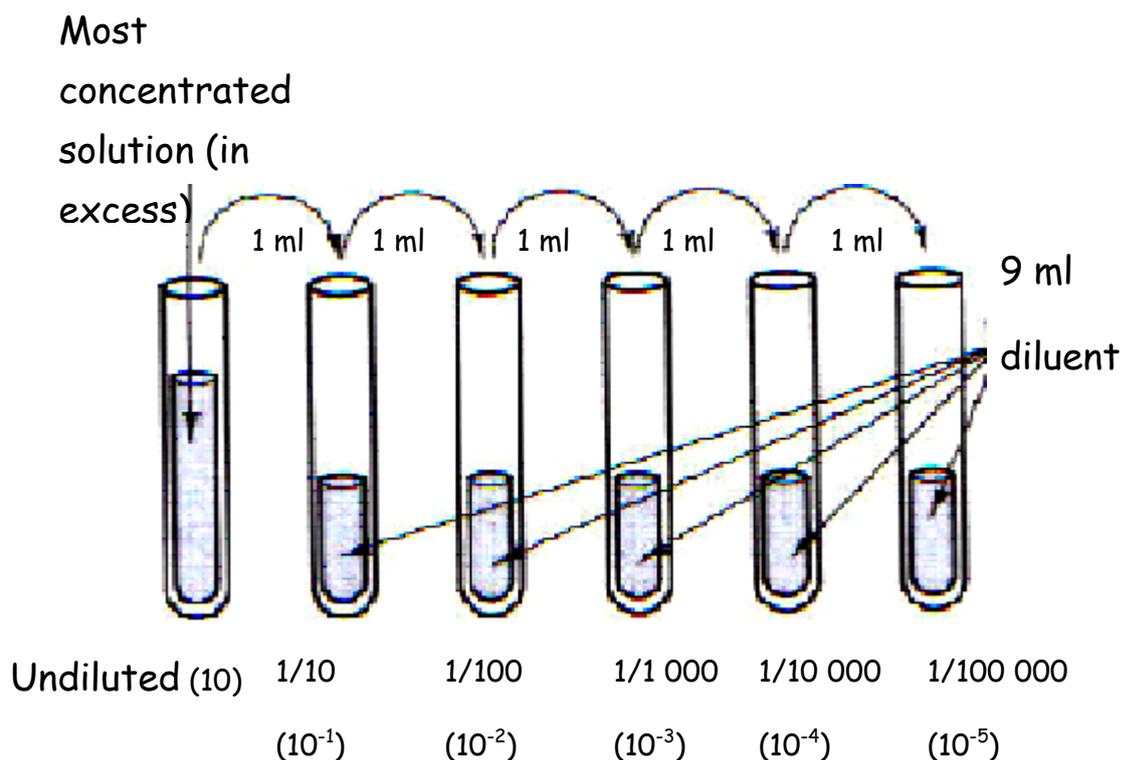
Preparing a dilution series

A series of dilutions is very useful for a wide range of procedures e.g.
- to investigate changing the concentration of substrate in an enzyme reaction
- to prepare calibration curves for colorimetry

How to make a dilution series:

- Always start with the most concentrated solution

Most concentrated solution (in excess)

1 ml   1 ml   1 ml   1 ml   1 ml

9 ml

diluent

Undiluted (10)   1/10   1/100   1/1 000   1/10 000   1/100 000

(10^{-1}) (10^{-2}) (10^{-3}) (10^{-4}) (10^{-5})

1. Decimal dilutions
   - Each concentration is one tenth that of the previous one ($\log_{10}$ dilution series)
   - Measure out the most concentrated solution with a 10% excess
   - Measure one-tenth of the volume required into a vessel containing nine times as much diluting liquid
   - Mix thoroughly
   - Repeat to obtain concentrations 1/10, 1/100, 1/1000, etc times the original
   - To calculate the actual concentration of solute multiply by the appropriate dilution factor

2. Doubling dilutions:-
   - Each concentration is half that of the previous one
   - Use twice the volume required of the first, most concentrated solution
   - Measure out half of this volume into a vessel containing the same volume of diluting liquid (e.g. distilled water)
   - Mix thoroughly
   - Repeat for as many doubling dilutions as are required
   - The concentrations obtained will be ½, ¼, ⅛, etc times the original

Potential Errors

(*Remember thinking about these can help you evaluate your procedure*)
- Contamination from syringes; rinse between use or use new syringes for each solution to avoid carry-over of solutions of the wrong concentration
- *(Note that when transferring a range of prepared dilutions from sample pots into test tubes, you should start with the lowest concentration and, if you rinse the syringe in the next concentration before dispensing it, you can use the same syringe or pipette)*
- Inaccuracy in measuring volumes; any slight inaccuracy will lead to compounded inaccuracies, so the most dilute solutions have huge errors in concentration (see precautions in measuring liquids above)

- Label tubes carefully to ensure correct solutions are transferred
- Mix solutions before transferring to ensure the correct amount of solvent is added to the next tube

Colorimetry

The spectrophotometer measures the absorption of radiation in the visible and uv regions of the electromagnetic spectrum. The spectrophotometer allows precise measurement at a

particular wavelength. A colorimeter is simpler, using filters to measure broader wavelength bands (e.g. green, red or blue light).
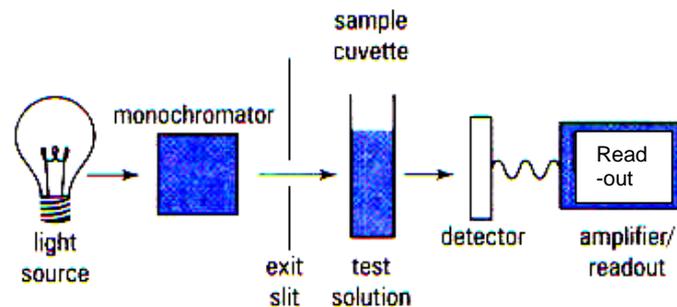
Principles of light absorption

- The absorption of light is exponentially related to the number of molecules of the absorbing solute in the solution i.e. [C], solute concentration
- Absorbance at a particular wavelength is often shown as a subscript e.g. A_{550} = absorbance at 550 nm
- The proportion of light *passing through* the solution is known as transmittance (T) and is calculated as the ratio of the emergent and incident light intensities. It is usually expressed as a percentage
- The colorimeter has 2 scales:-
  - An exponential scale from zero to infinity, measuring absorbance
  - A linear scale from 0 – 100, measuring (per cent) transmittance
- For most practical purposes you should use absorbance, which is linearly related to the solute concentration [C]

Calibration Curves

By preparing a set of standard solutions, each containing a known amount or concentration of a substance, and then measuring the absorbance of each solution, a "calibration curve" or "standard curve" can be produced. (Note that at high concentrations, the relationships above do not hold true, and the straight line relationship shown may become curved.) The line can be used to estimate concentrations of solute in a test or unknown sample.
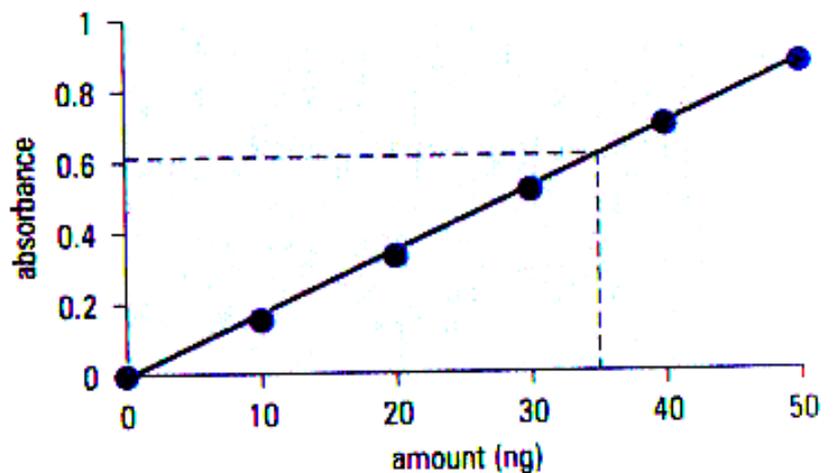
How to use the colorimeter

1. Switch on
2. Allow 15 minutes for the lamp to warm up and the instrument to stabilise
3. Select the correct coloured filter (It is best to use the filter which selects the range of wavelengths most strongly absorbed by the sample because this will give the maximum reading). The most suitable filter colour is usually the complementary colour to the solution being tested:-

COLOUR OF SOLUTION	FILTER COLOUR
Violet	Yellow-green
Blue	Yellow
Blue-green	Red
Green	Purple
Yellow-green	Violet
Yellow	Blue
Orange	Green-blue
Red	Blue-green
Purple-red	Green

4. Insert a reference blank cuvette
5. Check the reading is zero (zero if necessary)
6. Analyse samples
7. Check the scale is zero at regular intervals using a reference blank e.g. after every 10 samples
8. Check the reproducibility of the instrument; measure the absorbance of a single solution several times during analysis. It should give the same value

Inaccuracies may be due to:-

- Incorrect use of cuvettes
    i. Dirt
    ii. Fingerprints
    iii. Test solution on the outside of cuvettes
- Condensation on cold samples (allow cold samples to equilibrate to room temperature)
- Air bubbles in samples (tap gently to remove)
- Insufficient solution (refraction of light at meniscus)
- Cloudiness of sample (decant off supernatant to test, after allowing precipitate to settle)

Microscopy

Problems in light microscopy and possible solutions:-

No image; very dark image
- microscope not switched on
-
- objective nosepiece not clicked into place over a lens
- lamp failure

Image blurred and cannot be focused
- dirty objective
- dirty slide
- slide upside down
- slide not completely flat on stage
- fine focus at end of travel

Dust and dirt in field of view
- eyepiece lens dirty
- objective lens dirty
- slide dirty
- dirty on lamp glass or upper condenser lens

Setting up and using the light microscope

1. Select low power lens. Make sure the lens clicks into position.
2. Examine prepared slide without the microscope and note position, colour and rough size of specimen.
3. Place slide on stage, coverslip uppermost, viewing it from the side. Position it with stage adjustment controls so that the specimen is lit up.
4. Focus using first the course and then the fine focusing controls. Use <u>both hands</u> to alter the focusing controls; this helps keep the controls working properly and not going out of alignment.
   Note: The image will be reversed and upside down when seen by viewing the slide directly.
5. For higher magnifications, swing in the relevant objective lens carefully checking there is space for it. Adjust the focus using the fine control only. If the object is in the centre of the field of view with x10 objective, it should remain in view with the x40 objective.
6. When you have finished using the microscope,
   - Turn the objective lens back to x10
   - Lower the stage
   - Remove the last slide and return to correct section in tray
   - Clean the stage if necessary
   - Check eyepiece lenses and objective lenses are clean
   - Unplug cable and store tidily
   - Replace dust cover
7. General care of the microscope:-
   - Never force any of the controls
   - Never touch any of the glass surfaces with anything other than clean, dry lens tissue
   - When moving the microscope, hold the stand above the stage with one hand and rest the base of the stand on your other hand
   - Always keep the microscope vertical (or the eyepiece may fall out)
   - Do not touch lenses with your fingers

- Do not allow any solvent to come in contact with a lens
- Do not wear mascara when using the microscope; it marks the eyepiece!

Establishing Scale

Magnification used to observe a specimen = objective magnification x eyepiece magnification.

However, this is fairly meaningless when drawing any image, which could be drawn at any size. For this reason, it is essential to add a scale to your drawing. You can:
- Provide a bar of defined length e.g. 100 µm or
- Give an estimated size of the object

Methods of estimating size

1. Compare the size of the image to the diameter of the field of view:-
    a. Focus on the millimetre scale of a transparent ruler, using the lowest power objective
    b. Estimate the diameter of this field directly
    c. Use the information to work out the field diameters of higher powers e.g. if the field at x40 is 4 mm, at a magnification of x100 will be 40/100 x 4 mm = 1.6 mm

2. Greater accuracy can be obtained if an eyepiece graticule (micrometer) is used:-
    a. Graticule carries a fine scale and fits inside an eyepiece lens
    b. Graticule is calibrated using a stage micrometer i.e. a slide with a fine scale in it (*You will do this yourself*).

Magnification x40

Eyepiece

graticule

0 10 20 30 40 50 60 70 80 90 100

40   50   60   70

Stage

Andrew Todd
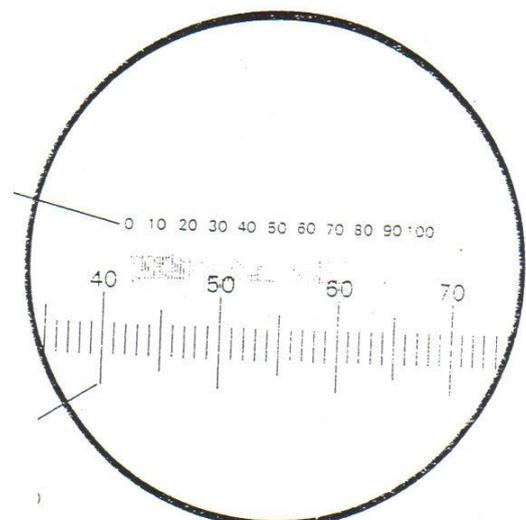
Micrometer

c. Once you calibrate your eyepiece graticule for each objective lens used, you can use it to measure objects
d. In the example below, the scale reading is multiplied by 2.65 µm to give the value in micrometres.
e. Avoid putting too many significant figures in any estimates of dimensions; there may be quite large errors involved

Recording by biological drawings

The making of accurate drawings is an essential skill for Advanced level biology students. Students are required to make drawings of external features of whole specimens, parts of specimens, dissections and prepared slides.

Drawings must be done **on white paper using sharp pencil**.

Students should note the following points:

1. A drawing should be genuine and accurate record of what has been seen. Do not copy diagrams from books with little resemblance to the specimen, dissection or prepared slide under investigation.
2. For low power drawings, only the outline of the structures or tissues needs to be drawn. Individual cells are required only for high power drawings.
3. Draw with bold, dark, smooth lines. Shading and the use of crayons are not encouraged.
4. Avoid making drawings on both sides of a paper.
5. In making a drawing, first decide what you want to show.   Then plan your drawing to fit on the page. It is important that the various parts of the structures are drawn in proportion.
6. Drawings should be large, neat and tidy.
7. All relevant structures should be fully and **clearly labelled using pencil.** Each label should be connected to the appropriate part of the drawing by a clear labelling line. The labelling lines should run as horizontal as possible and should not intersect with one another. Do not label too close to the drawings, and do not write on the drawing itself. Distribute the labels well around the drawing so that the labelling lines can be kept reasonably short.
8. Every drawing should include a title, the magnification power, and the plan of view of the specimen (such as transverse section, longitudinal section).
9. Make additional drawings if important details are too small to be shown in a low power drawing. This can be done by making a simple drawing of the main features, and other drawings on details of small parts only. For example, the recording of a transverse section of a stem should include a low power plan of the section

and high power drawings of different cells types as required showing the detail of the cells.

10. If suffices to show the internal structural details in a small representative sector of the specimen which shows repetitive features.

11. It is sometimes appropriate to make short succinct notes close to the labels. Such annotated drawings are particularly valuable as they combine a record of structures with related functions and/or biological interests. You may want to label an organelle to describe staining e.g. nucleus (darkly stained); starch grain (blue-black)

Labelling Species

- Record the species **full** scientific names (latin) with correct spelling
- Genus must have an uppercase first letter and all other letters to be lowercase
- Species must have all letters to be lowercase

Annotations

Whilst a label might be the name of a tissue, an annotation adds a descriptive quality such as shape of structures or cells, size of structures or cells, colour of stain, darkness of stain, number of nuclei, arrangement of nuclei (regular/irregular).

Annotations should include;

Shape of structure / cells

Colour of stain

Number of nuclei

Arrangement of nuclei