

"General Laboratory Safety Rules"

Basic safety rules for the laboratory conduct should be observed whenever working in a laboratory. Many of most important rules are listed below.

1. Know locations of laboratory safety showers, eyewash stations and fire extinguishers. The safety equipment may be located in the hallway near the laboratory entrance.
2. Know emergency exit routes.
3. Avoid skin and eye contact with all chemicals.
4. Minimize all chemicals exposure.
5. No horseplay will be tolerated.
6. Use equipment only for its designated purpose.
7. Never leave containers of chemicals open.
8. Avoid adding solid to the hot liquids.
9. Determine the potential hazards and appropriate safety precautions before beginning

any work.

10. Do not taste or intentionally sniff chemicals
11. Dress for the lab
12. Know what to do with lab accidents.
13. Washed exposed areas of skin prior to leaving the laboratory.

practical 01:

preparation of Dry Mount slides

Material:

- * Glass slide
- * Cover slips

Dry mounts work best for samples like

- * pollen

- * Hair

- * Feathers

- * or even dust particles caught in a microfilm filter.

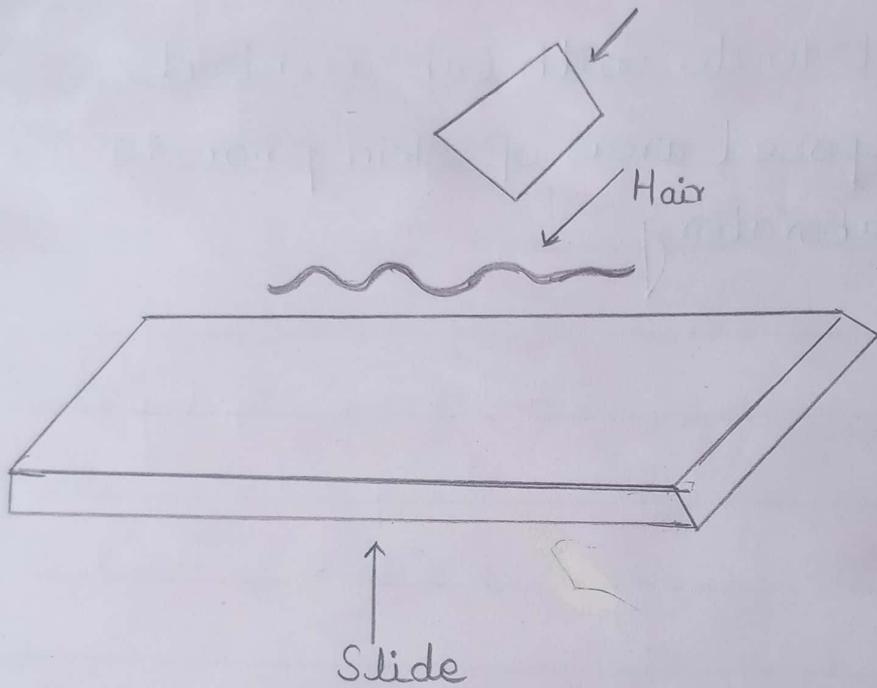
Steps:

- * place the sample on slide and cover.

The cover will protect both your sample and lenses.

Note:

These mounts are temporary unless you seal the cover slip in some way. Each mount has its own pros and cons.



preparation of Dry mount slides

Practical 02:

Preparation of wet Mount slides

Material:

- * Glass Slide
- * Cover Slips

procedure:

1. place a few drops of your desired liquid on slide
2. Add the specimen to your liquid.
3. Then place a few drops on top of it. This ensure that the specimen will be covered in the liquid.

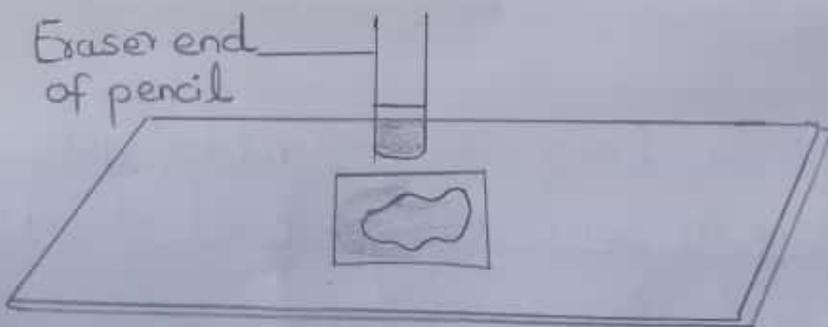
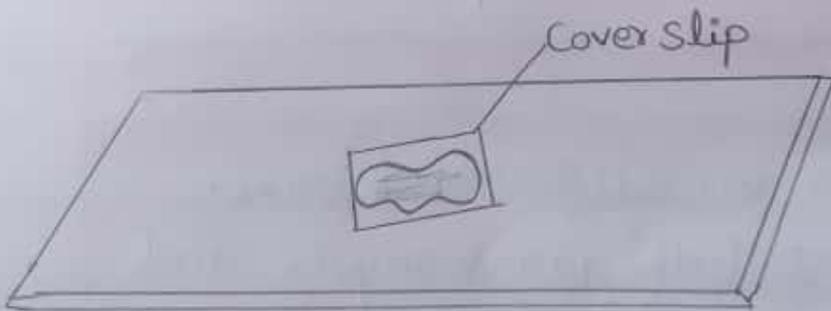
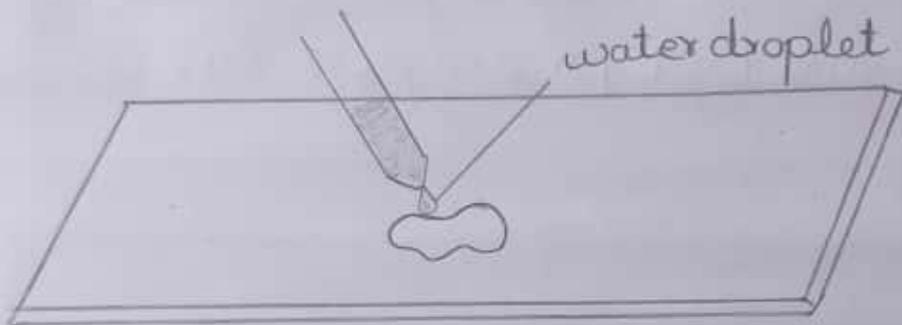
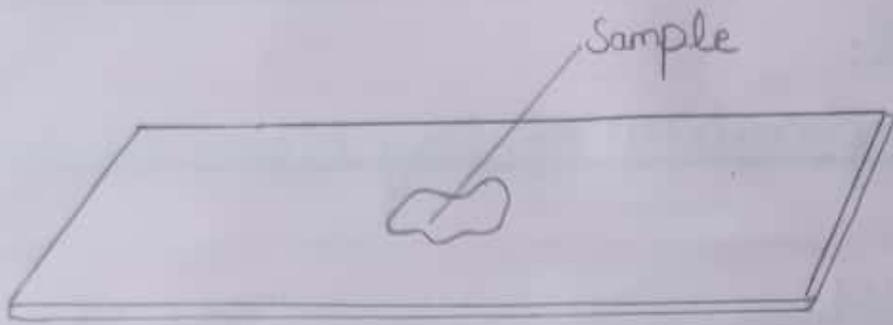
Looking at the aquatic specimen use the water in which specimen is exist in.

4. Next apply the cover slip. This is done very softly in order to avoid the air bubbles. Slowly lower the cover down at an angle, and it will ultimately be held in place by surface tension. If the cover slip is instable on the bottom half of slide you have used too much liquid.

Variety of liquids are suitable for making wet mount, from tap water to glycerin. Be sure to choose liquid sensibly to match your sample.

Note:

Wet mounts have weakness. Finding a moving specimen can be a problem. The slides also incline to dry out under the light of the microscope. If wet mounts are drying before you are ready, apply an extra drop of liquid under the cover slip.



preparation of wet mount slides

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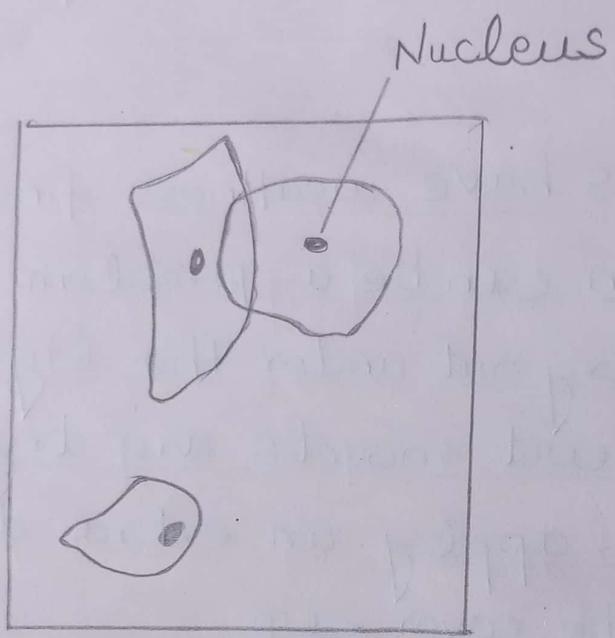
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Human cheek cells

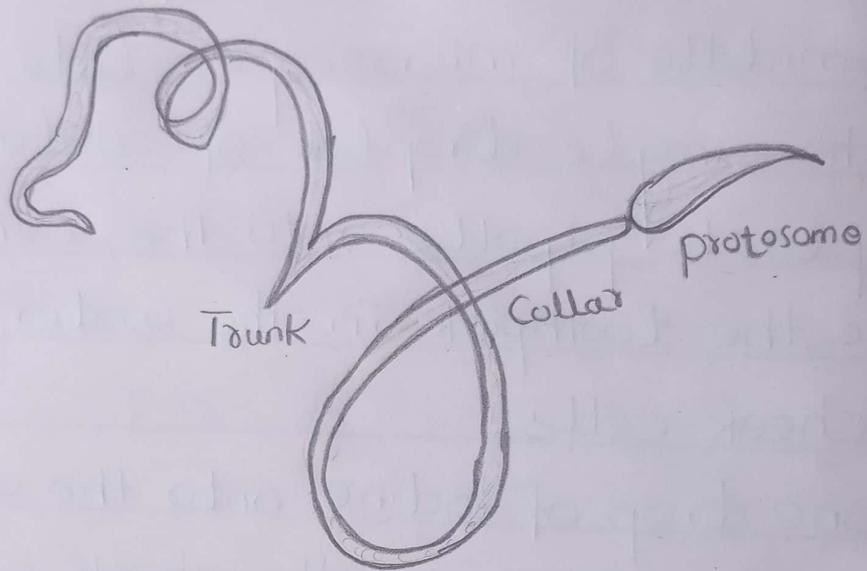
Practical 04 preservation of Representative Animals of Various phyla (Vertebrates)

Hemichordata:

- * For narcotization place specimens in containers of sea water and permit them to expand completely
- * Then add drop by drop over a period of an hour or more enough absolute alcohol to make 10% solution.
- * Complete relaxation usually takes 4 to 6 hours. When insensible the specimen may be formalin 5% will kill.
- * Specimens may be preserved either in 70% ethyl or isopropyl alcohol or 5% formalin.

Urochordata:

- * For narcotization put specimen in sea water then add a small quantity of chromacetic fixative. This will kill specimen within 30 minutes to 24 hours.
- * Then 5% formalin is added for its preservation.



Hemichordate

Fishes

Killing fishes:

- * Leave the fish in high dose of anaesthetizing solution.
- * Specimen is placed in the large mouthed glass.
- * First insert the fish head in jars. Do not pack many fishes in one jar, so that specimen should float freely.
- * Optimum fixation times for fish under six inches in length is one to two weeks.

Washing:

- * Specimen are put into 50% isopropyl alcohol. The decanted formalin can be reused. The specimen can be rinsed by putting the jar under running water. Alternatively the jar is filled with water. After several hours the water is decanted and fresh water is added.

Presevation:

- * Alcohol such as ethanol and isopropyl alcohol are also commonly used to fix and preserve fish specimens.
- * Then freeze in dry ice or liquid nitrogen.

Amphibia:

Fixative: Formalin

Washing in alcohol to soak out the formalin before transfer. The alcohol should be changed after 24 hours.

preservation:

5% formalin, 70% ethyl alcohol, 40 to 50% isopropyl alcohol.

Reptiles Snakes and Lizards

Killing:

- * Drowned in warm water but this is slow process.
- * injecting them with 10% ether solution requires from 48 hours to 1 week.

Small Lizards:

Formalin injected in the body cavity or through a cut made on the left ventral side of body.

Larger Lizards:

Should also be injected in each leg segment and just underneath skin

Snakes:

injected with 10% formalin every inch along the length of cavity, or else they are cut on the left ventral surface of the body. These cuts should measure 1 and 2 inches in length, should penetrate into body cavity and should be about 1 inch apart.

Reptiles - Turtles and Crocodile Turtles

- * The specimen is killed first.
- * For preserving small specimens when the specimen is dead the tail, head and limbs are pulled out of the shell so that they are exposed.
- * The specimen is placed directly in a jar with 10% formalin.

Crocodylians:

Small specimens should be positioned, fixed and preserved like lizards. Large specimens are skinned.

Birds:

Study skin: The most traditional preparation is study skin in which almost all of the body

inside the skin is removed and replaced with cotton.

Complete skeleton:

An skeleton all bones, muscle, digestive and other soft tissues is carefully removed and feathers and skin are stretched flat and dried.

ROM: A most recent preparation method pioneered by the Royal Ontario Museum removes all bones for a complete skeleton while also producing a white skin without bill or legs.

Shmoos:

If one set of wing and leg bones remain with the skin the preparation is called shmoos.

Mammals:

- * Entire fluid-preserved animals
- * Study skin with accompanying skulls / partial skeleton or freeze-dried specimens.
- * Entire skeleton, for studying anatomy, geographic variation or for age determination.

Field techniques:

- * In field the specimen is preserved in 10% buffered formalin or 70-90% alcohol.
- * For preservation in formalin the body cavity can be filled with formalin solution by injection until it is turgid and firm.

Long term storage in fluid:

- * After fixation in formalin, transfer into alcohol is the most desired alternative for permanent liquid storage of specimen in alcohol.
- * After fixation in 10% buffered formalin solution the specimen is to be washed by keeping it in slowly flowing water for 24 hours.
- * Next specimen is kept in distilled water for about 30 minutes.
- * For long term storage in a collection, a final transfer into 80% alcohol is recommended.

Practical 05

preservation of Representative Animals of various phyla (Invertebrates)

Fixation:

- * The fixation of biological specimen involve the coagulation of cell contents into insoluble substances with the purpose to prevent the autolysis and degradation of tissues.
- * Formalin is preferred fluid. Invertebrates require only 4% formalin.

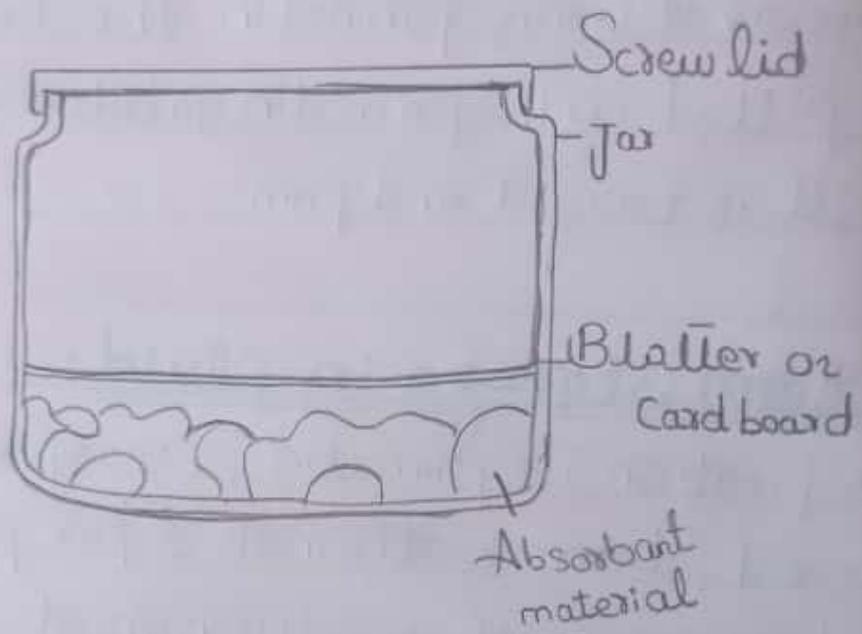
Preservation:

- * preservation is the process that serve to keep the dead body of organism from decay.
- * The preferred fluid is alcohol.
- * The standard is 70-75% ethanol.
- * 40-50% isopropyl alcohol is used on some animal taxa
- * Soaking solution (1.5 parts propylene phenoxetol, 5.0 parts propylene glycol, 10 parts formaldehyde and 83.5 parts distilled water for long term storage

Invertebrate Killing methods:

Killing Jars:

- * Liquid Killing agents are ethyl acetate, ether, chloroform and ammonia water. They are extremely volatile and flammable.
- * Solid Killing agents are cyanides, potassium cyanide, sodium cyanide or calcium cyanide.
- * Absorbant material plaster of paris / cotton.



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Practical 6:

Spectrophotometric estimation of Glucose

Materials:

Spectrophotometer (340-600nm), 0.1, 1.0, 10ml serological pipettes, 15x125 mixing tubes cuvettes, 0.1N Hydrochloric acid, Glucose kit (Sigma-115A), 500 mg/dl Glucose standard (Sigma G3761), Grape cool Aid, Test solution of blank 1, Blank-2 Grape cool-Aid Glucose.

Procedure:

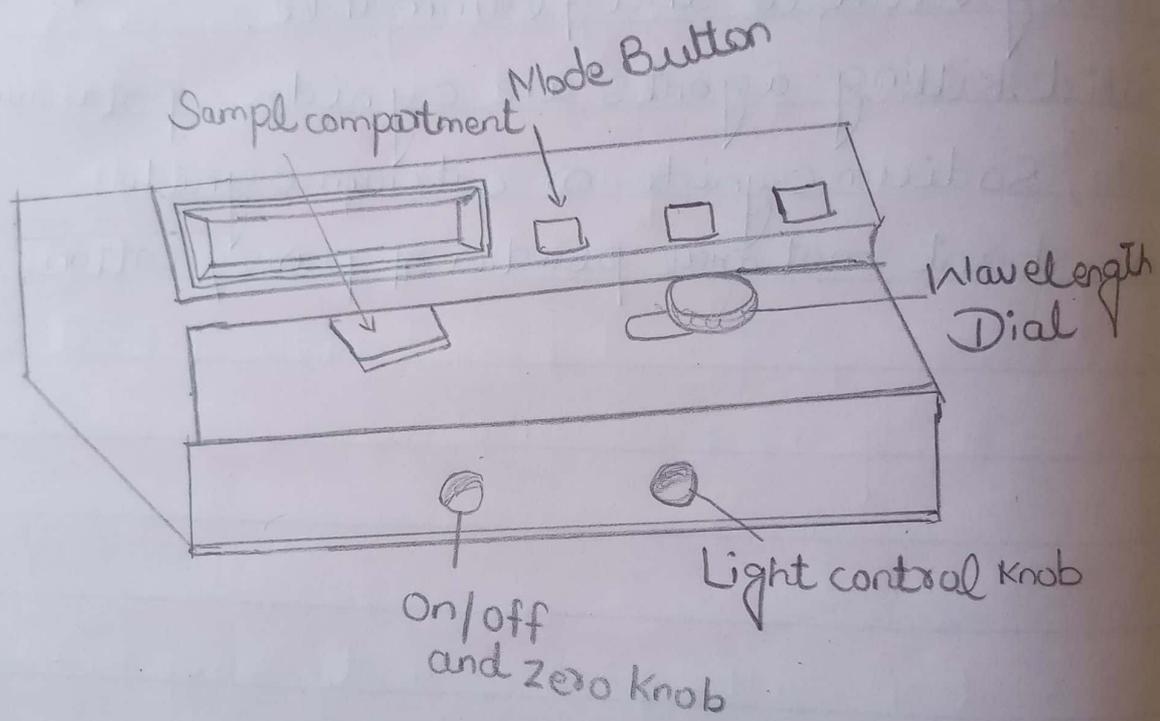
- 1 plug in spectrophotometer and turn it on by turning the left dial clockwise. Allow the spectrophotometer to warm up at least 5 minutes before proceeding.
- 2 Set the wavelength dial to 600nm.
- 3 With no vial in spectrophotometer, the light path is closed. Therefore no light is transmitted.
- 4 Now fill a cuvette with Blank 1. This solution should contain all the constituents except

the substance to be measured. Since we are measuring Grape cool Aid, Blank 1. contain the other constituents, Sugar and water in same proportion as are found in Grape Kool-Aid.

5 Insert the cuvette containing Blank 1 into sample chamber. As you do light path will be opened. This Blank solution does not contain any Grape cool Aid and the absorbance should be set to zero.

6 Next fill the cuvette with Grape-cool Aid test solution. Insert it into spectrophotometer and record its absorbance in question paper.

7 Next fill a cuvette with Blank 2 solution and repeat steps 5 and 6.



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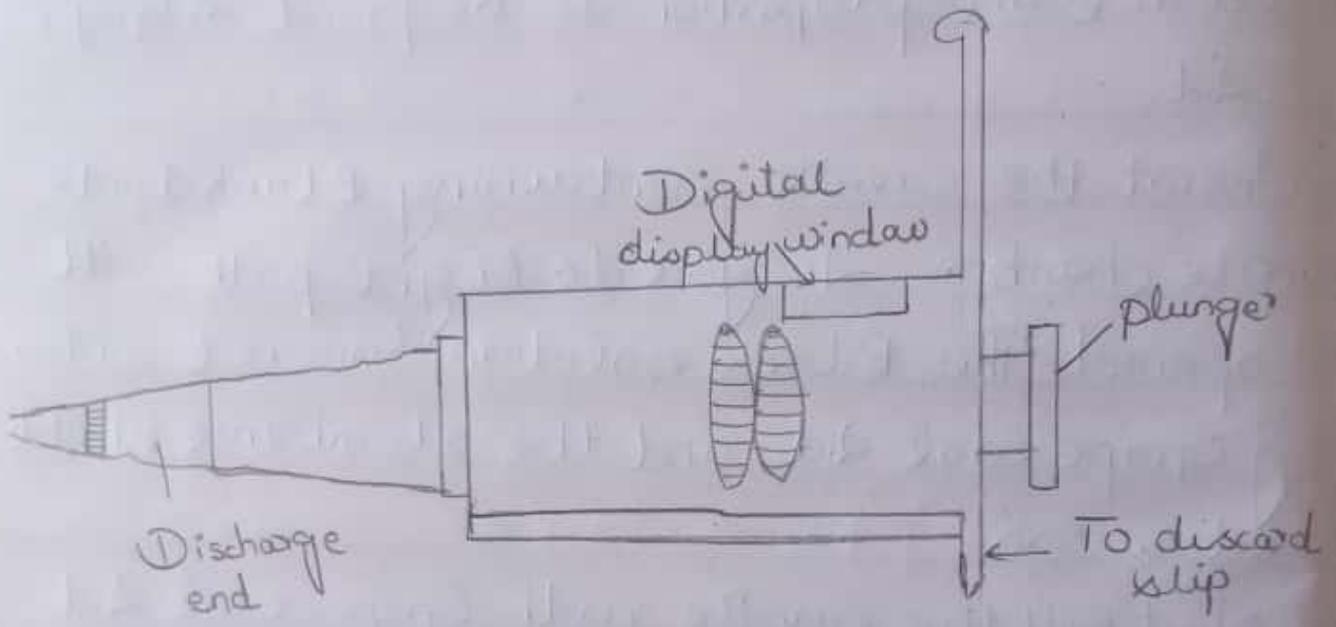
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practical 7:

Liquid Handling: proper use of pipettes.

The micropipette is used to transfer small amounts (1ml of liquids) The scales on micropipette are in microliters. ($1000\mu\text{l} = 1\text{ml}$).

The brand of micropipette we will be using is made by Rainin and called a 'pipetman'. These are very expensive, delicate instrument costing **\$250-300** a piece. We have four sizes identified by the number on the round button on the plunger. The value is the maximum value in microliters that can be transferred with that size pipette.



proper use of pipettes

practical 08

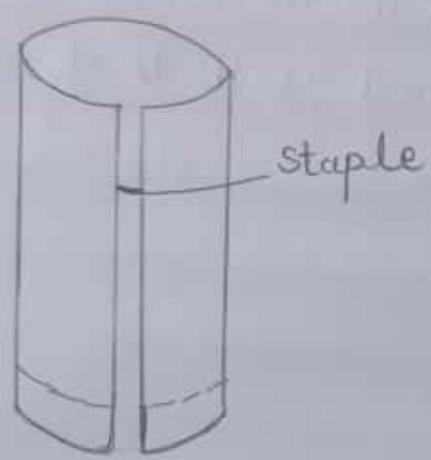
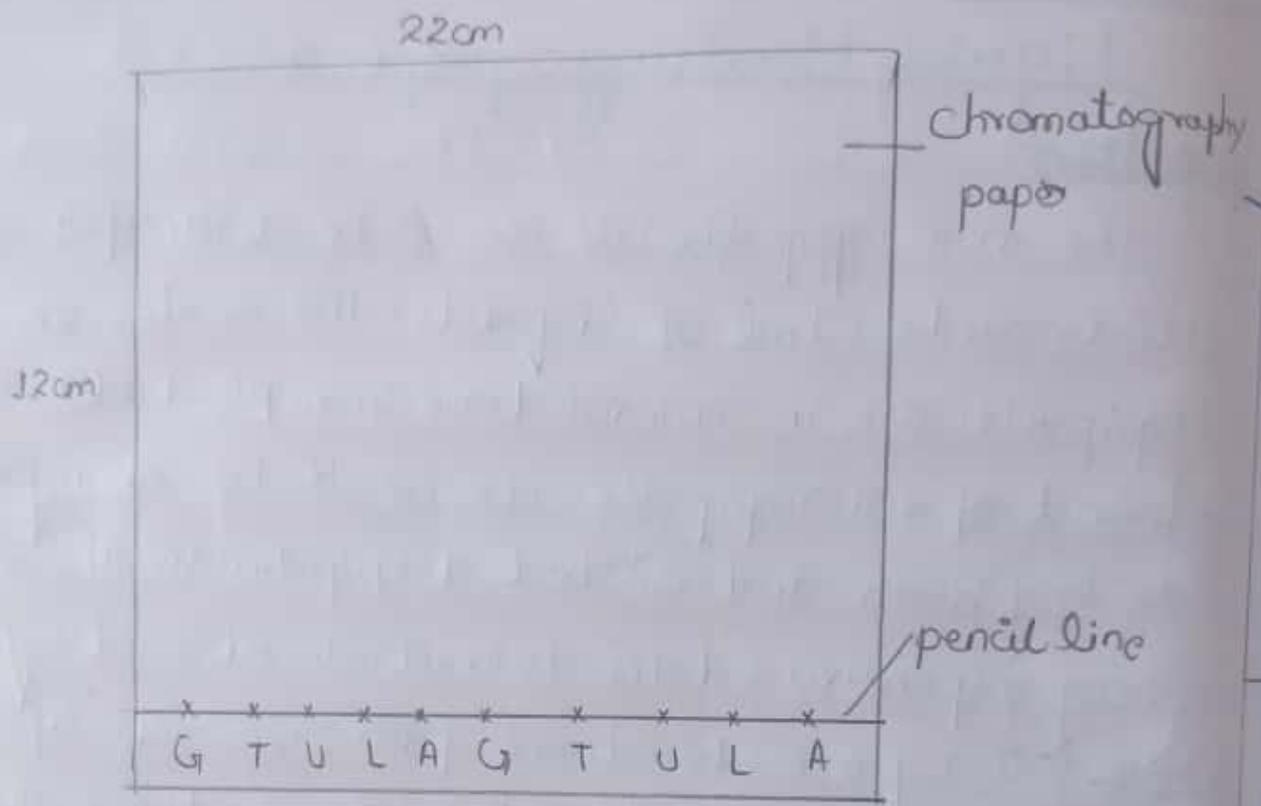
paper chromatography of amino acids

Materials required:

Whatman filter paper (12 x 22 cm), Butan-1,ol, Acetic acid, ninhydrin spray (2% solution of ninhydrin in ethanol), Capillary tube, beaker (500ml), oven.

procedure:

- 1 place enough chromatography solvent in tank or beaker to achieve a depth of about $\frac{1}{2}$ inch. Cover tank and allow solvent to saturate the atmosphere in the tank for at least 30 minutes.
- 2 Draw a line across the bottom of the chromatography sheet about 2.5 cm from the bottom edge of the chromatography paper with laid pencil.
- 3 Take a small amount of amino acid into capillary and deposit into paper by touching the capillary to line drawn. The spot should not be larger than $\frac{1}{4}$ inch.
- 4 After drying, roll the paper into cylinder and staple so that the edges do not touch.



paper chromatography of amino acids

practical 09

Thin layer Chromatography of Amino acids.

Reagents:

- 2% solution of individual amino acids
- solvent mixture of normal butanol, acetic acid and water in the ratio of 12:3:5 by volume
- Ninhydrin reagent

Requirements:

TLC plate

TLC chamber

Capillary tube

Reagent spray bottle

Conical flasks

Beakers.

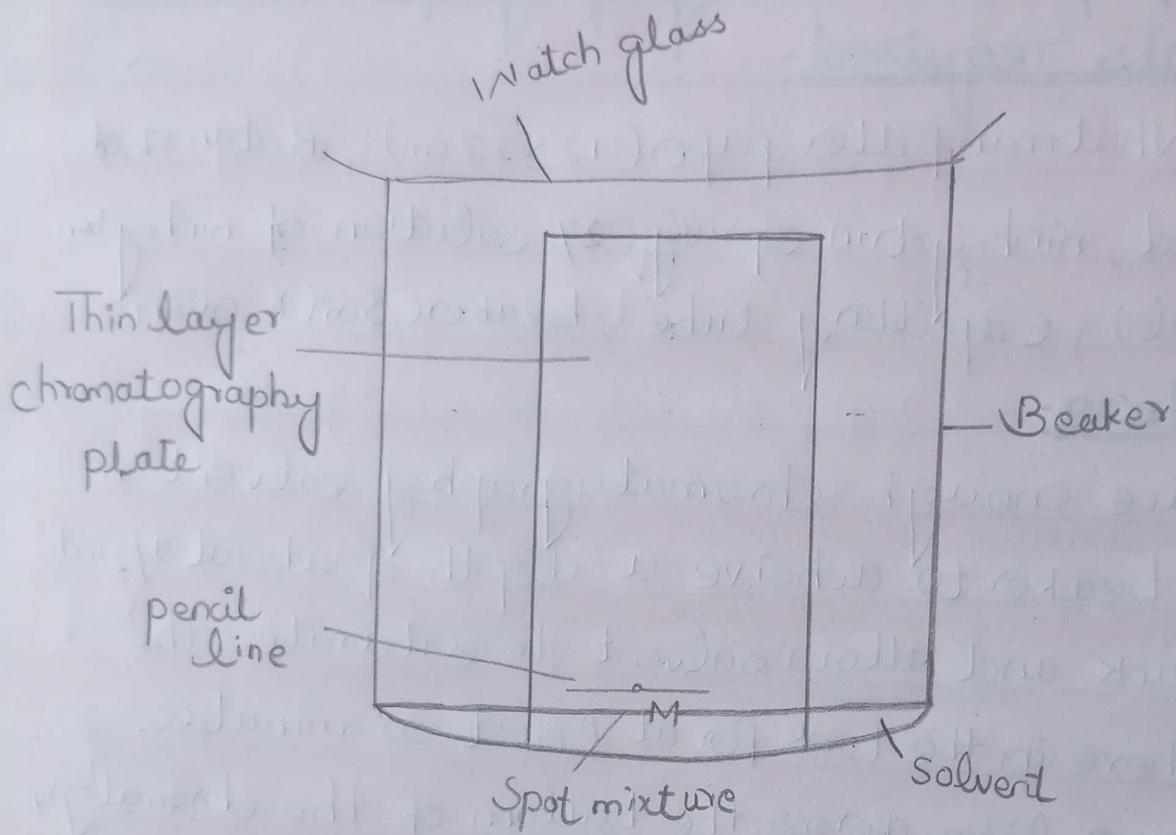
procedure:

- 1 pour the mixture into TLC chamber and close the chamber.
- 2 The chamber should not be disturbed for about 30 minutes so that the atmosphere in the jar become saturated with the solvent.

- 3 Cut the plate to the correct size and using a pencil gently draw a straight line across the plate.
- 4 Using a capillary tube, minute drop of amino acid is spotted on line.
- 5 Allow the spot to dry.
- 6 Spot the 2nd amino acid on the plate.
- 7 Repeat the above step for spotting the unknown acid.
- 8 place the plate in TLC chamber as evenly as possible and lean it against the slide
- 9 Remove the plate and immediately draw a pencil line across the solvent top.
- 10 Under a hood dry the plate with the aid of dryer.
- 11 Spray the plate with ninhydrin reagent.
- 12 Calculate R_f values

$$R_f = \frac{\text{Distance moved by the substance from origin}}{\text{Distance moved by solvent from origin}}$$

The R_f values are follow alanine 0.24, glutamic acid 0.25, glycine 0.2, valine 0.4, tyrosine 0.42.



Thin layer chromatography

Practical 10

Measurement of prokaryotic cell size

Components of prokaryotic cells:

All cells have four key components.

1 plasma membrane:

It is the outer covering that separates that cell interior from its surrounding environment.

2 Cytoplasm:

Consists of jelly like cytosol inside the cell, plus the cellular structures suspended in it.

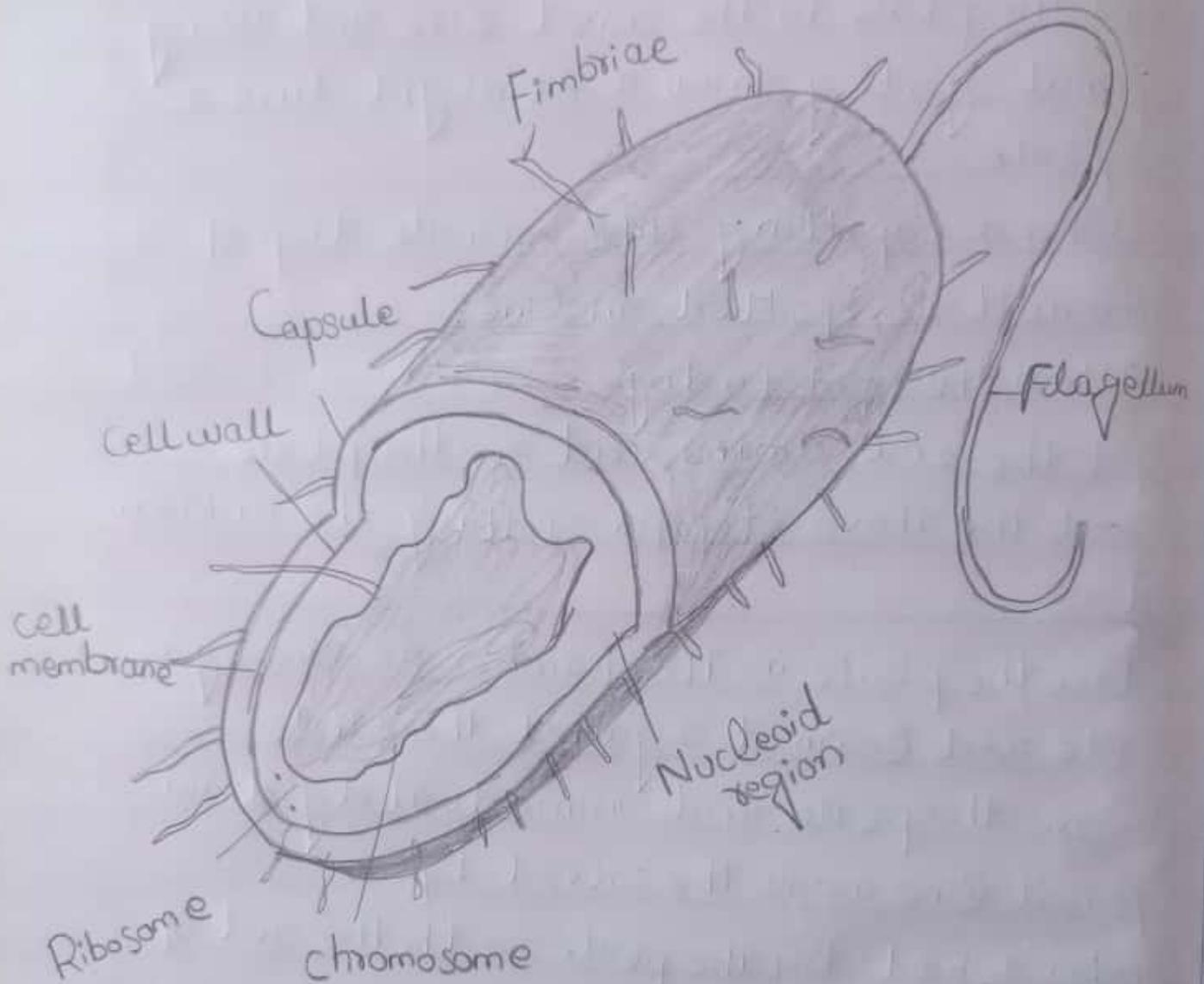
3 DNA:

It is the genetic material of the cell.

4. Ribosome:

Molecular machine that synthesize proteins.

Despite these similarities prokaryotes and eukaryotes are differ in many ways. A prokaryote is a simple single celled organism that lack nucleus & membrane organelles



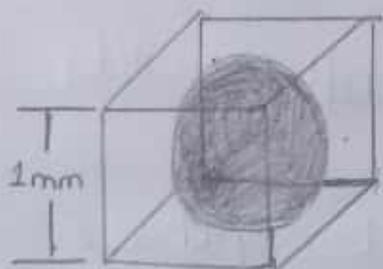
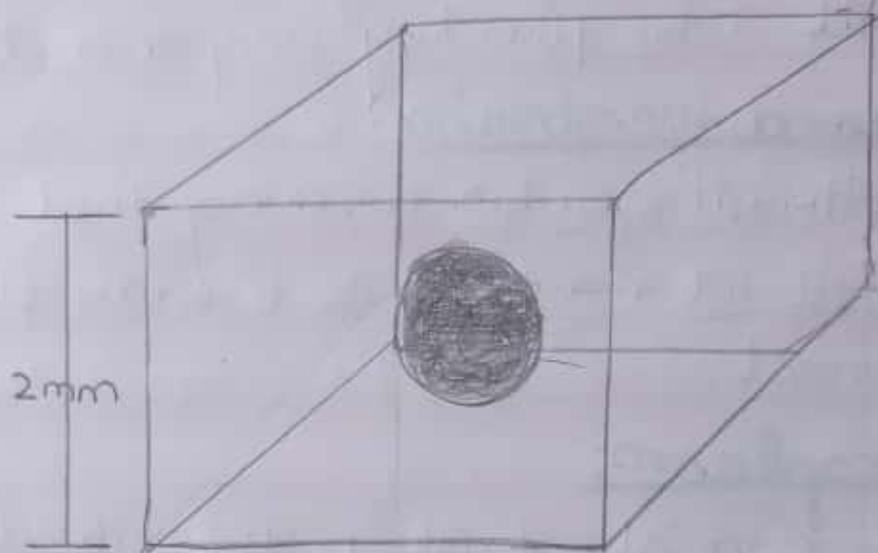
Cell size:

1. Typically prokaryotic cell ranges from 0.1 to 0.5 micrometers.

2. They have diameter ranging from 10 to 100 μm .

3. When cell gets bigger its surface area to volume ratio drops. For example the cube shaped cell on the left has volume of 1mm^3 (3 start superscript, 3 end subscript) and a surface area of 6mm^2 (2 start subscript, 2 end subscript) with a surface area to volume ratio of six to one, whereas the cube shaped cell on the right has volume of 8mm^3 (3 start subscript, 3 end subscript) and a surface area of 24mm^2 (2 start subscript, 2 end subscript) with a surface area to volume ratio of three to one.

4. Some cells are long and thin or have many protrusions from their surface, features that increase surface area relative to volume (3 start subscript, 3 end subscript).

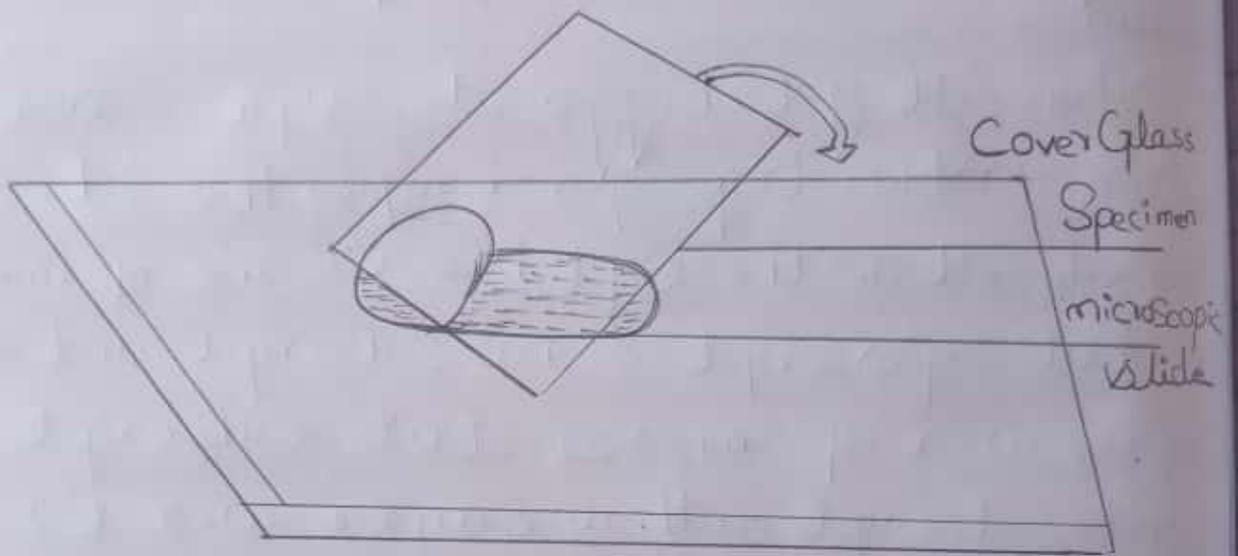


Practical 11

Measurement of cell size : Eukaryotic (Human Cheek Cell) cell size

Estimating the sizes of cell:

1. You will observe various types of cells, you may use prepared slides or you may make your own slides. To make wet amount: Obtain a clean glass slide.
2. place the object you wish to observe on the slide
3. You may use forceps or a toothpick to manipulate small objects into a paper orientation.
4. Use a pipette to add a drop of water (in some case you may add stain instead of water).
5. Now put a edge of a cover glass into the edge of the drop of water.
6. By carefully lowering the cover glass into the drop of water you should be able to prevent any air bubbles from being the formed.



preparation of wet mount on
microscopic slide

Practical 12

Recording of microscopic observations with the help of camera Lucida.

The Lucida camera when attached with the compound microscope, helps to draw microscope image of paper objects. It works on a simple optical principle that reflect light rays with prism and plane mirror.

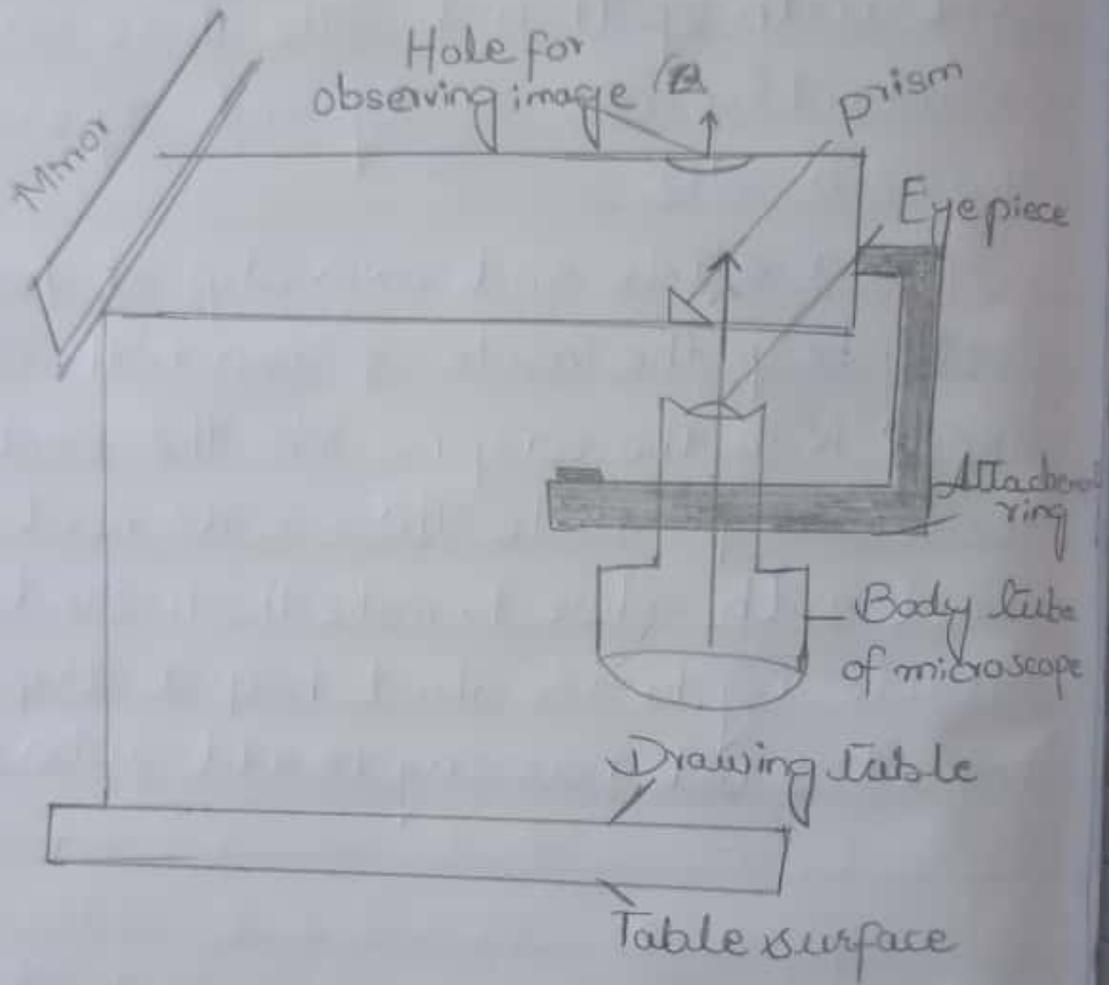
A small image of object is displayed by the prism on the plane screen and when from the image is displayed on the plane paper.

There are three main parts of the camera lucida.

- 1 Ring attachment
- 2 Prism
- 3 Mirror

The attachment ring attaches the lucida of camera and body tunnel to the microscope.

The prism sits above the eyeball when the tool is attached to microscope.



Diagrammatic representation of
Camera Lucid

practical 13

Liquid Handling proper use of Micropipette

Using a Micropipette:

Never exceed the upper or lower limit of pipettes

1. p10: 1.0 - 10.0 μ l
2. p20: 2.0 \rightarrow 20.0 μ l
3. p200: 20 \rightarrow 200 μ l
4. p1000: 200 - 1000 μ l

Load the sample:

\rightarrow The plunger will stop at two different positions when it is depressed. push the plunger down slowly to the point of the first resistance: this is the load volume.

\rightarrow While loading the plunger at the load volume first point: put the tip into the solution, so that it is immersed.

\rightarrow Slowly release the plunger to draw up the liquid. making sure to keep the tip immersed.

→ Visually inspect the load to make sure that it is correct. there should be no space in the distal end tip.

→ Deliver the sample. The second stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with body of the pipette. This second stopping point is used for the complete discharging of solution from the plastic tip.

→ You should not reach this second step when drawing liquid into pipette, only when expelling the last drop. To deliver volume

1 place the tip into the receiving vessel.

2 Depress the plunger into the point of initial resistance.

3 Wait 1 second.

4 Continue to press the plunger all the way to the bottom. - this expels all liquid.

* Discharge the tip place the discharge slider on the back of the grip.

