Lab manual / Standard Operating Procedures (SOPs)

for the practicals conducted under

DBT STAR COLLEGE Scheme

Progressive Education Society's
Modern College of Arts, Science and Commerce,
Ganeshkhind, Pune – 411 016

1) Title Study of Mitosis in plant material

Introduction :

Mitosis is equational cell division. It occurs in all meristimatic cells of plants. Root tips are the most actively growing organ and is ideal to study mitosis.Roots consist of different regions. The root cap functions in protection. The apical meristem is the region thatcontains the highest percentage of cells undergoing mitosis. The region of elongation is the area in which growthoccurs. The region of maturation is where root hairs develop and where cells differentiate to become xylem,phloem, and other tissues.

Materials:

Plant material used: freshly grown onion root tips

Chemicals-Stain Aceto-carmine ,1-M HCl, Fixative-Acetic acid :Alcohol (1:3)

Miscellaneous -slides and coverslips, Bunsen burner, compound light microscope, filter paper

Protocol:

1. Fix the onion root tips in the fixative for 24 hrs.
2. Remove tips from fixative, wash with distilled water and keep in 1N HCl at 50^0 for 10 min. Wash thoroughly with distilled water .
3. Take the tip on the slide and put 1-2 drops of acetocarmine stain. Warm gently. Repeat the process a few times to allow the stain to permeate the tissues.
4. Gently place a coverslip on the tip. Tap gently using back, flat side of the pencil between folds of filter paper to allow spread of cells in a single layer.
5. Observe under 10x and 45X of the microscope to visualize the chromosomes and various stages.
6. Identify the different stages and record in the journal with diagrams.

Observations:

1. The nondividing cell is in a stage called **interphase.**The nucleus may have one or more dark-

stained nucleoliand is filled with a fine network of threads, the chromatin. During interphase, DNA replication occurs.

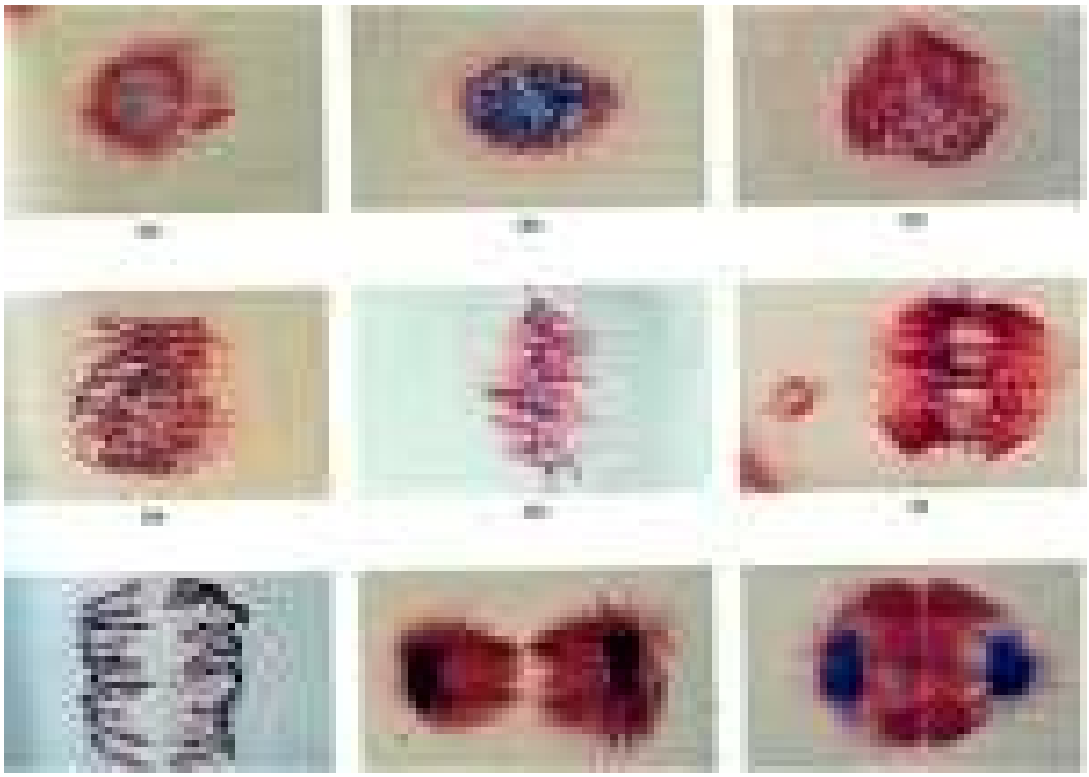
2. The first signs of a division is**Prophase,** in which a thickening of the chromatin threads occurs. Thickeningcontinues until it is evident that the chromatin has condensed into chromosomes. With highermagnification you may be able to see that each chromosome is composed of two chromatids joined at acentromere. As prophase continues, the chromatids continue to shorten and thicken. In late prophase thenuclear envelope and nucleoli are no longer visible, and the chromosomes are free in the cytoplasm. Justbefore this time the first sign of a spindle appears in the cytoplasm; the spindle apparatus is made up ofmicrotubules, and it is thought that these microtubules may pull the chromosomes toward the poles of the cell where the two daughter nuclei will eventually form.

3. At **Metaphase**, the chromosomes have moved to the center of the spindle. One particular portion of eachchromosome, the centromere, attaches to the spindle. The centromeres of all the chromosomes lie at aboutthe same level of the spindle, on an imaginary plane called the metaphase plate. At metaphase you should beable to observe the two chromatids of some chromosomes.

4. At the beginning of **Anaphase**, the centromere regions of each pair of chromatids separate and are moved bythe spindle fibers toward opposite poles of the spindle, dragging the rest of the chromatid behind them. Oncethe two chromatids separate, each is called a chromosome. The daughter chromosomes continue polewardmovement until they form two compact clumps, one at each spindle pole.

5. **Telophase**, the last stage of division, is marked by a pronounced condensation of the chromosomes, followedby the formation of a new nuclear envelope around each group of chromosomes. The chromosomesgradually uncoil to form the fine chromatin network of interphase, and the nucleoli and nuclear envelopereappear.

6**. Cytokinesis** may occur. This is the division of the cytoplasm into two cells. In plants, a new cellwall is laid down between the daughter cells. In animal cells, the old cell will pinch off in the middle along acleavage furrow to form two new daughter cells.



2) Title Study of Meiosis in plant material

Introduction :

Meiosis is reductional cell division. It occurs in pollen mother cell present in anther and also in megaspore mother cell lies within ovules. Flower buds are taken to study meiosis

Materials:

Plant material used: *Tradescantia /* Onion flower buds

Chemicals-Stain Aceto-carmine ,

Miscellaneous -slides and coverslips, Bunsen burner, compound light microscope, filter paper

Protocol:

1. Remove floral buds from fixative, wash with distilled water. Arrange anthers with filament according to size on a slide.

2. Take the anther on the slide and put 1-2 drops of acetocarmine stain. Warm gently. Repeat the process a few times to allow the stain to permeate the tissues.
3. Gently place a coverslip on the anther. Tap gently using back, flat side of the pencil between folds of filter paper to allow spread of cells in a single layer.
4. Observe under 10x and 45X of the microscope to visualize the chromosomes and various stages.
5. Identify the different stages and record in the journal with diagrams.

Observations:

First meiotic Division:

I)**Prophase:** It is the first stage of division in which a thickening of the chromatin threads occurs. Thickeningcontinues until it is evident that the chromatin has condensed into chromosomes. Prophase has the following stages:

1. **Leptotene:**This phase differs only slightly from the early stages of mitosis. The cells and nuclei of meiotic tissues are usually bigger than that of their neighbouring tissues and they do often seem to be longer and are longitudinally structured. At regular intervals thickenings can be found, like beads on a string: the **chromomeres**. Their number, size and positioning is constant in each species..

2.Zygotene. During this phase the pairing of homologous chromosomes begins. It is also called **synapsis** and the resulting structure **synaptic complex**. Directly after initiation of the process the pairing spreads like a zipper across the whole length of the chromosome.

3. Pachytene. During the pachytene the pairing stabilizes. The number of synaptic complexes corresponds to the number of chromosomes in a haploid set of the respective species. The pairs are also called bivalents.

3. **Diplotene**. The bivalents separate again. During this process it emerges that each chromosome is built of two chromatids, so that the whole complex harbours four strands during the separation. Normally the separation is not accomplished, but the homologous

chromosomes stick together at certain points, the chiasmata (sing. chiasma). This state is marked by the formation of cross-like structures, single or multiple loops.

4. **Diakinesis:** is the continuation of the diplotene. It is usually difficult demarcate both states. The chromosomes condense and become more compact.

II) Metaphase: From now on the processes resemble that of mitosis again. The nuclear membrane is completely dissolved, the mitotic spindle fully developed. The homologous chromosomes do still stay together.

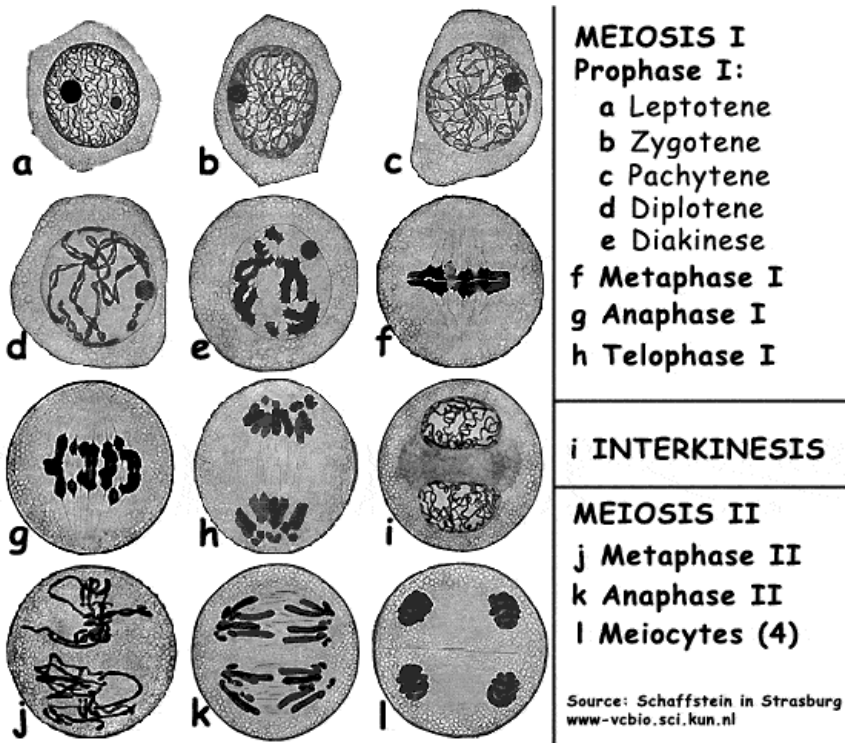
III)Anaphase: During anaphase the homologous chromosomes are separated from each other.

IV)Telophase: This follows anaphase.Two daughter cells are formed with each daughter containing only one chromosome of the homologous pair.

Second division of meiosis:

- **Prophase 2**: DNA does not replicate.
- **Metaphase 2**: Chromosomes align at the equatorial plate.
- **Anaphase 2**: Centromeres divide and sister chromatids migrate separately to each pole.
- **Telophase 2**: Cell division is complete. Four haploid daughter cells are obtained.

One parent cell produces four daughter cells. Daughter cells have half the number of chromosomes found in the original parent cell and with crossing over, are genetically different.Meiosis differs from mitosis primarily because there are two cell divisions in meiosis, resulting in cells with a haploid number of chromosomes.



3) Production of Mushroom at Laboratory scale

Aim: To cultivate Mushrooms at laboratory scale.

Mushrooms are highly nutritious food product. They contain high water content and low fat content. The mushrooms are saprophytic and can be grown on the organic wastes. Usual procedure for cultivation of mushrooms is to use sterilized agricultural wastes like rice bran or hey as a medium. The spawn or the 'seed' is then inoculated and the medium is incubated at 25-30^{o}C. Care is to be taken to keep the moisture constant. The mycelium growth is evident in a week's time. After 14 days of cultivation, the fruiting bodies appear on the surface of the medium. They grow in the size in next 24-48 hours and ready for the harvest.

At laboratory level the protein contents for the each batch is estimated by Lowry method

Procedure:

1. Spawn (seed) of the mushroom is collected from Agricultural college
2. Wheat bran and other agricultural waste are collected and sterilized in autoclave
3. The sterile waste is moistened and inoculated with spawn.
4. It is incubated at 25-28^{o}C in a room kept humid.
5. The mycelial growth is expected to cover the whole waste.
6. The mushrooms appear on the surface after 12 days of incubation.

4) Title- Production of Biofertizer

Aim- To produce BGA biofertilizer

Principle

Blue Green Algae is Cynobacteria which fixes nitrogen non symbiotically ; as they have Nitrogenase enzyme. Hence if soil is inoculated with BGA culture , organism fixes atomospheric nitrogen and make it available for plant growth.

Materials

Soil (carrier)- 2-3 Kg, superphosphate , plastic tray of size (30cmx 30cmx 15cm) , BGA biofertilizer culture.

Methods

1) Take a clean tray , fill it with soil to make layer of 10cm
2) Mix 20-25gm% super phosphate & also inoculate culture of BGA
3) Fill tray with water, keep tray in sunlight for 8-10 days
4) BGA will grow on surface and get dried up
5) Mix growth with soil , fertilizer is ready to use.

MUSHROOM CULTIVATION

Aim: To cultivate edible mushrooms like oyster mushrooms.

Mushrooms are also called `white vegetables' or `boneless vegetarian meat'. Agaricus bisporus, Pleurotus ostreatus, P. sajor-caju, Lentinus edodes, Volvariella vovacea etc. are common edible mushrooms.

Pleurotus ostreatus, the oyster mushroom (Dhingri) is tropical edible mushroom belongs to basidiomycetes. The systematic approach of Dhingri cultivation was undertaken by Dr. Janbaie in 1974. The oyster mushroom is considered a medicinal mushroom, since it contains statins such as. The oyster mushroom is one of the more commonly sought wild mushrooms, though it can also be cultivated on straw and other media. The mushroom has a broad, fan or oyster-shaped cap spanning 5-25 cm growing laterally. The flesh is white, firm, wavy and varies in thickness due to stipe arrangement. The gills are white to cream, and descend on the stalk.

Procedure:

1. Preapration of pure culture of *Pleurotus*
2. Preparation or procurement of spawn
3. Substrate preparation
4. Spawning of substrate

Preapration of pure culture of *Pleurotus:*

Requirements: Inoculum is fruiting body of *Pleurotus.*

Glasswares: Petri plates, test tubes, conical flasks, glass rod.

Other requirements: non absorbent cotton, Aluminum foil, Autoclave,

cabinate.

Chemicals: Glucose, Agar-Agar powder, Distilled water, alcohol etc. Procedure:

1) Petri plate glass rods and other glass wares are sterilized at

minutes at 120°C in autoclave.

2) Pleurotus ostreatus is taken as an inoculum.

Preparation of PDA (Potato Dextrose Agar): Composition of PDA:

1) Boil 250 gms of peeled poatao till they become soft.

2) Filter through muslin cloth and the filtrate is taken.

3) To this filtrate 20 gms of Agar and 20 gms of dextrose sugar is added and mixed well.

4) Above mixture is taken in conical flask and flask is sterilize at 151b pressure for

20 minutes after pluging the mouth of conical flask with cotton and aluminium foil.

5) After autoclaving the medium is passed in previously sterilized petri plates.

6) The petri plates are inoculated with innoculum pieces of fruiting body under aseptic condition i.e. in laminar fresh air flow cabinate

2. Spawn preparation:

A pure culture of *Pleurotus* sp. is needed for inoculation on sterilized substrate. It takes 10-15 days for mycelial growth on cereal grains. It has been reported that jowar andbajra grains are superior over wheat grains.

Procedure:

1) Grains are boiled with equal volume of water. Due to boiling grains become soft.

2) The grains are spread on blotting paper so as to remove excess water.

3) 2% CaCO3 powder is added to the grains and mixed the easily CaCO3

helps to maintain the pH and also absorb excess water.

4) The mixture is the filled in a wide mouth bottle or polythene bag and plug

highly it is then sterilized in the autoclave at 15 lb pressure for 20 min.

5) After sterilization the bottle is allowed to cool down completely and then bottle

or bag was inoculated with the pure culture in lamina fresh air flow cabinate.

6) After 15 days the grains get covered with white mycelium and then it is termed as spawn. Spawn is generally supply in glass bottle or polythene bag.

2. Plant tissue culture

Aim -- To produce a single cell culture

Method 1 st— 1) The callus is separated and transferred to a fresh medium to get more tissues.

2) Pieces of un-differentiated calli are transferred to liquid medium, which is continuously agitated to obtain a suspension culture.

Method 2nd --- Cells are suspended in a liquid medium culture medium with agar 1% is cooled and maintained at 350c in a water bath. Equal volume of liquid and agar media are mixed rapidly and spread in a petri-plate so that cells are evenly distributed in a thin layer after solidification. The plates are incubated in dark at 25 C and cell colonies developing from marked single cells are used to obtain single cell cultures.

Class **:** F.Y.B.Sc. Chemistry

Aim **:** To determine the molar refractions of methanol, ethanol, propanol and hence the molar refraction of $–CH_2$ group.

Apparatus **:** Abbe refractometer, dropper, cotton plugs, specific gravity bottle.

Chemicals **:** Methanol(MA), ethanol(EA) and propanol(PA)

Procedure **:**

1) Determine the density of all alcohols by using the 10 ml specific gravity bottle with distilled water as the reference liquid using the formula,

$$\text{Density(d)} = \frac{\text{Wt of liquid+bottle} - \text{Wt of empty bottle}}{\text{Wt of water+bottle} - \text{Wt of empty bottle}}$$

.

2) Clean the prism faces inside the prism box in the refractometer with a cotton plug moistened with alcohol.
3) Place few drops of liquid on surface of lower prism and close the prism box to enclose a film of liquid between the two prisms.
4) Focus the cross wire of the telescope in refractometer by rotating the eyepiece. Adjust the mirror so as to get maximum illumination.
5) Move the prism box backward and forward till the field of vision becomes partly light and partly dark.
6) Adjust the compensator screw till the coloured fringes disappear and a sharp boundary is seen.
7) Rotate the prism box till the sharp edge coincides with intersection of cross – wire in the telescope.
8) Read the refractive index directly from the scale.

9) Repeat the readings three times and calculate the mean refractive index value (n).
10) Repeat the above steps to note the refractive indices of other liquids too.
11) Calculate specific refraction (r) of each liquid using the formula,

$$\mathbf{r} = \frac{(n^2-1)}{(n^2+2)d} \text{ ml/g}$$

12) Calculate molar refraction (R_M)of each liquid using the formula,

R_M = Molecular wt. x **r**

13) Determine [$R_{M(EA)} - R_{M(MA)}$] and [$R_{M(PA)} - R_{M(EA)}$] values.

Results :

The values of $[R_{M(EA)} - R_{M(MA)}]$ and $[R_{M(PA)} - R_{M(EA)}]$ are same indicating that molar refraction possesses additive and constitutive properties. The regular increase in the molar refraction values is due to the $-CH_2$ group.

Class : T.Y.B.Sc. Chemistry

Aim : To determine the amount of copper present in the given solution of copper sulphate by colorimetric titration method using standard solution of EDTA.

Apparatus : Colorimeter, microburette, pipette, conical flask.

Chemicals : 0.04M copper sulphate, .1M EDTA, acetate buffer solution

Procedure :

Part I: Determination of max of Cu-EDTA complex

1. Standardize the given spectrophotometer as per the instructions given by the manufacturer.
2. Take 10 ml of 0.04 M copper sulphate solution and 20 ml acetate buffer solution and add large excess of 0.1M EDTA solution to it. Shake the solution well and use it for determination of λ_{max} of Cu-EDTA complex. Try all the filters available in the laboratory and select one with which the experimental solution has the maximum absorbance. This can be done by plotting absorbance (A) values against λ in nm and selecting wavelength corresponding to the maximum absorbance viz. λ_{max}.

Part II: Estimation of copper

1. Pipette out 10 ml of given solution of copper sulphate in a conical flask and add 20 ml of acetate buffer solution.
2. Fill a burette with 0.1 M solution of EDTA. Add 0.2 ml of EDTA solution from burette in to the copper solution and shake the solution well. Transfer small portion of this solution in the cuvette and find the absorbance of it at its λ_{max} using water as a blank.
3. Transfer the solution in the cuvette back in to the conical flask add further 0.2 ml of EDTA solution from burette and find the absorbance of the solution as described above.
4. Continue the titration by adding 0.2 ml of EDTA solution at a time. After equivalence point constant readings of absorbance are obtained. Note 5 - 6 fairly constant readings.

5. Plot a graph of absorbance against ml of EDTA added. Two straight lines with different slope values are obtained. The intersection of two lines gives equivalence point of the titration gives the value of 'x'ml.

Calculations:

1000 ml 1 M EDTA = 63.54 g of Cu^{2+}

'x' ml 0.1 M EDTA = $\frac{63.54\ x}{1000}$ g of Cu^{2+} = W g

10 ml of given $CuSO_4$ solution = W g of Cu

100 ml of given $CuSO_4$ solution = (W x 10) = A g of Cu

Results:

1.	λmax of Cu EDTA complex	λ_{max} nm
2.	Concenteration of Cu in given solution	A g/L

Class : T.Y.B.Sc. Chemistry

Aim : To determine the dissociation constant of given acid-base indicator by colorimetric method.

Apparatus : Colorimeter, filters, pH-meter, burettes (50 ml), volumetric flasks (50 ml), hard glass tubes, calibrated pipette.

Chemicals : Methyl red, 0.2 M Na_2HPO_4, 0.1 M citric acid

Procedure :

Part I : Preparation of Solution

1. Take 12 flasks of about 50 ml capacity and label them from 1 to 12.
2. Take two burettes and fill them with 0.2 M Na_2HPO_4 and 0.1 M citric acid solution

3. Withdraw following volumes of Na_2HPO_4 and citric acid solutions in the above flasks to get desired buffer solutions.

Flask No.	Expected pH	0.2 M Na_2HPO_4 (ml)	0.1 M citric acid (ml)
1	3.4	11.40	28.60
2	3.8	14.20	25.80
3	4.2	16.56	23.44
4	4.6	18.70	21.30
5	5.0	20.60	19.40
6	5.4	22.30	17.70
7	5.8	24.18	15.82
8	6.2	26.44	13.56
9	6.6	29.10	10.90
10	7.0	32.94	7.06
11	7.4	36.34	3.66
12	8.0	38.60	1.10

4. Take 12 hard glass test tubes and label them from 1 to12. From the above prepared buffer solutions, withdraw 10 ml from each and place in respective test tube.
5. Dissolve 20 mg of methyl red in 60 ml of ethanol and dilute to 100 ml with water to get indicator solution.
6. Add 1 ml of indicator with a help of calibrated pipette in each of the solution in the test tubes and shake well.

Part II : Determination of pH buffer solutions

1. Standardize the pH meter using 0.05 M potassium hydrogen phthalate solution at pH = 4.
2. Take buffer from flask no.1 in a beaker and insert the cleaned glass and calomel electrodes into the solution in the beaker so that the bulb of the glass electrode is completely immersed into the solution. Record the scale reading as pH of the solution.
3. Repeat the procedure for remaining solutions. Call the readings as observed pH of the solutions.

Part III: Determination of Absorbance

1. Determine the λ_{max} value by using dense colored methyl red solution.
2. Place the appropriate filter corresponding to λ_{max} value in the filter compartment.
3. Take the blank solution [buffer solution 1 without methyl red] in the cuvette, clean it from outside and insert it into sample compartment.
4. Adjust the meter reading to 100% transmittance with the help of coarse and fine set knobs.

5. Remove the cuvette, wash it with distilled water. Put the solution from test-tube no.1 in it, clean it from outside and insert it into sample compartment. Read the absorbance and % transmittance of the solution on the meter scale.
6. Repeat the procedure for remaining solutions to record their absorbance and % transmittance values.
7. Prepare an observation table as follows:

Solution from test tube no.	Observed pH	Absorbance A	%T	ΔA	ΔpH	$\Delta A / \Delta pH$	Mean pH
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

8. Plot the graphs of (a) absorbance against observed pH and (b) $\Delta A/\Delta pH$ against pH. The point of inflection of the first plot or peak position of the second plot indicates [MR-]/ [HMR] = 1.The pH value at these positions indicates pK value of the indicator.

Calculations :

Calculate the value of dissociation constant K of the indicator using pK= -log K

Results:

λ_{max}	pK	K

Class : T.Y.B.Sc. Chemistry

Aim : To determine the amount of NaCl in the given solution by potentiometric titration between NaCl and $AgNO_3$.

Apparatus : Beakers, silver electrodes, platinum electrode, saturated calomel electrode, magnetic stirrer, potentiometer, burette, salt bridge.

Chemicals : 0.05 M $AgNO_3$, 0.01 M $AgNO_3$, 0.01 M NaCl , distilled water.

Procedure :

Part I - Standardisation of Potentiometer

1. Adjust the emf on potentiometer to 1.0185 volt which is equal to emf of Weston standard cell.
2. Move the two way key on the potentiometer to bring the standard cell in the circuit.
3. Adjust the rheostat with the help of coarse and fine knobs so as to get null point.
4. Do not disturb the adjustment of potentiometer throughout the experiment.

Part II – Estimation of sodium chloride

1) Pipette out 20 ml of 0.01M NaCl solution in a 100 ml beaker and dilute with 30 ml distilled water.
2) Dip the silver electrode (-ve terminal) in it.
3) In another beaker, take 20 ml of 0.01M $AgNO_3$ and 30 ml distilled water. Dip another silver electrode (+ve terminal) in it.
4) Connect both the solutions using KNO_3 / NH_4NO_3 salt bridge.
5) Keep both beakers on a magnetic stirrer and stir the NaCl solution well.
6) Connect the cell to the respective EMF terminals of potentiometer.
7) Find the e.m.f. of the cell without adding $AgNO_3$ solution and record it.
8) Fill the burette with 0.05 M $AgNO_3$ solution.
9) Add 0.3 ml of 0.05 M $AgNO_3$ solution from burette into NaCl solution.
10) Stir the solution and note the e.m.f. along with the volume of added $AgNO_3$.
11) Continue the addition of 0.3 ml of $AgNO_3$ solution each time, till the end point exceeds by 2ml. Record the emf for each addition.
12) At the end point, the emf observed is minimum.
13) Prepare the observation table as:

Obs. No.	ml of $AgNO_3$ added (V ml)	E.m.f. E (volts)	ΔE (volts)	ΔV	$\Delta E/\Delta V$	MeanV (ml)
1.	0.0			–	–	–
2.	0.3			0.3		0.15
3.	0.6			0.3		0.45
4.	0.9			0.3		0.75
5.	1.2			0.3		1.05
.	.			.		.
.	.			.		.

14) Plot the graphs of (a) Emf against ml of $AgNO_3$ added (b) $\Delta E/\Delta V$ against Mean V and determine the volume of 0.05M $AgNO_3$ required at equivalence point 'x' along with the strength of NaCl solution.

Calculations :

1) To calculate the normality of NaCl :

$N_1 \times V_1 = N_2 \times V_2$
(NaCl) ($AgNO_3$)
$N_1 \times 20 = 0.05 \times$ 'x' (From graph)
$N_1 = (0.05$ 'x') / 20

=…..Exact normality of of NaCl

2) To calculate the strength of NaCl :

Strength of NaCl = Normality × Eq. wt.
= $N_1 \times 58.5$
= A g / L

3) **To calculate the amount of NaCl :**
Amount of NaCl in 1000 ml of solution = A g / L
Amount of NaCl in 20 ml of solution = (A × 20) / 1000
= B g

Results :

1.	Amount of 0.05 M $AgNO_3$ required to precipitate the halide completely	= 'x' ml
2.	Exact normality of NaCl solution	= N
3.	Strength of NaCl solution	= g / L
4.	Amount of NaCl in 20 ml solution	= g

Class **:** T.Y.B.Sc. Chemistry

Aim **:** To determine dissociation constant of oxalic acid by pH-metry titration with strong base.

Apparatus **:** pH- meter, calomel and glass electrodes (or combined electrode), burette, stirrer, beakers.

Chemicals **:** 0.1N oxalic acid, 0.5 N NaOH, 0.05 N potassium hydrogen phthalate

Procedure **:**

Part I - Standardisation of Potentiometer

1) Standardize the pH meter using 0.05 M potassium hydrogen phthalate solution (pH = 4).
2) Take 20 ml oxalic acid solution and 30 ml water in 100 ml beaker.
3) Place the solution on stirrer and dip calomel-glass electrodes into the solution.
4) Fix burette containing the strong base i.e. 0.5N NaOH above the beaker.
5) Measure the pH of the acid solution.
6) Add 0.3 ml of 0.5 N NaOH solution from burette to the acid solution, stir well and note the pH solution.
7) Repeat the procedure by adding 0.3 ml NaOH each time. Record the pH of the solution at each time and continue the addition of NaOH solution till the end point exceeds by about 4 ml.
8) At the equivalence point there is sharp increase in pH of solution.
9) Prepare the observation table as:

Obs. No.	Volume of 0.5 N NaOH added (V ml)	pH	Δ pH	ΔV	Mean V	Δ pH/ ΔV
	0.0			-	-	
2	0.3			0.3	0.15	
3	0.6			0.3	0.45	
4	0.9			0.3	0.75	
5	1.2			0.3	1.05	
.	.			.	.	
.	.			.	.	
.	.			.	.	

10) Plot the graphs of (a) pH against V ml of NaOH added and determine the $(pH)_1$ value at 0.5 x_1 ml and $(pH)_2$ value at 1.5 x_2 ml (b) Δ pH / ΔV against Mean V and determine the equivalence points 'x_1' and 'x_2' corresponding to first and second dissociation of acid respectively.

Calculations :

From the graph,

$(pH)_1 = pK_1$ and $(pH)_2 = pK_2$.

Calculate the first dissociation constant K_1 and second dissociation constant K_2 using relations: $pK_1 = -\log K_1$ and $pK_2 = -\log K_2$

Results :

First dissociation			Second dissociation		
x_1 ml	pK_1	K_1	x_2 ml	pK_2	K_2

These practicals are based on theory courses for the respective classes and will help in understanding the subject better. It will improve the students' practical skills.

Experiment No. 1

Title of the Experiment:

Determination of Least Count, Capacity and Range of various instruments used in the laboratory – glass pipettes, micropipettes, weighing balance etc.

1. **Least Count & Capacity of Instruments**

Pipettes

Liquids are dispensed in the laboratory by means of pipettes.

Pipettes used in the laboratory:

1. Glass pipettes
2. Micropipettes

Glass pipettes:

The following glass pipettes were studied:

1. 0.2 ml
2. 1 ml
3. 2 ml
4. 5 ml
5. 10 ml

Capacity, Least Count and Range of the pipettes are to be determined:

Least count is the highest degree of accuracy of measurement that can be achieved by that instrument.

It is determined in the following manner:

a) Count the number of divisions between '0' ml marking and the immediate next volume marking, say 'x' ml: if 'y' are the number of divisions between '0' ml and 'x' ml,
b) Least Count = x/ y ml

Capacity of a pipette is the maximum measurable volume that can be dispensed by that pipette in one transfer operation.

Range of an instrument is the range between minimum and maximum volumes that can be measured by that instrument.

Results and observation:

Sr. No.	Capacity	Least Count	Range*	Information inscribed on pipette
1	0.2 ml	0.1 ml/ 10 divisions= 0.01 ml	0 to 0.2 ml	Borosil Ex. 27^0C 2/ 10 ml in 1/100
2	1 ml	0.1 ml/ 10 divisions= 0.01 ml	0 to 1 ml	Borosil Ex. 27^0C 1 ml in 0.01
3	2 ml	0.2 ml/ 10 divisions= 0.02 ml	0 to 2 ml	Borosil Ex. 27^0C 2 ml in 1/ 50
4	5 ml	0.5 ml/ 10 divisions= 0.05 ml	0 to 5 ml	Borosil Ex. 27^0C 5 ml in 1/20
5	10 ml	1 ml/ 10 divisions= 0.1 ml	0 to 10 ml	Borosil Ex. 27^0C 10 ml in 1/10

*Though range of a pipette is given from 0 to maximum capacity of the pipette, range of measurable volumes that can be dispensed is from least count to capacity of that pipette.

2. WORKING WITH MICROPIPETTES

Introduction:

Laboratory work in molecular biology and biotechnology is usually done in minute quantities. The unit of measure used for setting up reactions is the **microliter (µl)**. One microliter is one millionth (10^{-6}) of a liter.

So: 1 L = 1,000,000 µl, and 1 ml = 1,000 µl

Practice these conversions:

1. Convert the following to ml: 100 µl 500 µl 3,000 µl 10 µl

2. Convert the following to µl: 5 ml 0.5 ml 0.004 ml 0.000001 ml

The **micropipette** is an instrument that allows us to accurately measure µl volumes of reagents. Micropipettes are delicate, very expensive, and the cornerstone of our work with DNA. In this lab, you will learn to properly use and care for micropipettes. A micropipette uses suction to draw up specific amounts of liquid. Its parts allow you to control how much liquid to suck up and dispense. It is essentially a hollow barrel with an adjustable plunger through it. On the left is a diagram of a micropipette and its specific parts.

The *control button*, or *plunger*, allows the user to suck up and dispense liquid.

The *eject button* allows ejection of micropipette tips after use.

The *volume knob* allows the user to dial the amount of liquid to be measured.

The *number window* shows the amount dialed.

The *tip* of the micropipette is where the micropipette tips are placed. The entire white part is called the *barrel*.

MICROPIPETTING

Damaging these instruments can be avoided by following a few simple rules:

- **Never rotate the volume knob beyond the upper or lower range of the micropipette.**
- **Never use a micropipette without a tip in place.**
- **Never lay down a micropipette with a filled tip.**

Sr. No.	Capacity	Least Count	Range
1	50 µl	0.5 µl	2 – 50 µl
2	200 µl	1 µl	20-200 µl
3	1000 µl	10 µl	200-1000 µl

- **Never allow plunger to snap back after ejecting fluid.**
- **Never immerse barrel of micropipette in fluid.**
- **Never flame micropipette tips.**

Micropipettes are of various types based on volumes they dispense:

1. Fixed volume pipettes e.g. 10 µl volume
2. Variable volume pipettes: the following **ranges** were studied:

Experiment No. 2

Title of the Experiment:

Microscopic observation of fungi using Lactophenol Cotton Blue.

Introduction:

Microbiologists use the term fungi / molds to include eukaryotic, spore bearing organisms with absorptive nutrition, no chlorophyll and that reproduce sexually and asexually. Fungi are primarily terrestrial organisms, although a few are freshwater or marine. Many are pathogenic and infect plants and animals. Fungi also form beneficial relationship with the other organisms, for e.g. mycorrhiza form association with the roots of many plants. Lichens are associations of fungi and algae. Identification of fungus, as soon as it is isolated in a pure form, is often made by recognition of characteristic structures seen in culture which includes Colony morphology, hyphae, presence or absence of septa, asexual spores, sexual spores, reproductive bodies and arrangement of conidia.

Economic importance:

1. Production of antibiotics e.g. Penicillin produced by *Penicillium notatum.*
2. Important role in industries such as in alcohol fermentation, production of organic acids such as oxalic acid, citric acid etc.
3. Gibberellin produced by *Gibberella fujikuroi* is used as growth hormone accelerating plant growth.
4. Many of them are pathogenic to plants, animals and humans.

Principle:

Lactophenol Cotton Blue is a stain used for microscopic observation of fungi. It stains the fungal cytoplasm and provides a light blue background, against which, walls of hyphae which remain colorless can be easily seen.

It contains 4 constituents:

i. Phenol: a fungicide
ii. Lactic acid: clearing agent
iii. Cotton blue: stains the cytoplasm

iv. Glycerine: osmolyte, provides protection, gives semi-permanent preparation

Materials :

1. A young culture of fungus e.g. *Penicillium, Aspergillus*.
2. Lactophenol cotton blue
3. Mounting needles
4. Pointed forceps
5. 70 % ehanol
6. Glass slides
7. Coverslips
8. Microscope

Procedure:

1. Place a drop of lactophenol cotton blue on a clean slide.
2. Transfer a small tuft of fungus, preferably with spores and spore bearing structures, into the drop with the help of flame sterilized forceps.
3. Gently tease the material using two needles.
4. Place a cover-glass over the preparation taking care to avoid trapping air bubbles in the stain.
5. Observe the slide under microscope

Observations:

1. Examine the preparation under low-power and high- power objectives.
2. Describe the type of hyphae, conidiophores, conidiogenous cells, conidia and their arrangement on conidiophores/ conidiogenous cells.
3. Draw a representive microscopic field under low power and high power magnification.
4. Identify the mold on the basis of characteristic features produced.

References:

1. K. R. Aneja (2009) Experiments in microbiology, Plant pathology and Biotechnology, 4th ed. New age international ltd. New Delhi.
2. J. G. Cappuccino and Natalie Sherman , Microbiology –A laboratory manual, 4th ed, Addison Wesley Longman, Inc.

Experiment No. 3

Title of the Experiment:

Elevated temperature test for the determination of fecal contamination of drinking water

Introduction:

Presently, coliforms are used as **indicator organisms** in water analysis . **Coliforms** are defined as gram negative, non-spore forming rods, facultative anaerobic, that ferment lactose with the production of acid and gas within 24 to 48 hours at 35^0 C ± 2^0 C.

At the end of the completed test in water analysis, we come to know about the presence or absence of the coliforms. Further for differentiation between fecal and non fecal coliforms the **Elevated Temperature** test is performed.

Requirements:

1. Positive test tubes from completed test.
2. Sterile Brilliant Green Lactose Bile Broth (BGLB) tubes, with inverted Durham's tube.
3. Water bath adjusted to **45.5^0C**

Method:

1. A loopful of sample from positive MPN tubes was inoculated in to BGLB medium and incubated at 45.5 ^{0}C for 24 hrs.

Observation:

The tubes were observed for turbidity and gas production, if any.

References:

A.J.Salle Fundamental Principles of Bacteriology.

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Testing the potability of drinking water in college

Aims and Objectives:-

1) Everybody in the college will be assured of being provided with good quality drinking water.
2) The undergraduate syllabus of microbiology includes topics related to water quality and water analysis in theory as well as practicals. So while participating in this activity the students will get exposure to the techniques which are commonly used in many water testing laboratories which are certified under BIS (Bureau of Indian Standards). They will gain an understanding of their responsibilities as future employees in this or similar fields. This experience will definitely add to their qualifications.

Method: Determination of the most probable number (MPN) of coliform bacteria.

Principle:

The presumptive test is specific for detection of coliform bacteria. Because coliform bacteria are capable of using lactose as a carbon source (The other enteric organisms are not), their detection is facilitated by use of lactose broth medium. In this test, known volumes of water are added to lactose broth tubes and production of acid and gas from the fermentation of lactose is a positive test for coliform bacteria. In addition to lactose, the medium also contains, bile salts, used to suppress the growth of organisms other than coliform bacteria. Instead of lactose broth medium McConkey's broth can also be used which is selective medium for coliform bacteria.

Tubes of the medium are inoculated with 10 ml, 1 ml and 0.1 ml aliquots of water sample. This test contains at least three groups or set of tubes each containing three or five tubes. Tubes are then inoculated with the designated volume of the water sample as described in procedure. Greater the number of tubes per set, the greater is sensitivity of the test. Development of turbidity and gas in any(as well as colour change from red to pink and further yellow)of the tube after 24 to 48 hrs of incubation at 35^0 c is presumptive evidence of the presence of coliform bacteria in the sample.

The presumptive test also enables the microbiologist to obtain some idea of the number of coliform organism present by means of the most probable number test (MPN). The MPN is estimated by determining the number of positive tubes after incubation and referring to the **McCrady's Table.**

Requirements:-

1. Water sample
2. Five tubes of sterile double strength McConkey's broth (2X), 10 ml each with inverted Durham's tube.
3. 10 tubes of sterile single strength McConkey's broth (1X), 5 ml each with inverted Durham's tube.
4. Sterile pipettes of 10 ml, 1 ml and 0.1 ml capacity.

Procedure:-

Each test requires total 15 tubes (in three sets), for each tube label the volume of sample inoculated. Sample inoculation is done as follows using sterile pipettes.

Set 1→ 5 tubes → 2X, 10 ml medium → sample added 10 ml each.

Set 2→ 5 tubes → 1X, 5 ml medium → sample added 1 ml each.

Set 3→ 5 tubes → 1X, 5 ml medium → sample added 0.1 ml each.

Incubate all tubes for 24 to 48 hrs at 35^0C.

References:

1) A.J.Salle Fundamental Principles of Bacteriology.

2) Indian Standards Specifications for drinking water – IS 10500: 1991

3) K. R. Aneja (2009) Experiments in microbiology, Plant pathology and Biotechnology, 4^{th} ed. New age international ltd. New Delhi.

Practical No: 1

WHITE BLOOD CELL COUNT

a. Principle. A sample of whole blood is mixed with a weak acid solution that lyses nonnucleated red blood cells. Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted.

b. Reagent. White-count diluting fluid. Either of the following diluting fluids may be used:

(1) Two percent acetic acid. Add 2 ml glacial acetic acid to a 100 ml volumetric flask. Dilute to the mark with distilled water.

(2) Onepercenthydrochloric acid. Add 1 ml hydrochloric acid to a 100 ml volumetric flask. Dilute to the mark with distilled water.

c. Procedure.

(1) Draw well-mixed capillary or venous blood exactly to the 0.5 mark in a white blood cell diluting pipet. This blood column must be free of air bubbles.

(2) Wipe the excess blood from the outside of the pipet to avoid transfer of cells to the diluting fluid. Take care not to touch the tip of the pipet with the gauze.

(3) Immediately draw diluting fluid to the "11" mark while rotating the pipet between the thumb and forefinger to mix the specimen and diluent. Hold the pipet upright to prevent air bubbles in the bulb.

(4) Mix the contents of the pipet for 3-5 minutes to ensure even distribution of cells. Expel unmixed and relatively cell-free fluid from the capillary portion of the pipet (usually 4 drops).

(5) Place the forefinger over the top (short end) of the pipet, hold the pipet at a 450 angle, and touch the pipet tip to the junction of the cover glass and the counting chamber.

(6) Allow the mixture to flow under the cover glass until the chamber is completely charged. Similarly, fill the opposite chamber of the hemacytometer.

NOTE: If the mixture overflows into the moat or air bubbles occur, clean and dry the chambers, remix the contents of the pipet, and refill both chambers.

(7) Allow the cells to settle for about 3 minutes. Under low-power magnification and reduced light, focus on the ruled area and observe for even distribution of cells.

(8) Count the white cells in the four 1 sq mm corner areas corresponding to those marked A, B, C, and D of Figure 5-1 in each of two chambers.

(9) Count all the white cells lying within the square and those touching the upper and right-hand center lines. The white cells that touch the left-hand and bottom lines are not to be counted. In each of the four areas, conduct the count as indicated by the "snake-like" line in figure 5-1. A variation of more than 10 cells between any of the four areas counted or a variation of more than 20 cells between sides of the hemacytometer indicate uneven distribution and require that the procedure be repeated.

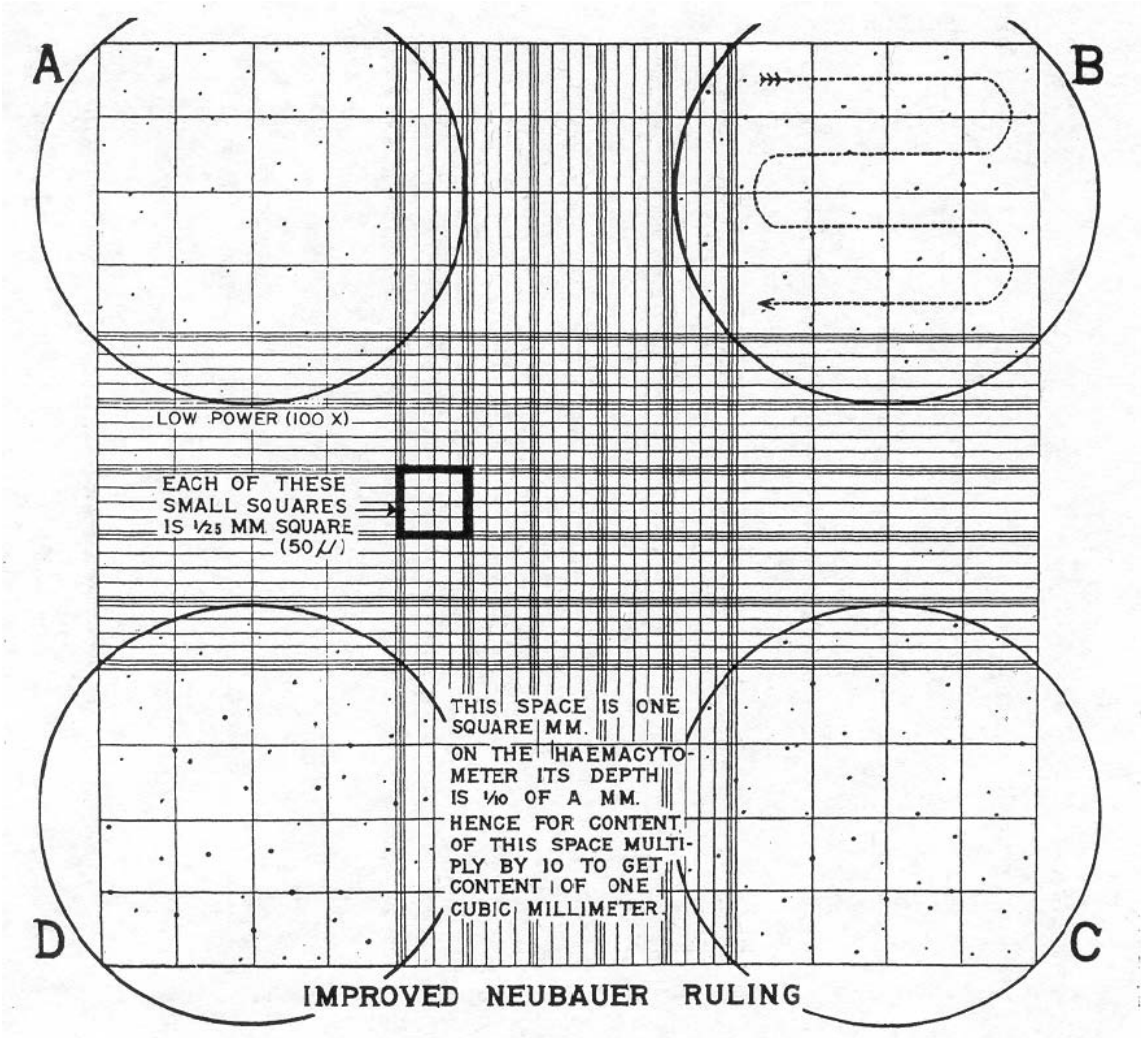

Figure 5-1.Hemacytometer counting chamber (WBCs). Areas marked A, B, C, and D are used to count white blood cells.

d. Calculation.

(1) Routinely, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Therefore, 0.5 volumes of blood are contained in 10 volumes of diluting fluid. The resulting dilution is 1:20. (These figures are arbitrary and refer strictly to dilution and not to specific volumetric measurements.)

(2) The depth of the counting chamber is 0.1 mm and the area counted is 4 sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore, 4 x 1.0 sq mm = a total of 4 sq mm). The volume counted is: area x depth = volume. Four sq mm x 0.1 mm = 0.4 cu mm.

(3) The formula is as follows:

$$\text{WBCs per cu mm} = \frac{\text{Average number of chambers (2) WBCs counted x dilution (20)}}{\text{Volume (0.4)}}$$

(4) For example:

First Chamber Cells counted in each square	Second Chamber Cells counted in each square
35	45
40	37
44	36
39	44
158 WBCs counted	162 WBCs counted

Calculate the average number of WBCs per chamber:

$$\text{Ave num WBCs} = \frac{158+162}{2} = 160$$

Calculate the number of WBCs per cubic mm:

$$\text{WBCs per cu mm} = \frac{160 \times 20}{.4} = 8000$$

e. Sources of Error.

(1) Improper collection of blood specimens causes variable results.

(2) Wet or dirty pipets.

(3) Poor condition or inaccurate calibration of pipets. Pipets must be in good condition and calibrated to have maximum error of ±1 percent.

(4) Poor pipetting technique causes high or low counts. Poor pipetting technique includes:

(a) Undershooting desired line with blood or diluting fluid.
(b) Overshooting desired line with blood or diluting fluid.
(c) Air bubbles in the column on bulb.
(d) Failure to wipe tip free of blood.
(e) Too slow manipulation following the withdrawal of the specimen thus, allowing some of the blood specimen to coagulate.
(f) Failure to mix the blood and diluent properly.

(5) Failure to expel 2 or 3 drops in the pipet tips before charging the hemacytometer.

(6) Overfilling the chamber of the hemacytometer, which causes erroneously high counts.

(7) Wet or dirty cover glasses and hemacytometers.

(8) Uneven distribution of cells in the counting chamber causes erroneous results.

(9) Inaccuracy or carelessness in marking counts.

(10) Diluent that which is cloudy or contains debris.

(11) Failure to mix anti-coagulated blood thoroughly before use.

f. Discussion.

(1) The available error when four large squares are counted is +20 percent. Counting eight large squares decreases the error to +15 percent.

(2) The importance of clean, dry diluting pipets cannot be stressed too much as the greatest source of error in the counting of WBC is the use of wet and/or dirty pipets.

(3) The counting chamber must be scrupulously clean and free of debris that might be mistaken for cells.

(4) The minimum blood sample recommended for performing routine white blood cell counts is that obtained using one pipet and counting two chambers as previously outlined.

(5) In cases where the WBC count is exceptionally high, as in leukemia, the dilution should be made in the red blood cell diluting pipette. The blood is drawn to the “1.0” mark and the diluting fluid is drawn to the “101” mark. The resulting dilution is 1:100.

(6) In cases of leukopenia, the white pipette should be filled to the “1.0” mark and diluted to the “11” mark with 2 percent acetic acid. The resulting dilution is 1:10.

(7) If nucleated erythrocytes are present, the count is corrected by the following formula:

$$\text{correct count} = \frac{\text{observed count} \times 100}{100 + \text{percent nucleated erythrocytes}}$$

The percent nucleated erythrocyte is obtained from the differential count, which is discussed in another course.

g. Normal Values.

(1) Adults (both sexes): 4,500-11,500 WBCs per cu mm.

(2) Childhood: 6,000-14,000 WBCs per cu mm.

(3) Birth: 9,000-30,000 WBCs per cu mm.

h. Unopette Procedure for White Blood Cell Count.

(1) Follow procedure described in para 2-5 for blood dilution.

(2) Prepare diluted specimen for count.

(a) Mix diluted blood by inverting reservoir to resuspend cells.

(b) Convert to dropper assembly by withdrawing pipet from reservoir and reseating securely in reverse position.

(c) To clean capillary more, invert reservoir, gently squeeze sides, and discard first three to four drops.

(d) Carefully load hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.

(e) Place hemacytometer in moisture chamber, let stand for 3 to 5 minutes (10 minutes for platelets) to allow cells to settle.

(3) Counting and calculation of leukocytes.

(a) Under 100X (low power) magnification, count leukocytes in all nine large squares of the counting chamber.

(b) Add 10 percent of count to total number of cells counted. This step simplifies the calculation that actually entails dividing the number of cells by the number of squares counted and multiplying by 10 to correct for the depth of the chamber.

(c) Multiply this figure by 1000 to get total leukocyte count.

Practical No: 2 White blood cell differential count

Definition:-

It also known as differential leucocyte count ,white blood cell morphology, it is performed to determine the relative number of each type of WBC in the blood.

Objective:-

To estimate each type of WBC in whole blood.

Methods:-

1. Manual method.
2. Electronic method.

Manual Differential WBC Count

Materials and Instruments

1) Whole blood drawn from finger.
2) Glass slides.
3) Microscope.
4) Alcohol 70%.
5) Lancet.
6) Leishman's stain.
7) Oil immersion.

Procedure:-

a) Preparing blood smear.

- Clean two slides, one to be covered with the blood film and one to be as spreader.

Prick the finger with lancet to obtain a drop of blood. Make a fine touch of one end of a slide with drop of blood.

- Place the edge of other slide on the surface of the first one at angle of 45 degree.
- Draw spreader back until contact the drop of blood.
- Push spreader slowly to the other end of the slide.
- Allow the film to dry at room temperature.

b) Staining the blood smear

- Blood smear should be stained within one to two hours.
- Drop Leishman's stain on to blood film.
- Stay for 1-2 minutes.
- Add distilled water to the stain.
- The diluted stain should act for 15-30 minutes.
- Wash it with Dis. Water.
- Shake off excess water and allow drying at room temperature.

c) Examination of the stained blood smears

- Put the slide on the microscope stage.
- Examine the blood smear using low power objective. Choose area with plenty of WBC.
- Place a drop of immersion oil on the selected area and use oil immersion objective.
- Perform the differential cell count, and examine the morphology of WBC's.

d) Calculations .Count each WBC seen and record on a dif. cell counter until 100 WBC's have been counted.

Results:-

The normal range of the different types of WBC is as follows :-

Neutrophils 62%

Eosinophils 2.3%

Basophils 0.4%

Monocytes 5.3%

Lymphocytes 30%

Discussion

The WBC differential assesses the ability of the body to respond to and fight infection. It also detects the severity of allergic reaction, parasitic and other types of infection, and drug reactions. It can also identify some types of leukaemia or lymphoma.

The WBC differential is normally run as apart of the full blood count, which is requested for many different conditions.

The results indicate the percentage of each type of WBC that is present.

Neutrophils can increase response to bacterial infection, inflammatory disease, steroid medication .

Decreased neutrophil levels may be the result of sever infection or other conditions such as responses to various medications or therapy.

Eosinophils can increase in response to allergic disorders, inflammation of the skin , and parasitic infections. They can also occur in response to some infections or to various bone marrow malignancies.

Basophils can increase in case of leukaemia, long standing inflammation, the presence of a hypersensitivity reaction to food, or radiation therapy.

Lymphocytes can increase in case of bacterial or viral infection, leukaemia, lymphoma, or radiation therapy. Decreased lymphocyte levels are common in later life but can also indicate steroid medication, stress, lupus, and HIV infection.

Monocyte can increase in certain leukaemias, in response to infection of all kinds as well as inflammatory disorders.

Decreased monocyte levels can indicate bone marrow injury or failure and some forms of leukaemia.