Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist. Raigad, 410206.

## DEPARTMENT OF ZOOLOGY

# Practical Manual

**Practical to be performed under**

## DBT STAR COLLEGE SCHEME

**(Academic Year: 2023-24)**

Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.- Raigad

## DEPARTMENT OF ZOOLOGY

**(ACADEMIC YEAR: 2023-24)**

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

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## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:**

**Roll Number**

## DETECTION OF HEALTH-HAZARDOUS ADULTERANTS FROM MILK SAMPLES

**AIM: To test adulterants (Starch and Glucose) in the given milk samples.**

### 1. DETECTION OF STARCH AS AN]9999 ADULTERANT

**Principle:** Since starch reacts with iodine to form a blue coloured complex, Lugol's iodine can be used as detecting agent.

**Requirements:** Lugol's iodine, 5% starch solution-boiled and cooled, Pure sealed packet of milk (sample A), milk adulterated with 5% starch solution (50:50 proportion) (sample B).

**Procedure:**

- Take two test tubes. In test tube A take 2 ml of sample A and in test tube B take sample B.
- To each test tube add few drops of Lugol's iodine and shake the test tube. If needed add few more drops of Lugol's iodine.

**Observation:** If the milk turns blue, it indicated that the milk is adulterated with starch which is observed in the sample 2. If the milk remains white as in sample 1, it is unadulterated.

**Result:**

_____

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### 2. DETECTION OF GLUCOSE AS AN ADULTERANT

**Background information:** Glucose is added to increase the density of milk. After water is added to milk, the density of milk decreases and lactometer reading also decreases, therefore glucose is added and the lactometer reading is increased. The quality of glucose added to milk is low. Quality control tests for milk are very important to assure adulterant

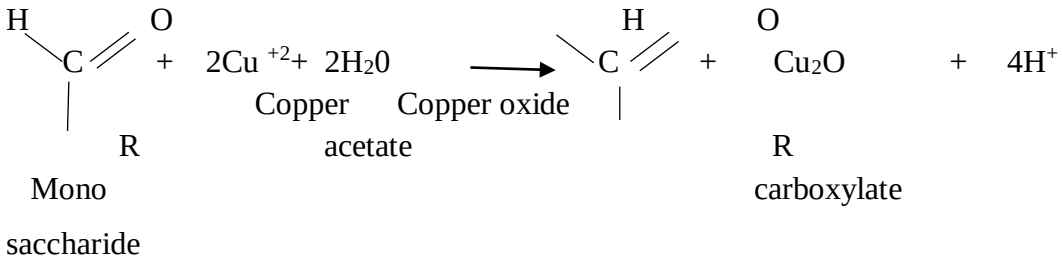
free milk for consumption. There are test available to detect milk adulterated with glucose.

## Barfoed Test:

## Principle:

Glucose is a monosaccharide. All monosaccharides are reducing sugars as they have a free reactive aldehyde group. Barfoed's reagent is a mixture of acetic (ethanoic) acid and copper acetate. Barfoed's reagent is combined with the test solution containing monosaccharide and boiled. In this reaction, glucose reduces copper acetate to copper oxide (Cu20) and forms a brick-red precipitate. Thus, Barfoed's reagent gets reduced to yellow or red cuprous oxide by glucose, depending on the concentration of sugars.

$$\underset{\text{Mono saccharide}}{RCHO} + 2Cu^{2+} + \underset{\text{copper acetate}}{2H_20} \xrightarrow{\text{Boil}} \underset{\text{carboxylate}}{RCOOH} + \underset{\text{copper oxide}}{Cu_2O} + 4H^+$$




## Procedure:

1) Take 3 ml of milk in a test tube and add 3 ml Barford's reagent,
2) Mix it thoroughly. Then keep it in a boiling water bath for 3 min and cool under running tap water.
3) Add 1 ml of phosphomolybdic acid and shake.
4) If blue colour is visible, then glucose is present in the milk sample.

## Results:

_____

_____

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**Class:** **Date:** **Time:**

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## STUDY OF NUTRITIONAL CONTENT OF FISHES AVAILABLE IN LOCAL MARKET.

**Aim: To estimate proteins contents of fishes by Folin- Lowry's method.**

**Principal:** Under alkaline condition the peptide bonds of proteins first react with cupric sulphate to produce cuprous ions ($Cu^+$) or Protin-$Cu^+$ complex. Subsequently the copper-treated protein reduces the Folin reagent (phosphomolybdotungstate) to heteropolymolybdenum blue. The colour intensity is primary due to the amino acids tyrosine and tryptophan. The optical density of the blue colour is read at 660 nm.

Alkaline condition

1) Peptide bonds of Protein +$CuSo_4$----------→ Protein- $Cu^+$ complex (involving tyrosine/tryptophan)

Protein-$Cu^+$ complex

2) Phosphomolybdotungstate ----------→Heteropolymolybdenum blue (in Folin-Ciocalteau reagent)

**Requirements:** Testtubes, test tube stand, pipettes, filter paper, colorimeter or spectrophotometer.

**Reagents:**

1. **Liver Homogenate (5%):** Homogenize 5gm liver in 100ml distilled water. Centrifuge the homogenate at 5000rpm for 10 minutes. Collect the clear supernatant and use it for estimation of proteins. (keep on ice to prevent protein degradation)
2. **Standard-Bovine Serum Albumin (BSA):** Stock solution (1mb/mL). Dilute the stock solution in ratio of 1:5 with distilled water (0.2mg/mL).
3. **Copper sulphate (Reagent A):** Prepare 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate solution.
4. **Alkaline Sodium carbonate (Reagent B):** Dissolve 2.0g of $Na_2CO_3$ in 0.1 N NaOH and make up the volume to 100ml with 0.1N NaOH.
5. **Alkaline copper sulphate (Reagent C) (prepared fresh):**Mix 1ml of Reagent A and 50 ml of Reagent B.

6. **Folin- Ciocalteau reagent:** Dilute the reagent with distilled water to 1N. (Prepared just before use)

**Procedure:**

Prepare three test tubes as shown in the observation table.

| Test tube | Distilled water (ml) | BSA std. (ml) 0.2mg | Liver homo-genate | Reagent C (ml) | Mix well and incubate at RT for 15 min | Folin-Ciocalt-eau reagent (ml) | Mix well and keep in dark for 10 min | Abs. 660 nm |
|---|---|---|---|---|---|---|---|---|
| **Blank** | 1.0 | - | - | 5.0 | | 0.5 | | |
| **Standard** | - | 1.0 | - | 5.0 | | 0.5 | | |
| **Test** | 0.5 | - | 0.5 | 5.0 | | 0.5 | | |

**Calculation:**

Amount of Protein= $\frac{\text{(Abs. Of Unknown x Conc. Of Standard)}}{\text{Abs. Of Standard}} \times \frac{1}{\text{Volume taken (0.5ml)}} \times 20$

$= \frac{\text{(Abs. Of Unknown x Con. Of Standard)}}{\text{Abs. Of Standard}} \times 40$

**Result:** The amount of protein is _____mg/gm of liver

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## STUDY OF PROKARYOTIC CELLS BY CRYSTAL VIOLET STAINING TECHNIQUES

**Aim: To study Prokaryotic Cells by Crystal Violet Techniques.**

### Background information

All living organisms are made up of cells. The cells can be classified into prokaryotic and eukaryotic based on the presence of a well defined nucleus. The prokaryotic cell is unicellular and lacks a nucleus 1 to 10 micrometers (μm). The genetic information is stored in the form of circular DNA called nucleoid which is attached to the plasma membrane. Membrane bound organelles are absent. Ribosome is present which take part in protein synthesis. Membrane is enclosed beneath cell wall. With the help of either long wave like or small hair like flagella

### Principle:-

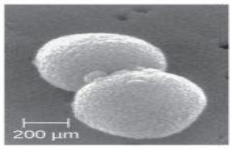
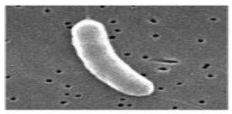
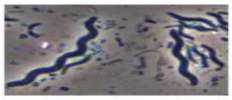
The chemical composition and structure of cell wall in gram positive and gram negative bacteria are different in gram positive bacteria cell wall has many layers of Peptidoglycan . gram negative bacteria cell wall is thin containing lipoprotein and Lipopolysaccharide. Crystal violet is a methane dye used as histological stain and in grams method of classifying bacteria crystal violet dissociates in aqueous solution into crystal violet and chloride ions. These two ions then penetrate through the cell wall and cell membrane of both gram positive and gram negative cells the crystal violet irons later interact with negatively charged bacterial components and stay in the bacterial cells purple.

Iodine acts as a mordant and as a trapping agent. A mordant is the substance that increases as the affinity of the cell wall for stain by binding to primary staining and insoluble complex which gets trapped in the cell wall at this stage all cells gram positive as well as negative will turn purple.

Alcohol or acetone dissolve the lipid outer membrane of gram negative bacteria thus leaving the peptidoglycan layer exposed and increase as the porosity of the cell wall. The crystal violet iodine complex is then washed away from the thin peptidoglycan like and layer, living gram negative bacteria colourless full stop on the other hand alcohol has a dehydrating effect on the cell walls of gram positive bacteria which causes the four of the cell wall to shrink.

The complex gets tightly bound into the multilayered highly cross link gram positive cell wall thus staining the cells purple. It will therefore stain gram negative bacteria into pink colour.

**Requirements:** Curd sample or any other bacterial culture, Crystal Violet stain, Counter stain- Saffranin, Gram Iodine, Decolorizing agent -95% ethyl alcohol, Distilled water, Microscope, Slides, Inoculating loop, Bunsen burner, Microscope with oil immersion objective, Test tubes etc

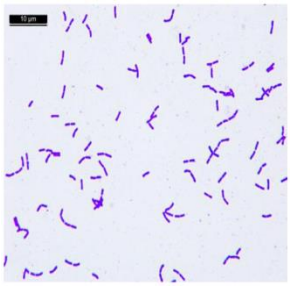
| Common Prokaryotic Cell Shapes | | | |
|---|---|---|---|
| **Name** | **Description** | **Illustration** | **Image** |
| Coccus (pl. cocci) | Round |  |   |
| Bacillus (pl. bacilli) | Rod |  |  |
| Vibrio (pl. vibrios) | Curved rod |  |  |
| Coccobacillus (pl. coccobacilli) | Short rod |  |  |
| Spirillum (pl. spirilla) | Spiral |  |  |
| Spirochete (pl. spirochetes) | Long, loose, helical spiral |  |  |

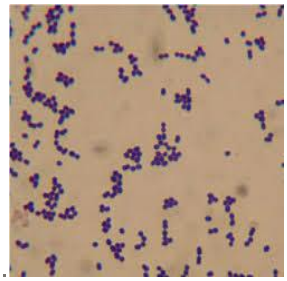
**Procedure:**

1. Take a glass slide. Mark the centre by keeping another slide in the centre perpendicular to it.
2. Take loop full of curd sample in one test tube containing 1 ml of sterilized saline (0.9 % Nacl) solution.
3. Mix it thoroughly.
4. Take a loop fully diluted sample and make a smear near on marked area of the slide.
5. Air dries the smear and flame it with Bunsen burner.
6. Put a drop of crystal violet once near and allow it to stain for 1 minute.
7. Drain off the crystal violet and put a drop of grams iodine and keep it for 5 minutes.
8. Rinse it with distilled water and then remove access stain with 95% alcohol.
9. Counter stain with saffranin for 2 to 3 minutes

**10.** Again rinse it with distilled water.
**11.** Observe it first under low power and then under oil immersion lens.

**Observation:**.





**Result:**

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## ASSESSMENT OF WATER QUALITY PARAMETERS FROM WATER RESOURCES AROUND PANVEL

**Aim: To estimate the water quality parameters from the given water sample.**

**Requirements:**

**Glassware's:** Burette, pipettes, conical flask, beakers and water samples.

**Reagents:**

- Winkler's reagent A: Dissolve 40 gm of $MnSO_4.4H_2O$ in 100ml of distilled water.
- Winkler's reagent B: Dissolve 50 gm of NaOH & 15gm of KI in 100m/ of distilled water.
- Sodium thiosulphate (0.0125 N): Dissolve 3.1 gm of sodium thiosulphate in 1000ml of distilled water. Add 0.4 gm of borax or pellets of NaOH as stabilizer. Keep in a brown glass stoppered bottle.
- Conc. $H_2S0_4$
- Starch solution (indicator): Dissolve I gm of starch in 100ml boiled distilled water. Starch Solution should be freshly prepared just before use.

**Procedure:**

1. Fill the water sample in a glass stoppered (BOD) bottle of known volume (250-300 ml) carefully, so as to avoid the formation of air bubbles. Stopper the bottle when filled up to the rim. Pour I ml of Winkler's A well below the surface and away from the walls.
2. Add I ml of Winkler's B at the bottom of the bottle with the help of pipette to prevent oxidation. Use a separate pipette for these two reagents.
3. Re-stopper the bottle and shake the contents well by inverting the bottle repeatedly. A precipitate will appear.
4. Keep the bottle standing for some time and allow the precipitate to settle down.
5. Add I -2 ml of concentrated H2SO. Shake well as mentioned above so as to dissolve the precipitate.
6. Transfer 100ml of treated sample to a conical flask for titration.
7. Titrate against sodium thiosulphate till the straw colour changes to pale yellow.
8. Then add a few drops of I % starch. A blue colour develops. Titrate further against sodium thiosulphate till blue colour changes to colourless.

## Observation Table:

1. Burette - 0.0125N $Na_2S_20_3$ solution
2. Conical flask - Treated sample water
3. Indicator - Starch (1 $^0$/0)
4. End point - Blue to colourless

| Readings | I (ml) | II (ml) | III (ml) | CBR(ml) |
|---|---|---|---|---|
| **Initial** | | | | |
| **Final** | | | | |
| **Difference** | | | | |

**Calculation:**

1 meq (milli equivalent) of $S_20_3$ corresponds to 8 mg of $O_2$

$$\text{Dissolved Oxygen (DO) content water sample in mg/L} = \frac{\text{Burette reading (BR) x Normality of } Na_2S_20_3 \text{ x 8 x 1000}}{100}$$

**Result:**

The oxygen content of the given water sample is =---------- mg/ml

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## DETECTION OF ABNORMAL CONSTITUTIONS OF URINE

**Aim: To detect the abnormal constituents of urine**

**Principal:** Normally urine consists of water and some impurities like urea, uric acid, ions and creatinine. However, some substances are there which are not present in easily noticeable amounts in urine of healthy individuals but are found in the urine of individuals suffering from certain disease. These are pathological or abnormal constituents of urine. This variable test will confirm the presence or absence of abnormal constituents of urine.

**Requirements:**

**Reagents:**

- Urine sample
- Benedict's reagent
- Sulphosalicyclic acid, Nitric acid, Ammonium sulphate, Sodium nitroprusside, Ammonium hydroxide

**Apparatus:** Test tubes, Beakers, Droppers

**Procedure: Glucose (Benedict's test)**

Take 5 ml of Benedict's reagent and to it add urine 0.5 ml and then boil it. Then based on following observation you can know about the presence of glucose.

- Blue colour is formed-No glucose
- A precipitate that is light green-0.1%-0.5% of glucose present.
- A precipitate that is green in colour is formed-0.5-1% sugar present.
- A precipitate that is yellow in colour-1-2% of glucose is present.
- Brick red precipitate-2% glucose present.

**Observation:**

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**Conclusion:**

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**Albumin:-**

**Sulphosalicyclic acid test**

- Take about 2 ml of urine sample and to it add one or two drops of sulphosalicyclic acid.
- If turbidity is formed then it indicates the presence of albumin.

**Observation:**

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**Conclusion:**

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**Heat coagulation test**

- Take a test tube and add urine sample to it so that test tube is filled up to 3/4th part of the test tube.
- To the upper part of the test tube which is filled with 1/3th part of urine sample, show it in flame or heat it.
- If turbidity appears in the place of heat then it is albumin positive.

**Observation:**

_____

_____

**Conclusion:**

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**Heller's nitric acid test**

- Take a test tube and to it add 3 ml of nitric acid.
- Then add 3 ml of urine sample in such a way that they will not mix.

- If a ring is formed in the junction area or the place where two liquids meet, then it is albumin positive.

**Observation:**

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**Conclusion:**

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**Ketone bodies**

**Rothera's test**

- Take 5 ml of urine sample and saturate it by mixing with ammonium sulphate and shaking it vigorously.
- Then take 2 drops of 5% solution of sodium nitroprusside and ammonium hydroxide 1 ml. Leave it in rack for few minutes.
- If a permanganate colour is formed just in the upper part of unmixed ammonium crystals, then it proves the presence of ketone bodies.

**Observation:**

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_____

**Conclusion:**

_____

_____

**Results:-**

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**Class:** **Date:** **Time:**

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## EVALUATION OF NUTRIENT CONTENTS OF TABLE EGGS FROM DIFFERENT SOURCES IN THE RETAIL MARKET

**Aim: To estimate the amount of protein in two different varieties of egg by Biuret Method.**

**Background information:**

Proteins are powerful macronutrients. They play a major role in the wear and tear of tissues in the body. Proteins are polymers of amino acids linked by peptide bonds. There are 20 amino acids, one additional being added to the list. Albumin is globular protein present in the egg.

**Principle:** In the Biuret test alkaline CuS04 reacts with protein containing two or more peptide bonds to give a violet-coloured complex called Biuret complex. The intensity of colour is directly proportional to the peptide bonds present in the protein sample.

**Requirements:**

1) Biuret reagent: Dissolve 3gm ofCuS04 and 9 gm of Na-K tartarate in 500ml of 0.2NNaOH add 5gm of potassium iodide and make up the volume to one lit with 0.2N NaOH

2) Standard protein solution (Bovine serum albumin (BSA) 5mg/ml)

3) Test samples:

a) Sample 1(E1) - Egg white of Farm egg (English) — such as white leghorn

b) Sample 2 (E2) - Egg white of country egg (Desi)

4) Egg white solution made in distilled water (l : 50 dilution).

5) Test tubes

6) Distilled water

**Procedure:**

l) Take four test tubes. Label them as blank (B), standard (S) and test sample I (T 1) and test sample 2 (T2).

2) In test tube labelled as 'B' take .0 1 distilled water.
3) In test tube labelled as 'S' take 0.2 ml of BSA solution + 1.8 ml Distilled water.
4) In test tube labelled 'Tl ' take 1.0 ml egg sample- E1 and in 'T2' take 1.0ml of egg sample – E2
5) To both tubes add I .0 ml Distilled water.
6) Add 3.0 ml of biuret reagent to each of the tubes and mix well.
7) Allow the tubes to stand at room temperature for 15 minutes and read the OD against the read blank at 540 mn.

**Observation table:**

| **Test Tubes** | **Distilled water (ml)** | **BSA (ml)** | **Sample (ml)** | **Biuret reagent (ml)** | **Incubate at room temp. (15 min)** | **OD (540)** |
|---|---|---|---|---|---|---|
| **Blank** | 2.0 | - | - | 3.0 | | |
| **Standard** | 1.8 | 0.2 | - | 3.0 | | |
| **T1** | 1.0 | - | 1.0 | 3.0 | | |
| **T2** | 1.0 | - | 1.0 | 3.0 | | |

**Calculations:**

1) Sample-I

Amount of protein (mg/ml) = $\frac{\text{OD of unknown x 0.2 (Conc. Of std) x 50(dilution factor)}}{\text{OD of standard x 0.2 (Volume of unknown taken}}$

2) Sample -2

Amount of protein (mg/ml) = $\frac{\text{OD of unknown x 0.2 (Conc. of std) x 50(dilution factor)}}{\text{OD of standard x 0.2 (Volume of unknown taken)}}$

**Result**: The amount of protein in Sample 1 is……..mg/ml and sample 2 is……mg/ml.

Therefore protein content in---------variety is more than……variety of egg.

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## Detection of soil fertility and texture by analysis of physical, chemical and Microbiological characteristics of soil.

**Aim: To determine moisture content of soil sample using gravimetric method.**

**Principle:** Gravimetric water content is the mass of water per mass of dry soil. It is measured by weighing a field moist soil (wet weight) and then either drying that soil (over a period of a week) or oven drying it for about 24 hours at a temperature of 105° C to remove all water. The soil should then be weighed again (to determine the dry weight). The deference between the wet and the dry weight is the amount of water in that soil.

**Apparatus:**

a. Drying oven
b. Analytical balance with 0.001 g accuracy
c. Desiccators
d. Aluminium cups or evaporating dishes

**Procedure:**

(1) Dry an aluminium cup (or evaporating dish) at 105 $^{o}$C for 2–3 hours in the drying oven; measure the constant weight (A gram).
(2) Put about 10 g of air-dried soil into the cup; weigh the cup with the soil in it (B gram).
(3) Dry the soil in the cup at 105$^{o}$C for 24 hours.
(4) Take out the cup from the drying oven, cool it in a desiccators, and weigh it (C gram).

**Calculation:**

(1) Soil moisture content of air-dried soil (%) = $[(B - C)/(B - A)] \times 100$

(2) Soil moisture correction factor (MCF) = $(B - A)/(C - A)$

The MCF is used to correct analytical results on air-dried soil to the dry weight basis

**Results:** Moisture content of the given soil sample ……..

**Aims: To determine the pH of soil samples using pH meter.**

**Principle:** Soil pH is a measure of the amount of acidity or alkalinity (basicity) that is present in the soil. Soil pH is a very import soil property due to its ability to determine the availability of nutrients for plant uptake. Different soil nutrients are available for uptake by plants at different soil pH levels. The Soil pH level that allows for a wider nutrient availability to crops is in the 5.5 to 7.5 range.

pH meter method uses an electrometric device, which consists of a glass electrode that insensitive to hydrogen ions (H+) – which are used to measure the level of acidity. The glass electrode contains a salt solution (sodium based – Na+) and when this glass electrode is immersed into the soil solution, there is an exchange of ions between the soil solution (H+) and the glass (Na+). A reference electrode that produces a constant voltage is also required, which when immersed with the glass electrode into the soil solution produces an electromotive force (voltage) or electrical potential that is measured by a mill voltmeter.

This estimates the soil's pH.

**Apparatus**

a. pH meter with glass electrode

b. Analytical balance

c. Plastics bottles (wide-mouth)

**Reagent:**

1) pH buffer solutions: acid (pH ≈ 4), neutral (pH ≈ 7) and alkaline (pH ≈ 9)

**Procedure:**

(1) Weigh 10 g of air-dried soil (accuracy 0.1 g) and put the soil in a 100 mL bottle.

(2) Add 25 mL of distilled water and cap the bottle.

(3) Occasionally shake the bottle for 1 hour.

(4) Before opening the bottle for measurement, shake it once again.

(5) Immerse the glass electrode of the pH meter in the soil suspension.

(6) Record pH when the reading becomes stable.

**Results:** pH of the given soil sample …….

**Aim: Enumeration of Soil Microorganism by spread plate method**

**Background Information:** Soil contains uncountable variety of microorganisms which are responsible for most of the nutrient release from organic matter. When microorganisms decompose organic matter, they use the carbon and nutrients in the organic matter for their own growth. They release excess nutrients into the soil where they can be taken up by plants. For e.g. Bacterial species of genus *Pseudomonas*, *Bacillus*, and *Mycobacterium* solubilise bound phosphorus and make it available to the plants. In the soil, organic sulphur is bound as hydrogen sulphide (H2S). Bacterial species of the genus *Thiobacillus* and *Beggiatoa* oxide H2S to elemental sulphur (S). Elemental sulphur aggregates as crystals inside the phototropic species of bacterium *Chromatium*. Other bacterial species of *Chlorobium*and *Ectothiorhodospira*also oxidize hydrogen sulphide but release elemental sulphur into the soil. Organically bound iron is attacked by bacteria of genus *Thiobacillus, Pseudomonas, Bacillus, Klebsiella, Streptomycetes*, and some filamentous fungal species.

Fertility of soil is directly related to the number of microorganisms in the soil; therefore, it is a "microbial number game". If the number in the soil is inadequate, microbes should be added along with the liquid fertilizers.

Isolation of soil microorganisms can be done by using serial dilution spread plate and pour plate method.

## Serial dilution method

### Principal

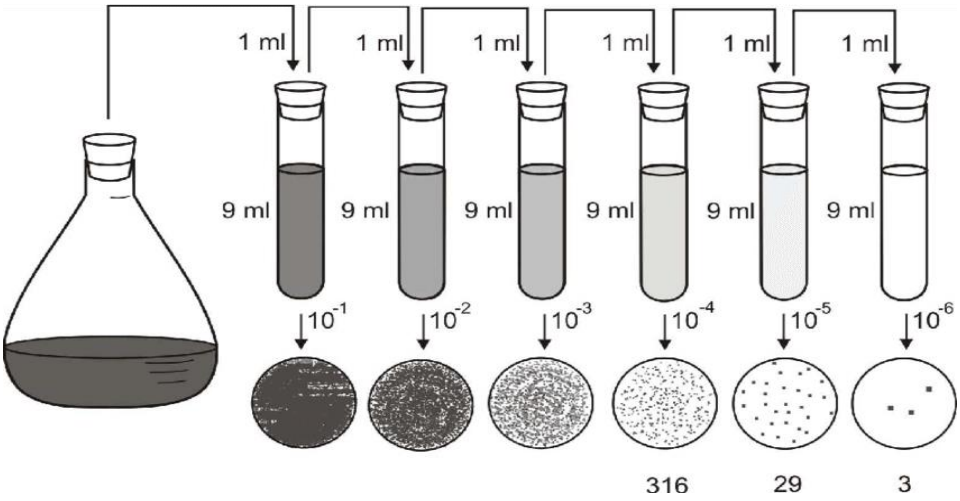
Serial dilution, as the name suggests, is a series of sequential dilutions that are performed to convert a dense solution into a more usable concentration. The density/number of cells is reduced at each step so that it is easier to calculate the concentration of microorganisms in the original sample by calculating the total dilution over the entire series.

It is used in microbiology to estimate the number of cells/organisms (bacteria, viruses and fungus) in a unknown sample using pour plate or spread plate technique. Dilution of sample systematically helps to obtained an easily countable number of colonies (around 30–100).

### Procedure for a ten-fold serial dilution

1. 1gm Solid sample or 1 ml Liquid sample/culture is taken in a test tube. If sample is solid then mix sample vigorously in 1 ml diluents in sterile test tube.
2. In sterile six test tubes, each with 9 ml of sterile diluents (D/W or 0.9% saline) is taken using sterile pipette.
3. 1 ml of properly mixed sample/culture is drawn into the sterile pipette.
4. The sample is then added to the first tube to make the total volume of 10 ml. This provides an initial dilution of $10^{-1}$.
5. The dilution is thoroughly mixed by shaking the test tube
6. Now, 1 ml of mixture is taken from the $10^{-1}$ dilution and is emptied into the second tube. The second tube now has a total dilution factor of $10^{-2}$.
7. The same process is then repeated for the remaining tube, taking 1 ml from the previous tube and adding it to the next 9 ml diluents.

8. At the end, 1 ml of last dilution should be discarded to make volume same in all the dilution tubes.
9. As six tubes are used, the final dilution for the bacteria/cells will be $10^{-6}$ (1 in 1,000,000).
10. The selection of dilution for plating in pour plate or spread plate is totally depending upon the sample microbial load




## Spread Plate Method

### Principal:

In this technique, a serially diluted specimen is spread over the solidified agar media plates with the help of a sterile L-shape glass rod (Spreader).The principle behind this method is that because of evenly spreading the culture, cells will be deposited onto the surface of the Agar media at a distance sufficient to allow the colonies that develop to be free from each other.

- The Spread Plate Technique, in conjunction with serial dilutions, is a valuable research tool.
- It is used to demonstrate the cultural characteristics of the bacteria (e.g. colour, texture, size, elevation, etc.) and also use to estimate the viable counts of the bacteria in the specimen.

### Procedure:

1. Make a dilution series from a sample.
2. Pipette out 0.1 ml from the appropriate desired dilution series onto the centre of the surface of an medium agar plate.
3. Dip the L-shaped glass spreader into alcohol.
4. Flame the glass spreader (hockey stick) over a Bunsen burner.
5. Allow to cool the spreader
6. Spread the sample evenly over the surface of agar, carefully rotating the Petri dish underneath at the same time.
7. Incubate the plate at 37°C for 24 hours
8. Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.

**Calculation:**

Colony-forming unit (CFU or cfu) is a measure of viable bacterial. The results are given as CFU/mL (colony-forming units per millilitre) for liquids and CFU/g (colony-forming units per gram) for solids.

Calculate the number of bacteria (CFU) per millilitre of sample by dividing the number of colonies by the dilution factor.

**The CFU/ml can be calculated using the formula:**

**cfu/ml = (no. of colonies x dilution factor) / volume of culture plate.**

For example, suppose the plate of the $10^{-6}$ dilution yielded a count of 130 colonies. Then, the number of bacteria in 1 ml of the original sample can be calculated as follows:

Bacteria/ml = (130) x ($10^{-6}$) /1

= $1.3 \times 10^{-8}$ or 130,000,000 CFU/ml

**Results:** Bacteria/ml =

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Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.- Raigad

## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:**

**Roll Number:**

## DETERMINATION OF BLOOD SUGAR BY GOD-POD METHOD

**Aim: To estimate plasma glucose by GOD-POD method**

GOD-POD methods are used to determine the blood glucose level in a specific serum/plasma.

**Background Information:**

Glucose is present in large amounts in the blood. Glucose is oxidized in the cell and is the body's primary source of energy. The estimation of glucose gives useful information regarding diabetes mellitus treatment management. Diabetes mellitus, hyperglycemia, hyperparathyroidism, renal failure, extreme stress, and cerebrovascular accidents all cause an increase in glucose concentration. Hypoglycemia, hypothyroidism, hypopituitarism, liver disease, and carbohydrate intolerance all have low glucose levels.

**Principle**: Glucose is oxidised to gluconic acid and hydrogen peroxide in the presence of glucose oxidase (GOD). In the presence of the enzyme peroxidase (POD), hydrogen peroxide combines with phenol and 4-aminoantipyrine to generate a pink-color edquinoneimine dye complex, the intensity of which is proportional to the amount of glucose in the sample. At 505nm, the absorbance of coloured dye is measured (490-550 nm)

**Reaction:**

$$\text{Glucose} + 02 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_20_2 + \text{phenol} + \text{4- aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_20$$

**Requirements:** Sample- serum/ plasma, clean dry glassware, micropipettes or glass pipettes, colorimeter

**Reagents:**

**Reagents 1:** Working glucose reagent: Add 20 mg of 4-amino phenazone and 30 mg of sodium azide in 100 ml of phosphate buffer PI 17.0, and add 6.5 ml of glucose oxidase, and 5 ml of peroxidase into it. Mix all the contents.

**Reagents 2.**

Glucose diluent: Mix 100 mg of phenol reagent in 100 ml D/W or use commercially available.

**Reagents 3.**
Glucose standard 100mg%: Dissolve 100 mg of glucose in 100 ml D/W.

**Working reagent preparation:**
**For kit method:** Prepare a working reagent by adding reagent 2 (Glucose diluent) to the reagent I vial as given in the kit. Dissolve the powder completely.
**For laboratory preparation:**
2 part of buffered enzyme (reagent I) and of phenol (reagent2) to get working reagent.
**Reagent stability:** The working reagent is light sensitive. It should be stored a 2-8$^0$ C in dark brown/ amber colour bottle provided, to protect it from light.

**Procedures:**
1.Prepare the three test tubes: Blank (B), Standard (S) and Test (T) as given in the observation table below.
2. Mix the contents well and incubate at 37$^o$c for 10 mins. Take the O.D. against blank at 530nm.

**Observation table:**

| Reagents | Blank (B) (ml) | Standard (S) (ml) | Test (T) (ml) |
|---|---|---|---|
| Serum/ Plasma | - | - | 0.01 |
| Glucose standard | - | 0.01 | - |
| Distilled water | 0.01 | - | - |
| Working glucose reagent | 1.0 | 1.0 | 1.0 |

**Calculations:**

Serum/plasma glucose in mg/ 100ml = $\frac{\text{O.D of test X Conc. of standard}}{\text{O.D of standard}}$

where concentration of standard is 100mg. = ---------- mg/100ml

**Result**: The glucose content in the given sample (Plasma/serum) = ----------mg/ 100ml

**Conclusion:**

The concentration of glucose in the given blood sample lies within / out of the normal range _____

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## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:**

**Roll Number:**

## SEPARATION OF PLASMA PROTEINS BY GEL ELECTROPHORESIS

**Aim**: **To separate plasma proteins of serum by Polyacrylamide Gel Electrophoresis (PAGE)**

**Requirement for Polyacrylamide Gel Electrophoresis:**

- Acrylamide solutions (for resolving & stacking gels).
- Isopropanol / distilled water.
- Gel loading buffer.
- Running buffer.
- Staining, destaining solutions.
- Protein samples
- Molecular weight markers.

The equipment and supplies necessary for conducting SDS-PAGE includes:

- An electrophoresis chamber and power supply.
- Glass plates (a short and a top plate).
- Casting frame
- Casting stand
- Combs

Stock Solution:

1. Monolayer solution (acrylamide, bis acrylamide)
2. 4X Running Gel Buffer - Tris Buffer
3. 4X Stacking Gel Buffer- Tris buffer
4. Initiator – Ammonium per sulphate
5. 6X Gel loading buffer (Glycerol, Bromophenol blue )
6. 25X Tank Buffer (Tris , Glycine)
7. Staining solution – (Coomassie blue R-250, methanol, acetic acid)
8. Distaining solution- (Methanol , acetic acid)

**Procedure:**

**Setting up the Unit:**

1) You are provided with an electrophoretic unit consisting of a lower tank, an upper tank- bearing one attached glass plate (with a notch), and one detachable glass plate.
2) On a suitable support, place the upper tank unit horizontally (flat), with the notch plate facing upwards.
3) Place one spacer each, on the extreme left and right side of the notch plate.
4) Place one rubber tube against each spacer.

5) Place one more spacer each in alignment with left and right tubes, such that, you now have one rubber tube on each side of the glass plate, and each rubber tube is flanked by two spacers.
6) Position the square glass plate on top of the notch glass plate.
7) Clamp the glass plates by using two clamps on either side.
8) Align the glass plates properly by raising it from the horizontal position and placing it vertically on a flat surface.
9) Press both the glass plates downwards, from upper end, and clamp the unit tightly.
10) Pour 2% molten agar in the front trough of lower tank, and place the upper unit in the flooded trough, vertically (approximately20 ml).
11) Allow the agar to solidify for two minutes.
12) The unit is now set and ready for addition of the gel solution.

**Preparation of the separating gel:**

13) Mix the various components of the separating gel solution, as indicated in the table, adding TEMED at the end, and swirling the contents gently.
14) Pour the above solution into the sandwich of two glass plates to a height of about 10 cms.
15) Apply a water overlay by adding a few drops on the surface of the gel solution.
16) Allow the gel to polymerise for 30 minutes. A line of demarcation between the gel and the water layer indicates completion of polymerisation.
17) Lift the entire assembly and turn it upside down to remove water. Rinse the gel surface with a few millilitres of distilled water and discard the water.

**Preparation of the stacking gel:**

18) Mix the various components of the stacking gel solution, as indicated in the table, adding TEMED at the end, and swirling the contents gently.
19) Pour the above solution into the glass sandwich up to the rim of the notch plate.
20) Insert the comb into the stacking gel to a desired depth, and allow the gel to polymerise for 30 minutes.(keep overnight)
21) Fill the upper tank and the lower tank with IX tank buffer (32ml of 25X dilute to 800ml), making sure that the steel clamps are well above the level of buffer in the lower tank.
22) Remove the comb gently. Now your unit is ready for sample loading.

**Sample loading in Electrophoresis:**

23) Prepare the protein samples as follows:

Take 1 ml of whole blood in an Eppendorf tube and centrifuge at 10,000 rpm for 5 minutes. Collect the supernatant. This is serum sample. To the pellet add 1 ml of distilled water. Mix and centrifuge at 10,000 rpm for 5 minutes. Collect the supernatant. This haemolysed sample. Take 100 ul of sample and mix it with 100 ul of 6X gel loading buffer. Load 10-50 ul of this in the wells, depending upon the concentration and sensitivity of the detection method.

24) Start the electrophoresis, first by applying 50 V for 5 minutes, and then increasing it to 100 V or 150 V.
25) Switch off the current as soon as the tracking dye reaches bottom.

## Staining, destaining and preservation:

26) Dismantle the unit by removing the electrical cords and clamps. Remove the extreme left and right spacers. Insert a spatula in the lower right corner and lift the upper plate by turning the spatula clockwise-gently.
27) The gel will stick to the lower or upper plate. Lift the gel by holding its lower ends and put it in a tray containing staining solutions for 1 to 6 hours.
28) Destain the gel by soaking it in a destaining solution. Change the destaining solution 2-3 times at intervals of 2-3 hours till the background becomes near courless. Use 50-100ml of 1X destainer at a time.
29) Preserve the destained gel by sandwiching it between two cellophane papers which have been pre-soaked in the destainer. Avoid creases and air bubbles in the cellophane. This is best done on a glass plate, folding the loose cellophane edges under the glass plate. Keep the gel for drying at 37 deg. Celsius, overnight.
30) The gel fuses with the cellophane, which can be detached from the glass plate and cut to the proper size.

**Signature of Student** **Signature of Teacher**

Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.- Raigad

## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**
**Name of Student:**
**Roll Number:**

## ENUMERATION OF TOTAL COUNT OF R.B.C. W.B.C & PLATELETS

**Aim: To enumerate the total R.B.C count from blood smear.**

**Principle:** The blood is diluted to facilitate the counting as the density of RBCs in the blood is high. The dilution is made in a ratio of 1:200 using an isotonic saline solution. Counting of RBCs is done in the Neubauer's counting chamber or haemocytometer. The haemocytometer consists of 9 counting squares with each square having an area of $1mm^2$. RBCs are counted in one central square area (1 mm x 1 mm) which is again divided into 25 smallest squares (0.20 mm x 0.20 mm). The RBCs are counted in the four small corner squares and one small central square.

**Requirements:** Haemocytometer, Cover clip, RBS diluting Pipette, Sterile lancet, Absorbent cotton, Blotting paper, Compound microscope.

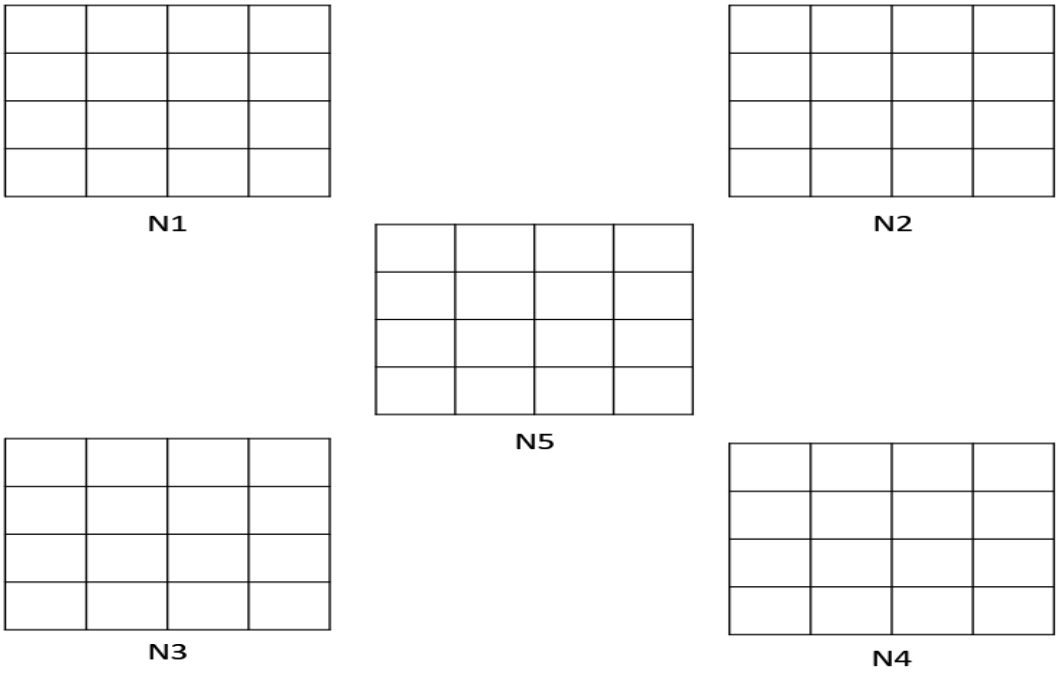
**Chemicals and Reagents:** Haymen's fluid / Gower's fluid (0.8% NaCL), 70% alcohol

**Procedure for RBC count:**

- Fill the RBC pipette up to the 0.5 mark with the blood specimen and wipe out the pipette externally to avoid false high results.
- Fill the same pipette with the RBC diluting fluid (preferably Hayem's Fluid) up to the mark 101.
- Be cautious that there should be no air bubble in the pipette bulb.
- Mix the Blood and Diluting fluid in the pipette by rotating the pipette (horizontally) between your palms.
- Take out the Neubauer's chamber / Haemocytometer from its case and clean it using a swab or gauze
- Now, put the RBC pipette, mix the solution present in it again and then discard 1-2 drops from the pipette before charging the chamber.
- Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.
- Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

- Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.
- After charging, wait for 3-5 min so that the cells settle down in the chamber & then focus the chamber under the microscope to calculate Red Cells.
- Observe under the high power of the compound microscope and count the cells in the central large RBC square

**RBC counting Squares**




**Calculations:**

Each smallest square has a side of 1/20 mm.

Therefore, the area of one smallest square is 1/20 x 1/20 = 1/400 sq. mm.

The depth of the counting chamber is 1/10 mm.

Therefore, volume of each smallest square = 1/400x 1/10 = 1/4000 cu. mm.

Therefore, volume of 80 smallest squares = 1/4000 x 80 = 1/50 cu.mm.

Number of RBCs in 5 groups of 80 smallest squares is "N" = $[N_1 + N_2 + N_3 + N_4 + N_5]$

Therefore, the number of RBCs in 1 cu. mm of blood = $\frac{\text{N x Dilution factor}}{1/50}$

$= \frac{\text{N x 200}}{1/50} =$ N x 200 x 50

= N X 10,000

= _____

**Normal Values:**

- Men: 4.5 – 6.0 x $10^6$ (million) cell/$mm^3$
- Women: 4.0 – 5.5 x $10^6$ (million) cell/$mm^3$
- Infant: 5.0 – 6.3 x $10^6$ (million) cell/$mm^3$

**Result:** the total number of RBCs in the blood is _____ cells/$mm^3$

**Precautions:**

- Wear gloves all times during the experiment.
- Disinfect the pricked region on the finger.
- Pipette and Neubauer chamber should be clean and dry.
- Avoid bubbles while pipetting and loading.

**Aim: To enumerate the total W.B.C. count from blood smear.**

**Principle:** Blood is diluted with WBC diluting fluid (Turk's fluid) which lyses the red blood cells and mildly stains the WBC, which can then be counted. Turk's fluid functions as follows- Glacial acetic acid present in the Turk's fluid haemolyzes RBCs and acts as a fixative while crystal/gentian violet stains the nuclei of the WBCs.

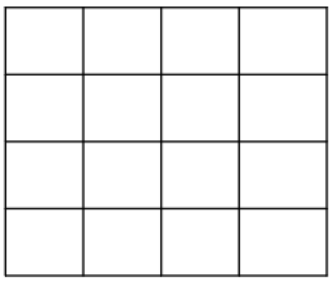
**Requirements:** WBC diluting pipette, Haemocytometer, Cover slip, Sterile lancets, Absorbent cotton, blotting paper.

**Chemicals & Reagents:** 70% alcohol, Turk's fluid (8 ml glacial acetic acid, 1 ml of 1% gentian violet and make the volume up to 100 ml with distilled water).

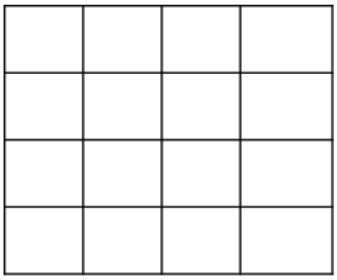
**Procedure:**

1. Sterilize the tip of finger with alcohol and allow it to air dry.
2. Gently squeeze near the tip of the finger and prick with lancet.
3. Gently draw blood in the WBC diluting pipette up to 0.5 mark.
4. Wipe the excess blood from the tip of the pipette with blotting paper.
5. Draw Turk's fluid in to the pipette up to 11 mark (i.e., 20 times dilution).
6. Rotate the pipette in between the palms of the hands to mix blood and diluting fluid.
7. Place the cover slip on haemocytometer and keep it ready for charging.
8. Discard first few (3-4) drops from the pipette (to remove the fluid in the stem not mixed with blood).
9. Load the haemocytometer by holding the WBC pipette at an angle such that the diluted WBCs are spread between the coverslip and the moat.
10. Allow the WBCs to spread and wait for 2-3 minutes to settle down.
11. Count the WBCs in the 4 corner large squares.

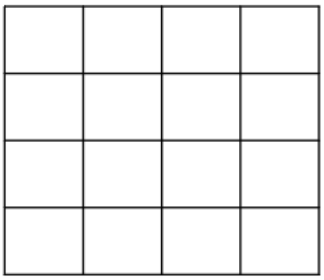
**WBC counting Squares:**



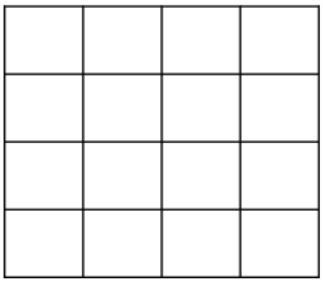
N1



N2



N3



N4

**Calculations:**

Number of WBCs/cu mm (µl) of the whole blood = $\frac{\text{Number of white cells counted x Dilution}}{\text{Area counted x Depth of fluid}}$

Where Dilution = 20

Area counted = 4 x 1 sq.mm = 4 sq.mm

Dept of fluid = 0.1 mm (constant)

Hence number of white cells per cu.mm (µl) of whole blood

= Dilution factor X $\frac{1}{\text{Volume}}$ X Number of cells counted (N)

= 20 x N

4 x 0.1

= 50 x N/cu.mm

= _____.

**Normal Values:**

Adults: -4000-10000 cell/$mm^3$

**Result:** the total number of WBCs in the blood is _____ cells/$mm^3$

**Signature of Student** **Signature of Teacher**

Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.- Raigad

## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:** **Roll Number:**

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## ESTIMATION OF PROTEIN BY LOWRY METHOD

**Aim: To estimate the amount of protein from given plasma sample**

**Reagents Required:**

1. Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.

2. Reagent B: 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate. Prepare fresh by mixing stock solutions.

3. Alkaline copper solution (Reagent C): Mix 50mL of reagent A and 1 mL of reagent B prior to use.

4. Diluted Folin's reagent (Reagent D): Dilute Folin-Ciocalteau reagent with an equal volume of 0.1 N NaOH

5. Standard: Dissolve 50mg BSA in 50mL of distilled water in a volumetric flask. Take 10mL of this stock standard and dilute to 50 mL in another flask for a working standard solution. One ml of this solution contains 200 µg of protein.

**Apparatus and Glasswares required**: Test tubes, Pipettes, Colorimeter, etc.,

**Procedure:**

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labelled test tubes.

2. Pipette out 1 mL of the sample in another test tube.

3. Make up the volume to 1 mL in all the test tubes. A tube with 1 mL of distilled water serves as the blank.

4. Now add 5 mL of reagent C to all the test tubes including the test tubes labeled 'blank' and 'unknown'.

5. Mix the contents of the tubes by vortexing / shaking the tubes and allow to stand for 10 min.

6. Then add 0.5 mL of reagent D rapidly with immediate mixing well and incubate at room temperature in the dark for 30 min.

7. Now record the absorbance at 660 nm against blank.

8. Then plot the standard curve by taking the concentration of protein along X-axis and absorbance at 660 nm along Y-axis.

9. Then from this standard curve calculate the concentration of protein in the given sample.

**Observation table:**

| Sr. No. | BSA (µg/mL) | Stock BSA (mL) | Distilled Water (mL) | Analytical Reagent (mL) | Incubate for 10 minutes at Room Temp. | Folin's Reagent (mL) | Incubate for 30 minutes at Room Temp. | (OD at 720 nm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | Blank | 1.0 | | | | | |
| 2 | 20 | 0.2 | 0.8 | | | | | |
| 3 | 40 | 0.4 | 0.4 | | | | | |
| 4 | 60 | 0.6 | 0.2 | | | | | |
| 5 | 80 | 0.8 | | | | | | |
| 6 | 100 | 1.0 | | | | | | |
| 7 | To be estimated | 1.0 | | | | | | |

**Result:** The given unknown sample contains ----------µg protein/ml.

**Signature of Student** **Signature of Teacher**

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## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

## EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:**

**Roll Number:**

## EXTRACTION OF DNA AND RNA FROM VARIOUS SAMPLES.

**Aim: Estimation of DNA by Diphenylamine reagent.**

**Principle**: Since the sugar moiety of DNA consists exclusively of 2-deoxyribose in all of its nucleotides, a colour reaction with this sugar would serve for the determination of DNA. Free as well as bound deoxyribose sugar of the DNA reacts with diphenylamine in a mixture of glacial acetic acid and sulfuric acid at 100° C. in the solution at low pH, the straight chain form of deoxypentose is converted to the highly reactive β – hydroxylevulin-aldehyde that reacts with diphenylamine giving blue colour which is stable and measured at 600 nm against a blank. (The sharp absorption maxima is at 595 nm).

**Reagents:**

1. 10% and 5% Trichloro Acetic Acid (TCA)
2. Acetaldehyde: 16mg/ml
3. Diphenylamine: Dissolve 105 gm of diphenylamine in 100 ml acetic acid.
4. Add 1.5 ml of conc. $H_2SO_4$. Just half an hour before use, mix 0.1 ml of acetaldehyde with 20 ml diphenylamine reagent.
5. DNA standard: 0.4 mg/ml in 5 mM NaOH. Store in freezer.

**Procedure:**

1. Homogenize 1 gm liver in 10 ml ice cold distilled water and prepare homogenate.
2. Take 1 ml of the homogenate and add 1 ml of 10% TCA
3. Mix the suspension and centrifuge at 2500 rpm for 10 min.
4. Discard the supernatant and wash three times in 2 ml of 5% TCA and each time discard the supernatant after centrifugation.
5. Add 2 ml of 5% TCA to the precipitate and mix well.
6. Heat the suspension for 20 min at 90° C. Cool the tube and centrifuge for 10 min at 2500 rpm.
7. For estimation of DNA, take 0.2 ml of supernatant add 0.8 ml of 5% TCA and 2 ml of diphenylamine reagent. Mix well and heat at 70° C for 15 min. Cool the tube and measure the optical density at 600 nm.

8. Preparation of tube for DNA Standard: Take 0.2 ml of (0.4 mg/ml DNA std. solution), add 0.8 ml of 5% TCA and 2ml of diphenylamine reagent. Mix well and heat at 70$^{o}$ C for 15 min. Cool the tube and measure the optical density at 600 nm.
9. Preparation of tube as Blank: Take 1 ml of 5% TCA and 2ml of diphenylamine reagent. Mix well and heat at 70$^{o}$ C for 15 min. Cool the tube and measure the optical density at 600 nm.

**Tabulation**:

| TUBE | Supernatant for estimation | DNA standard solution (0.4mg/ml) | 5% TCA | Diphenylamine reagent | O.D at 600nm |
|---|---|---|---|---|---|
| Blank | ----- | ----- | 1 ml | 2 ml | 0.0 |
| Standard | ----- | 0.2ml | 0.8 ml | 2 ml | |
| Unknown | 0.2 ml | ----- | 0.8 ml | 2 ml | |

**(Note:** O.D. is the same as Δ O.D, only when blank is adjusted to zero. If O.D. for blank cannot be adjusted to zero for some reasons, the reading for blank has to be subtracted from that of the standard and unknown.)

**Calculations:**

X = O.D. of standard
Y = O.D. of unknown (0.2 ml supernatant obtained from homogenate)
Z = Concentration of standard = 0.08 mg

$$\text{Amount of DNA in 0.2 ml of extract} = \frac{Y \times Z}{X} = D \text{ mg}$$

This 0.2 ml was taken from 2 ml of supernatant
∴ 0.2 ml supernatant contains **D mg. DNA**
∴ 2 ml supernatant contains **10 D mg. DNA**
This 2 ml supernatant was obtained from 1 ml of liver homogenate.
So, 1 ml of liver homogenate contains **10 D mg of DNA**
However, 1 gm of liver was homogenized to give 10 ml homogenate.
∴ 10 ml homogenate contains **100 D mg. of DNA.**
Express the final results as follows:

$$\text{Amount of DNA} = \left( \frac{\text{O.D. of unknown X conc. Of std}}{\text{O.D. of std}} \times 100 \right) \text{ mg/gm of tissue [liver]}$$

**Results: Amount of DNA= _____mg/gm of tissue**

**Aim**: **Estimation of RNA by Orcinol reagent**

**Principle**: Orcinol reaction or Bial reaction is a general reaction of pentose sugars. This reaction depends on the formation of furfural when the pentose (here ribose sugar) is heated with concentrated hydrochloric acid. Orcinol reacts with furfural in the presence of ferric chloride as a catalyst giving greenish-blue colour which is read at 660nm.

**Reagents:**

- 10% and 5% Trichloro Acetic Acid (TCA)
- 0.3 N KOH: 1:18 gm KOH in 100 ml distilled water.
- Orcinol Reagent: 210 mg of Orcinol and 1 gm of Ferric Chloride, dissolved in 100 ml of conc. HCL.
- RNA standard: 0.4 mg/ml in 5mM NaOH. Store in freezer.

**Procedure:**

1. Homogenize 1 gm liver in 10 ml ice cold distilled water and prepare homogenate.
2. Take 1 ml of the homogenate and add 1 ml of 10% TCA
3. Mix the suspension and centrifuge at 2500 rpm for 10 min.
4. Discard the supernatant and wash three times in 2 ml of 5% TCA and each time discard the supernatant after centrifugation.
5. Add 4 ml of 0.3 N KOH to hydrolyse the RNA.
6. Incubate at 37$^{o}$ C for 60 min.
7. Transfer the tubes to ice bath and after 5 min add 3 ml of 10% TCA. Leave the tubes at 0$^{o}$ C for 10 min.
8. Centrifuge the tubes at 2500rpm for 10 min and collect the supernatant.
9. Make the volume 10 ml by diluting supernatant with distilled water. (Note: supernatant should be clear.)
10. Use 2 ml of this diluted supernatant for RNA estimation as follows:
    2 ml supernatant + 1 ml distilled water + 3 ml Orcinol reagent mis well, heat in boiling water bath (100$^{o}$ C) for 15 min. Cool the tube and read Optical Density at 660 nm within a few min.
11. Tube for RNS standard:
    1 ml RNA std. solution + 2 ml 5% TCA + 3 ml Orcinol reagent mis well, heat in boiling water bath (100$^{o}$ C) for 15 min. Cool the tube and read Optical Density at 660 nm within a few min.
12. Tube as Blank:
    2 ml distilled water + 1 ml 5% TCA + 3 ml Orcinol reagent mis well, heat in boiling water bath (100$^{o}$ C) for 15 min. Cool the tube and read Optical Density at 660 nm within a few min.

**Tabulation**:

| TUBE | Supernatant for estimation | D/W | RNA standard Solution (0.4 mg/ml) | 5% TCA | Orcinol Reagent | O.D. at 660 nm |
|---|---|---|---|---|---|---|
| Blank | ----- | 2 ml | ----- | 1 ml | 3 ml | 0.0 |
| Standard | ----- | | 1 ml | 2 ml | 3 ml | |
| Unknown | 2 ml | 1 ml | ----- | ----- | 3 ml | |

**Calculations:**

X = O.D. of standard

Y = O.D. of unknown (0.2 ml supernatant obtained from homogenate)

Z = Concentration of standard RNA taken for estimation = 0.4 mg

$$\text{Amount of RNA in 2 ml of extract} = \frac{Y \times Z}{X} = R \text{ mg}$$

This 2 ml was taken from 10 ml of supernatant

∴ 2 ml supernatant contains **R mg. RNA**

∴ 10 ml supernatant contains **5 R mg. RNA**

This 10 ml supernatant was obtained from 1 ml of liver homogenate.

So, 1 ml of liver homogenate contains **5 R mg. RNA**

However, 1 gm of liver was homogenized to give 10 ml homogenate.

∴ 10 ml homogenate contains **50 R mg. RNA.**

Express the final results as follows:

$$\text{Amount of RNA} = \left( \frac{\text{O.D. of unknown X conc. Of std}}{\text{O.D. of std}} \times 50 \right) \text{mg/gm of tissue [liver]}$$

**Results: Amount of RNA = _____mg/gm of tissue**

**Signature of Student** **Signature of Teacher**

Rayat Shikshan Sanstha's

# Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.- Raigad

## DEPARTMENT OF ZOOLOGY

## (ACADEMIC YEAR: 2023-24)

### EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

**Class:** **Date:** **Time:**

**Name of Student:**

**Roll Number**

## ASSESMENT OF NEMATODE PARASITE FROM COMMERCIALLY IMPORTANT FISHES SOLD IN RETAIL MARKET

**Aim: To identify and assess the presence of nematode parasites in commercially important fish** species

**Principle:** Fishes that are economically important are consumed by people. However, the presence of parasites in the products may adversely affect consumer perception and/or pose a direct health hazard which can cause harm to the human beings and causes diseases.

**Requirements:** Freshly caught commercial fish, sterile containers, Dissection tools (scalpel, scissors, forceps) Glass slides and cover slips, Microscope.

**Procedure:**

### 1. Sample Collection

- **Select Fish**: Choose at least 2 different specimens of a commercial fish species.
- **Store Properly**: Place the fish in sterile containers and keep them on ice to preserve samples until dissection.

### 2. Dissection

- **Preparation**: Wear gloves and use sterilized tools to avoid contamination.
- **Open the Fish**: Using scissors make a ventral incision to open the body cavity carefully.
- **Inspect Organs**: Examine the gastrointestinal tract (stomach and intestines) and other organs (liver, muscles) for the presence of nematodes.

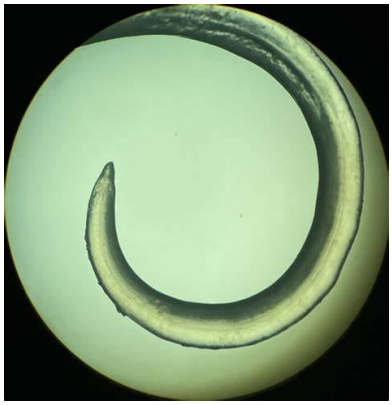
### 3. Isolation of Nematodes

- **Remove Nematodes**: Use forceps to extract any visible nematodes from the organs.
- **Place in Containers**: Place each nematode in separate sterile containers with a small amount of saline solution.

## 4. Nematode Identification

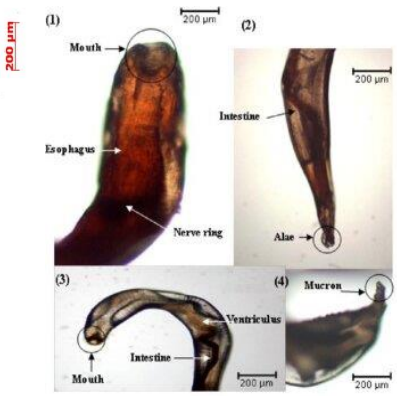
- **Prepare Slides**: Transfer nematodes to glass slides. Use a small drop of saline to keep them viable.
- **Microscope Examination**: Examine under a microscope at various magnifications.

## Observation:



Contracaecum sps.

Rhabdocona sps




Promallancunus sps

## Result:

_____

_____

_____

_____

_____

_____

**Signature of Student** **Signature of Teacher**