visualization, enabling them to handle complex datasets effectively in biomedical research contexts.

Practical Syllabus

ZOO-76P-304- Practicals based on Microbiology, Immunology & Biostatistics

INDEX

Microbiology

1. Preparation and use of culture media for microbes (NA and PDA).
2. Culture of microbes (bacteria and fungi) from food (curd/ spoiled food).
3. Gram's staining for bacteria.
4. Simple staining of bacteria.
5. Slide preparation and identification of any two fungi.

Immunology

6. Tests of blood grouping.
7. Widal test to identify any pathogen in blood.
8. Differential leucocyte count (DLC) in the blood sample.

Biostatistics

9. Representation of data by using bar diagram and pie charts.
10. Numericals based on biostatistical measurement: Mean, Mode and Median.
11. Numericals based on standard deviation, Student's T test and Chi square test.

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BSc 6 semester zoology practical paper (A) maximum mark 40

Q. 1 gram's standing for bacteria (6)

Q. 2 teste of blood grouping (4)

Q. 3 representation of data by using bar diagram and pie charts(4)

Q. 4 identification and comment on sport (1 to 8)=16

1 gram iodine

2 mono bacillus

3 agar

4 pi chart formula

5 gram safrinin

6 precipitation

7 RBC pipette

8 mean formula

BSc 6th semester zoology practical paper (B) maximum mark 40

Q. 1 preparation and use of culture media for microbes (6)

Q. 2 widal test to identify any pathogen in blood (4)

Q. 3 numerical based on chi square test (4)

Q. 4 identification and comments on spot (1 to 8)=16

1 mean formula

2 standard deviation formula

3 indicator media

4 gram alcohol

5 RBC pipette

6 Agar jail

7 gram iodine

8 Gram Negative bacteria

Q. 5 viva voce (5)

Q. 6 class record (5)

- **Observing fungal growth characteristics:** The PDA medium can be used to cultivate isolated fungal cultures, allowing for observation of colony morphology, pigmentation, and sporulation, which helps in fungal identification.
- **Preserving stock cultures:** PDA slants (medium solidified in test tubes at an angle) are used to maintain stock cultures of specific fungi over time.

PDA- Composition, Principle, Preparation, Results, Uses

Microbe Notes

Use in zoology practicals

In zoology, PDA is often used for studying fungi associated with animals.

- **Investigating animal pathogens:** If a practical involves examining animal samples (e.g., skin scrapings, fur) for fungal infections (dermatophytes), the sample can be inoculated onto a PDA plate.
- **Studying gut microbiota:** Some experiments might involve isolating and identifying the fungal component of an animal's gut flora. Fecal samples can be inoculated onto PDA plates to grow and characterize the yeast and mold species present.
- Observing fungal growth

1. **Prepare potato infusion:** Boil 200 grams of sliced, unpeeled potatoes in 500 ml of distilled water for 30 minutes. Strain the liquid through a cheesecloth to collect the potato extract.

2. **Combine ingredients:** Add 20 grams of dextrose and 17 grams of agar to the potato extract and add distilled water to make a final volume of 1 liter.

3. **Dissolve and sterilize:** Heat and stir until all components are fully dissolved. Autoclave the medium at 121°C for 15 minutes.

4. **Complete preparation:** Follow the remaining steps as for the dehydrated PDA (acidification, dispensing, and solidification).

2. **Boil:** Heat the mixture, stirring frequently, until the powder is completely dissolved.

3. **Autoclave:** Sterilize the medium at 121°C for 15 minutes.

4. **Acidify (optional):** To inhibit bacterial growth, cool the medium to 45–50°C and add sterile 10% tartaric acid to adjust the pH to 3.5. **Note:** Do not reheat after adding the acid, as this can prevent the agar from solidifying.

5. **Dispense:** Pour the medium into sterile Petri dishes in a sterile environment and allow it to solidify.

Using fresh potatoes

1. **Prepare potato infusion:** Boil 200 grams of sliced, unpeeled potatoes in 500 ml of

While PDA is primarily used in mycology (the study of fungi), it can be used in zoology practicals, particularly for studying the association of fungi with animal tissues or digestive systems. Here is how it is prepared and used:

Preparation of Potato Dextrose Agar (PDA)

PDA can be prepared from a commercial dehydrated powder or from scratch using fresh ingredients.

Using dehydrated PDA

1. **Suspend:** Measure 39 grams of PDA powder and suspend it in 1000 ml of distilled water.
2. **Boil:** Heat the mixture, stirring frequently,

and spores.

5. **Dispense into plates:** After autoclaving, let the medium cool to about 45–50°C. Pour the molten medium into sterile Petri plates under aseptic conditions, such as in a laminar flow hood, to avoid contamination.

6. **Solidify and store:** Allow the plates to solidify. Once solid, they can be stored in a clean, cool area, often inverted to prevent condensation from dripping onto the surface.

Preparation and use of PDA for zoology practicals

While PDA is primarily used in mycology (the study of fungi), it can be used in zoology

2. **Dissolve in water:** Add the weighed ingredients to distilled or deionized water. Mix thoroughly using a magnetic stirrer or by shaking. Gentle heating may be necessary to fully dissolve all components, especially agar.

3. **Adjust pH:** Check and adjust the pH of the medium as needed for the target microbes. A pH meter can be used, and the pH is corrected by adding a few drops of acid or base.

4. **Sterilize:** Dispense the medium into appropriate vessels (like flasks or bottles) and plug them with cotton. Sterilize the medium and containers using an autoclave at 121°C and 15 psi pressure for 15–20 minutes to kill all living microbes and spores.

Microorganisms are grown in a lab using a culture medium, which provides the necessary nutrients for growth. Potato Dextrose Agar (PDA) is a specific type of culture medium used primarily for growing yeasts and molds, and is a common component in microbiology and some zoology practicals.

General preparation of culture media for microbes

The preparation of any culture medium must be done with great care to prevent contamination. The general steps include:

1. **Weigh ingredients:** Accurately measure the required amounts of powdered medium and other ingredients using a precision balance.

Significance

A DLC is a valuable diagnostic tool in zoology and medicine. Deviations from the normal range for different species can help indicate:

- Infections (bacterial, viral, or parasitic)
- Allergic reactions
- Inflammation
- Certain blood disorders

After counting 100 white blood cells, the number you counted for each type directly represents its percentage.

Example:
If you counted 65 neutrophils, 25 lymphocytes, 5 monocytes, 3 eosinophils, and 2 basophils, the DLC result would be:

- **Neutrophils:** 65%
- **Lymphocytes:** 25%
- **Monocytes:** 5%
- **Eosinophils:** 3%
- **Basophils:** 2%

Significance

Lymphocyte	Small, if any.	Large, round, or slightly indented, and stains dark purple.	Scanty, sky-blue rim aroun the nucleus.
Monocyte	No granules.	Kidney-shaped, large, and stains light purple.	Abundant grayish-blue.

Calculation and result

After counting 100 white blood cells, the number you counted for each type directly represents its percentage.

Each of the five leukocyte types has distinct characteristics when stained.

Leukocyte Type	Granules	Nucleus	Cytoplas
Neutrophil	Fine, pinkish/purple granules.	Multi-lobed (2–5 lobes).	Pale pink/bluis
Eosinophil	Coarse, red or pink granules.	Usually bi-lobed.	Pale blue.
Basophil	Large, coarse, dark purple/blue granules that often obscure the nucleus.	Bi-lobed or S-shaped.	Blue.
Lymphocyte	Small, if any.	Large, round, or slightly	Scanty, sky-blue rim aroun

the area near the feathered edge where cells are evenly distributed.

- **Oil immersion:** Place a drop of immersion oil on the selected area and switch to the oil immersion objective (100x).
- **Counting:** Follow a systematic path, such as the "battlement" or serpentine method, to avoid counting cells twice. Count and identify the first 100 white blood cells you encounter. A differential cell counter can help keep a tally.

Identification of white blood cells

Each of the five leukocyte types has distinct characteristics when stained.

- **Staining:** Add an equal or double amount of distilled water to the stain on the slide. Gently mix by blowing on it or rocking the slide until a metallic sheen appears. Let the diluted stain sit for 10–15 minutes.
- **Washing:** Wash the slide gently with a continuous, indirect stream of distilled water until the film appears pink.
- **Drying:** Shake off excess water and place the slide upright to air dry.

3. Examining and counting the cells

- **Microscope setup:** Place the dried, stained slide on the microscope stage. Examine the smear under low power (10x) to find a suitable area for counting—the area near the feathered edge where

- **Spread the blood:** Hold a second "spreader" slide at a 30–45° angle. Pull it back to contact the blood drop, allowing the blood to spread along its edge.
- **Make the smear:** Push the spreader slide forward in one smooth, quick motion to create a thin, tongue-shaped film of blood. The smear should be thin enough to be translucent at the tail end.
- **Dry the smear:** Allow the blood film to air dry completely by waving it gently.

2. Staining the blood smear

- **Fixation:** Place the dried slide on a staining rack. Cover the smear completely with Leishman's stain and allow it to act for 1–2 minutes. The methyl alcohol in the stain will fix the blood cells

- **Other supplies:** Distilled water, staining rack, microscope, immersion oil, spirit, cotton swabs, and a disposable lancet.

Procedure

1. Preparing the blood smear

- **Clean and sterilize:** Use a cotton swab with spirit to clean the skin (typically the finger). Allow it to air dry.
- **Collect the blood:** Prick the finger with a sterile lancet. Wipe away the first drop of blood, as it may be contaminated with tissue fluid. Place a small drop of the second blood drop onto one end of a clean glass slide.

Materials required

- **Blood sample:** A small drop of fresh capillary blood (e.g., from a finger prick) or anticoagulated blood (mixed with EDTA).
- **Glass slides:** At least two clean, grease-free slides per sample.
- **Spreader slide:** A second slide with a smooth edge.
- **Stain:** Leishman's stain is common for this practical. It contains eosin (acidic, stains basic granules pink) and methylene blue (basic, stains acidic granules and nuclei blue) dissolved in acetone-free methyl alcohol.
- **Other supplies:** Distilled water, staining rack, microscope, immersion oil, spirit

To perform a Differential Leukocyte Count (DLC) in a zoology practical, you will **prepare and stain a blood smear to microscopically identify and count the different types of white blood cells (leukocytes)**. This allows you to determine the relative percentage of each leukocyte type present in the blood sample.

Aim

To prepare a stained blood film and determine the percentage of each of the five types of white blood cells: neutrophils, eosinophils, basophils, lymphocytes, and monocytes.

Materials required

- **False negatives:** Can occur if the test is done too early in the infection before antibodies have developed, or if the patient is on antibiotics.
- **Limited specificity:** The test cannot distinguish between a current infection, a past infection, or antibodies from a vaccination.
- **Time-consuming:** Obtaining a meaningful result, particularly a rising titer, requires taking paired samples over several days.
- **Not a standalone diagnostic:** Due to its limitations, blood culture is considered the gold standard for confirming a typhoid diagnosis.

AH/BH (S. paratyphi)	Suggests an infection with *S. paratyphi* A or B.

Note: The most definitive result is a four-fold increase in the antibody titer between two serum samples taken 7–10 days apart.

Limitations of the Widal test

While useful in resource-limited settings, the Widal test is not a foolproof diagnostic tool and should be used in conjunction with other clinical findings.

- **False positives:** Can result from previous infections, typhoid vaccination, or cross-reactivity with other bacteria (e.g., malaria, dengue).
- **False negatives:** Can occur if the test is

Interpretation of results

The interpretation of Widal test results requires careful consideration, especially in regions where typhoid is common, as baseline antibody levels can be elevated due to past infections or vaccinations.

Antigen	Interpretation of a significant titer (≥1:160)
TO (S. typhi O)	Suggests an active, recent infection. The O antibodies appear early and disappear relatively quickly.
TH (S. typhi H)	Can indicate a current or past infection. The H antibodies appear later and persist for longer periods.
AH/BH (S. paratyphi)	Suggests an infection with *S. paratyphi* A or B.

5. **Observe:** Gently rock the slide back and forth for about one minute and observe for visible clumping, or agglutination. The O antigen typically produces granular agglutination, while the H antigens result in floccular (fluffy) agglutination.

Quantitative (Tube) Method:
If the slide test is positive, a quantitative test using serial dilutions of the serum is performed to determine the antibody titer. Higher dilutions are used in test tubes, and the highest dilution that shows agglutination is recorded as the titer.

Interpretation of results

The interpretation of Widal test results requires careful consideration, especially in regions where typhoid is common, as

Steps:

1. **Labeling:** Place the clean, sterile slide or card on a flat surface. Use a pencil or marker to label separate reaction circles for O, H, AH, and BH antigens.
2. **Add Serum:** Place a single, uniform drop of the patient's undiluted serum in each of the four labeled circles.
3. **Add Antigens:** Add one drop of the specific commercial antigen suspension to the corresponding serum drop: O, H, AH (*S. paratyphi* A), and BH (*S. paratyphi* B).
4. **Mix:** Using a separate, clean applicator stick for each circle, mix the serum and antigen thoroughly, spreading the mixture over the entire area of the circle.

Agglutination Method)

The rapid slide agglutination method is often used in practical settings for its speed and simplicity.

Requirements:

- Widal test kit with commercial antigens (O, H, AH, BH)
- Sterile glass slide or specialized agglutination card
- Applicator sticks or mixing rods
- Micropipette or dropper
- Patient's serum sample
- Positive and negative controls (optional but recommended)

- **"O" (Somatic) Antigen:** Found on the surface of the bacterial cell wall. Antibodies against this antigen indicate an acute, active, or recent infection.
- **"H" (Flagellar) Antigen:** Found on the flagella (tail-like structure) of the bacteria. Antibodies against this antigen can signify a current infection, a past infection, or prior vaccination.

The test uses these commercial antigens to detect and measure the concentration (titer) of the corresponding antibodies in a blood sample. A higher titer suggests a more significant antibody response.

Procedure for a Widal test (Slide Agglutination Method)

The Widal test is a serological technique used in zoology or microbiology practicals to help diagnose enteric fever, specifically typhoid, caused by the bacterium *Salmonella enterica* serovar Typhi. It is based on the principle of agglutination, where antibodies in a patient's serum react with specific antigens from the *Salmonella* bacteria, causing visible clumping.

Principle of the Widal test

The body's immune system produces antibodies (agglutinins) against two main antigens of *Salmonella typhi* when an infection occurs.

- **"O" (Somatic) Antigen:** Found on the surface of the bacterial cell wall.

No	No	Yes	O
No	No	No	O

Precautions

- Always use new, sterile equipment for each person to prevent infection and cross-contamination.
- Dispose of all lancets, swabs, and slides in a designated biohazard sharps container.
- Ensure that the antisera are not expired and have been stored correctly.
- If a sample is hard to read, or if any result is unexpected, it should be retested.

Yes	No	Yes	A·
Yes	No	No	A·
No	Yes	Yes	B-
No	Yes	No	B-
Yes	Yes	Yes	Al
Yes	Yes	No	Al
No	No	Yes	O
No	No	No	O

Precautions

- Always use new, sterile equipment for each person to prevent infection and

particles (agglutination) indicates a positive reaction.

8. **Record and dispose:** Record the results and dispose of all materials properly in a biohazard container.

Interpreting the results

By observing which samples show agglutination, you can determine the blood type.

Agglutination with Anti-A	Agglutination with Anti-B	Agglutination with Anti-D (Rh)	Bl Ty
Yes	No	Yes	A·
Yes	No	No	A·

4. **Collect the blood sample:** Use a sterile lancet to prick the fingertip. Wipe away the first drop of blood with a cotton ball, as it may be contaminated with tissue fluid.

5. **Place blood drops:** Gently squeeze the finger to allow a fresh drop of blood to fall into each of the three labeled circles on the slide.

6. **Mix the samples:** Use a new, clean toothpick to thoroughly mix the blood and antiserum in each circle. Avoid cross-contaminating the samples by using a fresh toothpick for each mixture.

7. **Observe for agglutination:** Watch the mixtures for about a minute. The presence of visible clumping or granular particles (agglutination) indicates a

Procedure

1. **Prepare the slide:** Take a clean glass slide and label three separate sections or draw three distinct circles on it. Label one "A" (for Anti-A), one "B" (for Anti-B), and one "Rh" or "D" (for Anti-D).

2. **Add antisera:** Place a single drop of Anti-A serum in the circle labeled "A," one drop of Anti-B serum in the circle labeled "B," and one drop of Anti-D serum in the circle labeled "Rh".

3. **Prepare the finger:** Clean the fingertip (typically the ring finger) with an alcohol swab and allow it to dry completely.

4. **Collect the blood sample:** Use a sterile lancet to prick the fingertip. Wipe away the first drop of blood with a cotton ball

Materials required

- Clean glass slide or blood typing card
- Sterile lancet for finger pricking
- Alcohol swab or antiseptic wipe
- Three different antisera: Anti-A (typically blue), Anti-B (typically yellow), and Anti-D (Rh factor, often colorless)
- Three separate toothpicks or applicator sticks
- Sterile cotton balls
- Biohazard disposal container for sharps and waste

Procedure

1. **Prepare the slide:** Take a clean glass

Individuals with type A blood have A antigens, type B blood has B antigens, type AB has both, and type O has neither. Naturally occurring antibodies (agglutinins) circulate in the blood plasma: Anti-A antibodies in type B blood, and Anti-B antibodies in type A blood.

- **Rh system:** In addition to the ABO antigens, red blood cells may have the Rh (or D) antigen. If this antigen is present, the blood is Rh-positive (Rh+); if it's absent, the blood is Rh-negative (Rh-).

- **Agglutination:** When blood is mixed with an antiserum containing specific antibodies, the red blood cells will clump together (agglutinate) if the corresponding antigen is present.

is a hands-on experiment to determine the ABO and Rh blood types using an agglutination (clumping) reaction. The test relies on the principle that specific antigens on red blood cells will react with corresponding antibodies in test serums, causing the blood to clump.

Disclaimer: This procedure is for educational purposes only. It should only be performed under the direct supervision of a qualified instructor and with all necessary safety precautions in place.

Principle

- **ABO system:** Human red blood cells have antigens (proteins) on their surface. Individuals with type A blood have A

- Note the arrangement of the cells, such as in clusters (**staphylo-**), chains (**strepto-**), or pairs (**diplo-**).
- Record the color of the stained cells, which will be the same color as the stain used.

Precautions

- Always use a *thin* smear. A thick smear will show densely packed cells that are difficult to distinguish.
- Ensure the smear is completely air-dried before heat-fixing.
- Do not overheat the smear during fixation, as this can distort the bacterial cells.
- Always sterilize the inoculating loop

3. **Rinse:** Gently rinse the slide with a gentle stream of distilled water until the water runs clear. Do not rinse the smear directly to avoid washing it off.

4. **Blot dry:** Gently blot the slide with bibulous paper to remove excess water. Do not wipe the smear.

5. **Examine:** Place the stained slide on a microscope and observe it under the oil immersion (100x) objective.

Observation and results

- Observe the morphology (shape) of the bacterial cells, such as rod-shaped (**bacilli**), spherical (**cocci**), or spiral (**spirilla**).

- Note the arrangement of the cells, such

6. **Heat-fix the smear:** Once completely dry, pass the slide, smear-side up, through the Bunsen burner flame 2–3 times. This kills the bacteria and adheres them to the slide so they don't wash off during staining.

Part 2: Staining the smear

1. **Stain the smear:** Place the heat-fixed slide on a staining rack. Flood the smear with your chosen simple stain (e.g., Methylene Blue).
2. **Wait:** Allow the stain to sit on the smear for the appropriate time, typically 1–2 minutes.
3. **Rinse:** Gently rinse the slide with a gentle stream of distilled water until the water

4. **Make the smear:**
    1. **From a solid culture:** Use the sterile loop to pick up a *tiny* amount of a single bacterial colony. Mix the bacteria into the drop of water and spread it into a thin film.
    2. **From a liquid culture:** Transfer one or two loopfuls of the bacterial broth culture directly onto the slide and spread it out. You do not need to add extra water.
5. **Air dry:** Allow the smear to air dry completely. The smear will appear as a thin, hazy film.
6. **Heat-fix the smear:** Once completely

Procedure

Part 1: Preparing a heat-fixed smear

1. **Label the slide:** Label one end of a clean, grease-free glass slide with a wax pencil to identify the culture being used.
2. **Add water (if using solid culture):** If taking bacteria from a solid medium (agar plate), place a small drop of distilled water in the center of the slide.
3. **Transfer the bacteria:** Sterilize an inoculating loop by passing it through a flame until it is red hot. Allow it to cool for 15–20 seconds.
4. **Make the smear:**
    1. **From a solid culture:** Use the sterile loop to pick up a *tiny*

Materials required

- Bacterial culture (e.g., from a slant or broth)
- Clean glass slides
- Basic stain (e.g., Methylene Blue, Crystal Violet, or Safranin)
- Bunsen burner or microincinerator
- Inoculating loop
- Distilled water
- Staining tray or sink
- Bibulous paper or filter paper
- Compound microscope with an oil immersion (100x) objective

technique for observing the morphology, size, and arrangement of bacterial cells under a microscope. It involves using a single basic dye, which is positively charged and adheres to the negatively charged surface of bacterial cells.

Principle

The most common simple stains, such as crystal violet and methylene blue, are basic dyes. Since most bacterial cell walls and nucleic acids have a negative charge, the positively charged dye is attracted to and stains the cells themselves. This creates a contrast that makes the bacteria visible against a bright background.

Materials required

3. Observe and note the shape (e.g., cocci, bacilli) and arrangement (e.g., clusters, chains) of the bacteria.

Common errors and limitations

- **Poor decolorization:** Over-decolorizing can cause Gram-positive cells to appear Gram-negative, while under-decolorizing can cause Gram-negative cells to appear Gram-positive.
- **Old cultures:** As bacterial cultures age (typically over 24 hours), the cell walls of Gram-positive bacteria may deteriorate and give false Gram-negative results.
- **Inappropriate fixation:** Excessive

10. **Dry:** Blot the slide with bibulous paper or air-dry it completely.

3. Observe the results

1. Place a drop of immersion oil on the dried smear.
2. Examine the slide under the oil immersion (100x) objective lens of your microscope.
3. Record your observations:
    1. **Gram-positive bacteria** will appear **purple or blue**.
    2. **Gram-negative bacteria** will appear **pink or red**.
    3. Observe and note the shape (e.g., cocci, bacilli) and arrangement (e.g., clusters, chains) of the bacteria.

4. **Mordant:** Flood the smear with Gram's iodine for 60 seconds. This fixes the crystal violet to the cell walls.
5. **Rinse:** Gently wash with distilled water.
6. **Decolorize:** Hold the slide at a 45-degree angle and apply the decolorizer (ethanol or acetone) drop-by-drop until the run-off liquid is clear or has only a faint blue tinge. **This is the most critical step;** do not over-decolorize.
7. **Rinse:** Immediately wash with distilled water to stop the decolorization process.
8. **Counterstain:** Flood the smear with safranin for 30–60 seconds.
9. **Rinse:** Gently wash with distilled water.
10. **Dry:** Blot the slide with bibulous paper or

6. **Heat-fix** the smear by passing the slide quickly through a Bunsen burner flame three or four times. This kills the bacteria and adheres them to the slide.

2. Stain the smear

1. Place the heat-fixed slide on a staining rack.
2. **Primary stain:** Flood the smear with crystal violet for 60 seconds.
3. **Rinse:** Gently wash the slide with a gentle stream of distilled water for a few seconds to remove excess stain.
4. **Mordant:** Flood the smear with Gram's iodine for 60 seconds. This fixes the crystal violet to the cell walls.

Procedure

1. Prepare and fix the smear

1. Take a clean microscope slide and label the end.
2. If the bacteria are from a solid culture, place a small drop of sterile water on the slide. If the sample is from a broth culture, a drop of water is not needed.
3. Using a sterile inoculating loop, pick up a tiny amount of the bacterial culture.
4. Gently mix the bacteria into the water drop and spread the suspension to create a thin smear.
5. Allow the smear to completely air-dry.
6. **Heat-fix** the smear by passing the slide quickly through a Bunsen burner flame

- **Wash bottle:** With distilled water.
- **Bibulous paper or blotting paper:** To dry the slide.
- **Immersion oil:** For viewing the slide at 100x magnification.
- **Bacterial culture:** A mixed culture of Gram-positive (e.g., *Staphylococcus epidermidis*) and Gram-negative (e.g., *Escherichia coli*) bacteria is ideal for practice.
- **Stains and reagents:**
  - **Primary stain:** Crystal violet.
  - **Mordant:** Gram's iodine.
  - **Decolorizer:** 95% ethanol or acetone.
  - **Counterstain:** Safranin.

complex. The complex is washed away, leaving the cell colorless. A counterstain (safranin) is then added, which stains the colorless cells pink or red.

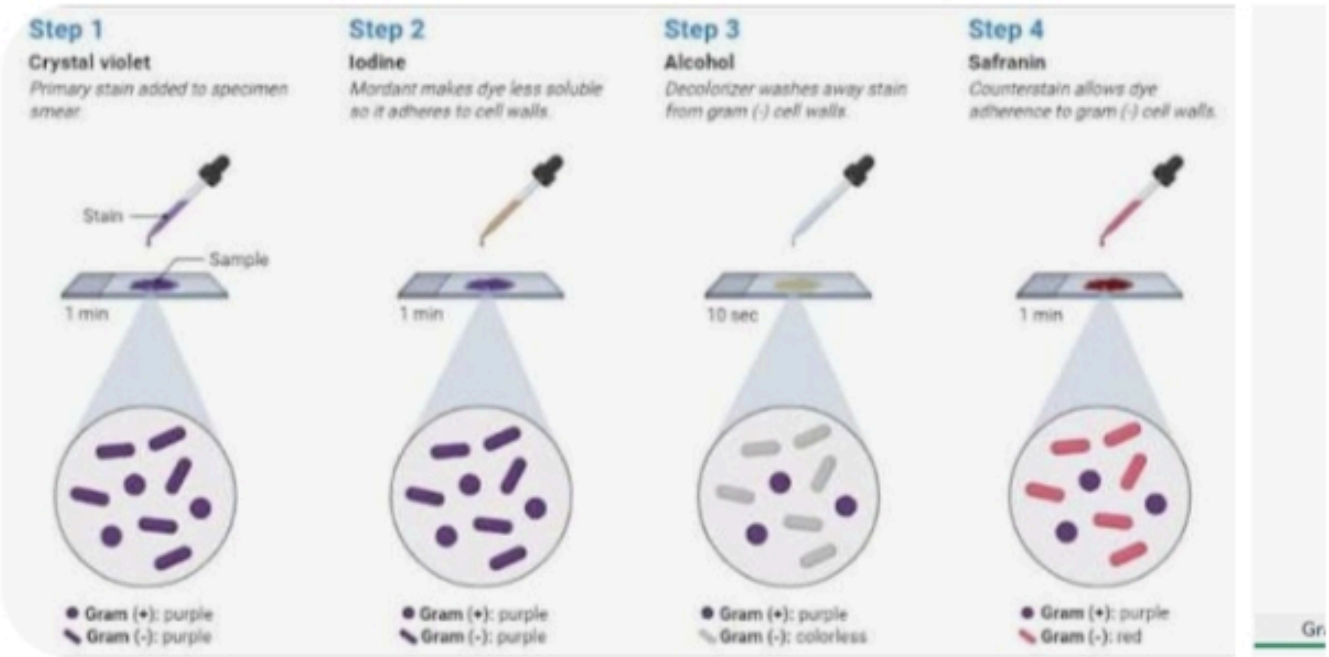
Materials and reagents

- **Microscope slides:** Clean and grease-free.
- **Bunsen burner:** For heat fixation.
- **Inoculating loop:** For transferring the bacterial sample.
- **Microscope:** With an oil immersion (100x) objective lens.
- **Staining rack:** To hold slides during the process.
- **Wash bottle:** With distilled water.

The difference in cell wall structure is the key to this staining method.

- **Gram-positive bacteria:** Have a thick, mesh-like peptidoglycan layer in their cell wall. When stained with crystal violet and treated with Gram's iodine, they form a large, insoluble crystal violet-iodine complex. During decolorization with alcohol, the thick cell wall is dehydrated, causing it to shrink and trap the complex inside, so the cells remain purple.
- **Gram-negative bacteria:** Have a thin peptidoglycan layer and an outer membrane with high lipid content. The alcohol in the decolorizer dissolves the outer membrane, and the thin cell wall cannot retain the crystal violet-iodine complex. The complex is washed away,

technique used to differentiate most bacteria into two groups: Gram-positive and Gram-negative. The distinction is based on the chemical and physical properties of the bacterial cell wall, which affects its ability to retain certain stains. This practical guide explains the procedure and the principles behind it.



Principle of Gram staining

Observations and results

- Record the microscopic observations of the shape, arrangement, and Gram staining results of the microbes from the direct smears.
- Document the different colony characteristics (e.g., color, texture, shape) seen on the cultured agar plates.
- Compare the diversity of microbes found in the curd versus the spoiled food. You should find primarily lactic acid bacteria in the curd and a wider variety of bacteria and fungi in the spoiled food.

Incubate fungal plates at room temperature for several days.

4. **Observe colonies**: After incubation, observe the plates for different types of microbial colonies.
    1. **Curd**: Colonies of *Lactobacillus* and *Streptococcus* will be visible.
    2. **Spoiled food**: Note the appearance of different bacterial and fungal colonies, recording their shape, color, size, and texture.
5. **Subculture for pure colonies**: Using a sterile inoculation loop, pick a single, isolated colony of interest and streak it onto a new, sterile agar plate to obtain a pure culture for further study.

samples onto both nutrient agar (for bacteria) and potato dextrose agar (for fungi) to get a comprehensive view of the microbial community.

3. Use a sterile spreader to distribute the sample evenly across the surface of the agar plate.

3. **Incubate the plates**:

    1. **Curd (MRS agar)**: Incubate at approximately 37°C for 24–48 hours, as this is the optimal temperature for many lactic acid bacteria.

    2. **Spoiled food**: Incubate bacterial plates at 37°C for 24–48 hours. Incubate fungal plates at room

Procedure

1. **Prepare serial dilutions**: Perform a serial dilution of both the curd and spoiled food samples into sterile distilled water to reduce the number of microbes for easier isolation. For curd, this can involve mixing 1g of curd with 9ml of sterile water, and then using this mixture for further dilutions.

2. **Perform spread plating**:

    1. **Curd**: Use a sterile pipette to transfer 0.1 mL of the diluted curd sample onto a specialized medium like MRS agar, which is selective for lactic acid bacteria.

    2. **Spoiled food**: Plate diluted samples onto both nutrient agar (for bacteria) and potato

- Curd and spoiled food samples
- Sterile Petri plates
- Sterile nutrient agar medium (for bacteria)
- Sterile potato dextrose agar (for fungi)
- Sterile distilled water
- Test tubes
- Micropipettes and sterile tips
- Bunsen burner
- Incubator

Procedure

1. **Prepare serial dilutions**: Perform a serial dilution of both the curd and spoiled food samples into sterile distilled water to

1. **Curd**: Examine the stained slide first under low power (10X), then high power (40X). Look for the rod-shaped *Lactobacillus* bacteria and spherical *Streptococcus* species.

2. **Spoiled food**: Observe the slide for different bacteria, yeasts, and molds. Fungi (like molds) often appear as filamentous structures, while bacteria will have distinct rod or cocci shapes.

Part 2: Culture microbes on nutrient agar plates

Materials

- Curd and spoiled food samples

5. **Stain the smear**:

    1. **Simple staining**: Cover the dried smear with methylene blue for about one minute. This will color all the microbial cells uniformly.

    2. **Differential staining (Gram staining)**: Follow the Gram staining protocol using crystal violet, Gram's iodine, alcohol, and safranin. This will differentiate bacteria into Gram-positive (purple) and Gram-negative (pink/red).

6. **Observe under the microscope**:

    1. **Curd**: Examine the stained slide first under low power (10X), then high power (40X). Look for the

a clean toothpick or sterile loop, take a very small amount of the curd's watery portion and mix it with the water to create a thin smear.

3. **Make the smear for spoiled food**: If the spoiled food has a soft, moist area, use a toothpick to transfer a small portion to a drop of water on a separate slide. For moldy bread, you can gently touch a clear adhesive tape to the mold and stick it to the slide.

4. **Dry and fix the smear**: Allow the smear to air dry completely. For a heat-fixed smear (bacteria only, not mold), pass the slide quickly through a Bunsen burner flame two to three times to adhere the microbes to the glass.

5. **Stain the smear:**

Materials

- Curd and spoiled food samples (e.g., spoiled fruit, moldy bread)
- Microscope slides and coverslips
- Distilled water
- Methylene blue or safranin stain
- Toothpicks or sterile inoculation loops
- Compound microscope

Procedure

1. **Prepare the slides**: Take a clean, dry glass slide.
2. **Make the smear for curd**: Place a small drop of distilled water on the slide. Using a clean toothpick or sterile loop, take a very small amount of the curd's watery

The zoology practical for culturing microbes from curd and spoiled food involves two main procedures: a direct microscopic examination of the samples and culturing microbes on a growth medium. The direct examination offers a quick look at the types of organisms present, while culturing allows for the isolation and study of individual microbial species.

Part 1: Microscopic examination of curd and spoiled food

Materials

- Curd and spoiled food samples (e.g., spoiled fruit, moldy bread)
- Microscope slides and coverslips

DATE .....

PIE CHRIS

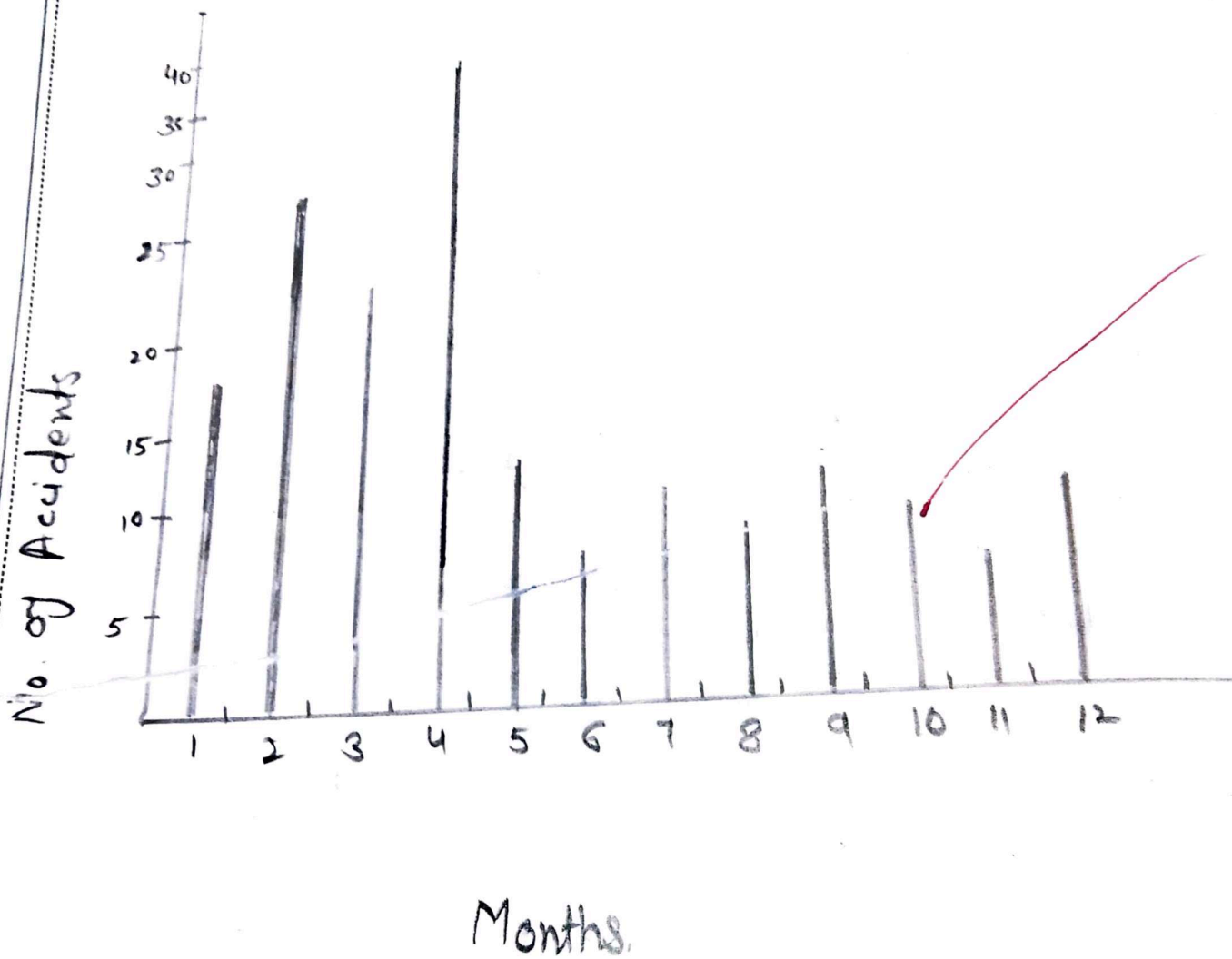
A pie chart is a circular Stastistical graphic, which is divided into slices to illustrate numerical proaportion. In a pie chart the arc lenght of each slice is proaortional to the quantity it rearesents When there is a wide range of data, a rearesentation of such data in the form of circle is called pie chart. for the construction of pie chart / diagram, square root is calculated. There after the radius calculated accoreding to their Square roats on the basis of which the Circles are drawn. Such Circles Should be drown only on the Surface and the distance among of ~~two types~~: these Should be eqtual Pie diagram are of two types:

a) Plane or Normal Pie diagram.
b) Subdivided Pie diagrams.

Example: The annual Productivity (Kg) of five areas is as ~~follo~~ follows:

A = 3600 , B = 1600 C = 900 , D = 100, E =

Drow the five plain pie diagrams and give their Scales also


No. of Accidents
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
Months.

DATE ....

Solution: Computation of the Pie-diagram:

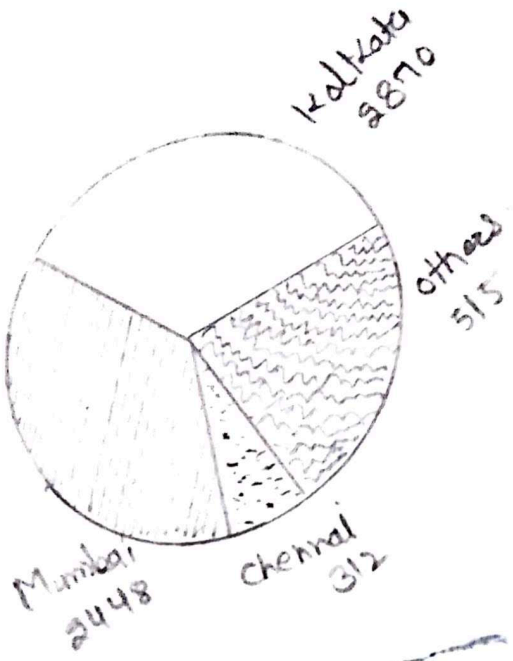
To Compute the Scales for a pie diagram, first of all area of the Circle should be calculated as shown in the following table.

[Kg]	~~3600~~ A	B	C	D	E
Sq	~~60~~3600	1600	900	100	
Square root	~~3~~60	40	30	10	75
Size of squares	3	2	1.5		8.66
[Dividing by 20]				0.5	0.4

The area of circle would be equal to πr^2 [hero pie $\frac{22}{7}$ is or 3.14]. In this figure the one 2270 circle, which be 22/7×9=198/7 Square cm which reflects the 3600 kg. productivity. Therefore, one square cm would be equivalent to [3600n.......27 kg approx].

SUB-DIVIDED PIE CHARTS:

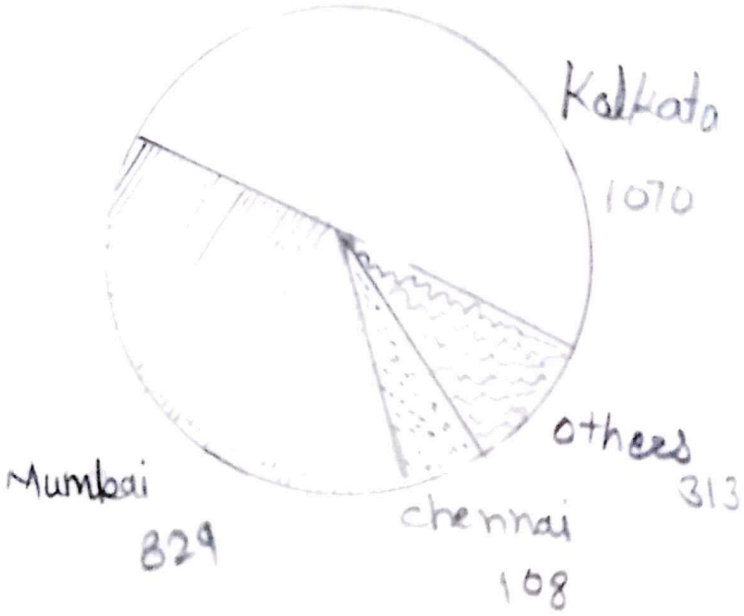
When there are several subdivisions of the facts or data, then the circle is further divided. The Centre of a Circle always makes an angle of 360°. Keeping this fact in view, the Sum total of all the values is decided which has an angle, being fraction of 360°. There fore, this is also called angular ~~diag~~ diagram.



AIDS patients
Numbers.

1992

Fig : Sub-divided pie diagram



1989

In Case two inter-divided pie diagrams are to be drawn then their radius are calculated in Proportion of the sum total of their Square roots.

Example: following is the data on the occurrence of AIDS Patients in two years. Draw a subdivided pie diagram.

Occurrence of AIDS - Patients

City	1989	1992
Kolkata	10,10	2610
mumbai	829	2443
chennai	312	312
others	313	515

Solution:- The above data requires two pie or circle diagrams to be sketched and these should also be comparable, for both the years, two different circles for different years are placed in proportion of the sum total of their Square roots, the angle should be worked out to sub-divide each circle, as they been depicted in table

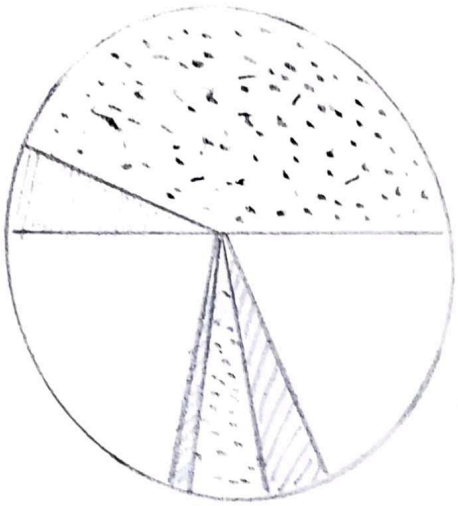
Signature

Herbivores

carnivores

Omnivores

chordates
2490



Non-chordates
1076

Sub: Divided pie Chart

CITY	1989 AIDS Patients	Degrees of Angles	AIDS Patients	1992 Degrees of Angles
KOLKATA	1,070	166	2,670	163
MUMBAI	829	128	2,443	149
CHENNAI	108	17	274	17
OTHER	313	49	515	31
TOTAL	2320	360	5,902	360
Square	4817	-	76.82	-
Radius	2.40	divided by 20	3.84	divided by 20

Scale Measurements: The area of 1989 year reflects an area for the total 2320 patients. Therefore, 1 square centimeter would be equivalent to 2320/18.03 = 128.16 patients

Two-in-one Pie Diagram:

Such a diagram is divided into two halves and each half includes the data which could have also been represented by two separate circle diagram.

Example: Draw a single two-in-one pie diagram to depict the following data.

Animal Types	Herbivores	Carnivores	Omnivores	Total
Chordates	711	1,469	310	2,490
Non-Cordates	334	673	69	1,076
Total	1,045	2142	379	3,566

Solution: The total 3566 is taken to be equivalent to 360° and it should be first of all divided Proportionally for the chordates and non-chordates groups.

Chordates will have a degree 2490/3566 × 360 = 250°

Non-chordates will have degree 1076/356 × 360 = 110

In the same manner 2490, taking 250, the subgroups of chordates will be calculated and taking 110 for the non-chordates all the sub-groups of Non-chordates will be calculated. Those have been depicted in the following table.

Animal Types	Herbivores	Carnivores	omnivores	total
Chordates	70	148	32	250
Non-chordates	34	68	8	110
Total	104	216	40	360

The following diagram will truly represent the data.

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DATE .....

STANDARD DEVIATION

The standard deviation or mean error is an absolute measure of dispersion of an individual series or a frequency distribution is the square of deviation of values from their arithmetic mean.

Standard deviation was derived by Pearson or root mean square deviation from mean. Standard deviation is also known as the mean square error or root mean square deviation from mean.

Standard deviation is represented by the small greek letter sigma (σ). While calculating σ signs are taken into consideration.

Calculation of standard Deviation (S.D.)

I) Calculation of S.D. in Individual Series:

In individual series S.D. is calculated by following two methods :-

A) DIRECT METHOD - Steps involved are as follows :-

i) First of all calculate the arithmetic mean ($\bar{X}$).

ii) Now get the difference of each observations from the arthimetic means.

iii) The square the differences of observations

DATE

for the arithmetic mean.

(iv) After that add the square values to find the sum of the squares.

(v) Divide by the total number of observations.

(vi) formula for S.D. $\sigma = \sqrt{\frac{\Sigma (x-\bar{x})^2}{N}}$ or $\sigma = \sqrt{\frac{\Sigma d^2}{N}}$

Where:

$d = (x - \bar{x})$

X = Individual value or variable value

$\bar{x}$ = Arithmentic mean

N = Number of observations.

Example: Calculate the standard deviation from the following data:

Heights (cms) 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83

Solution:

S.No.	Height in cms (x)	Deviation from the arthemetic Mean $d = (x - \bar{x})$	Square of the Deviation $d^2 = (x - \bar{x})^2$
1	71	$71 - 77 = -6$	36
2	72	$72 - 77 = -5$	25
3	73	$73 - 77 = -4$	16
4	74	$74 - 77 = -3$	9
5	75	$75 - 77 = -2$	4
6	76	$76 - 77 = -1$	1

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7	71	77 - 77 = 0	0
8	78	78 - 77 = +1	1
9	79	79 - 77 = +2	4
10	80	80 - 77 = +3	9
11	81	81 - 77 = +4	16
12	82	82 - 77 = +5	25
13	83	83 - 77 = +6	36
N = 13	$\Sigma X = 1001$		$\Sigma d^2 = 182$

Formula = Mean $(\bar{X}) = \frac{\Sigma X}{N} = \frac{1001}{13} = 77\text{cm}$

standard deviation (S.D.) $= \sigma = \sqrt{\frac{\Sigma d^2}{N}} = \sqrt{\frac{182}{13}}$

$= \sqrt{14}$

$\sigma = 3.74\text{ cm}$

B) SHORT CUT METHOD :- steps involved are as follows:-

i) When the mean of the given data comes out to be a fraction, the standard deviation is found by short cut method.

ii) Firstly, get the assumed mean (A) from given values.

iii) Deviation of the given values may be taken from assumed mean ($d = x - A$) and

then get the sum of the deviation (Σd)

(iv) Now squares of deviation are obtined and then get the sum of square of deviations (Σd^2)

(v) Σd^2 is the divided by the total number of observations (N)

vi) Formula for S.D -

$$\sigma = \sqrt{\frac{\Sigma d^2}{N} - \left(\frac{\Sigma d}{N}\right)^2}$$

where -

$d = X - A$

X = variable value

A = Assumed meas

N = Total number of observations.

Example :- Find the standard Deviation (S.D) from the given data :-

Marks :- 25, 27, 30, 24, 32, 33, 34, 35, 40, 38.

Solution :-

S.no	Height in cms (X)	Deviation from Assumed mean (A) $d = (X-A)$ $(A=34)$	Squares of the deviations $d^2 = (X-A)^2$
1.	25	$25 - 34 = 9$	81
2.	27	$27 - 34 = 7$	49
3	30	$30 - 34 = -4$	16

4	24	24-34 = -10	100
5	32	32-34 = -2	4
6	33	33-34 = -1	1
7	34	34-34 = 0	0
8	35	35-34 = +1	1
9	40	40-34 = +6	36
N=10	38	38-34 = -4	16
N=10		Σd = -33+11 = -22	Σd^2 = 304

Assumed mean = 34

Formula :- Standard deviation (S.D)

$$\sigma = \sqrt{\frac{\Sigma d^2}{N} - \left(\frac{\Sigma d}{N}\right)^2}$$

$$\sigma = \sqrt{\frac{304}{10} - \left(\frac{-22}{10}\right)^2}$$

$$= \sqrt{304 - 4.84}$$

$$= \sqrt{25.56}$$

$$\sigma = 5.05$$

II. Calculation of standard deviation (S.D) in Discrete so following methods are uses :-

i) Dived Method :- Following steps are involved :-

i) First of all calculate the arthmetic mean ($\bar{x}$)

ii) find the deviation from arithmetic mean.

(iii) squares of deviation are also obtained $(d)^2$

iv) Now d^2 are muliplied by their frequencies to

(1) find Σfd^2

formula : (S.D) $\sigma = \sqrt{\frac{\Sigma fd^2}{N}}$

where : $d = (x - \bar{x})$

$N = \Sigma f$ (total frequency).

Example : calculate the S.D from the following data

Haemoglobin values (Hb) (grms) (x)	7	8	9	10	11	12	13	14
No of patients (f)	2	3	9	13	16	4	7	8

Hb (grms) (x)	No. of patients (f)	Hb(grms) x No of patients (f) (fx)	Deviation from the Arthemetic Mean $d = (x - \bar{x})$	square of the deviation $d^2 = (x-\bar{x})^2$	Square of the deviation multiply by f $f(d^2)$
7	2	14	7 - 10.90 = -3.9	15.21	30.42
8	3	24	8 - 10.90 = 2.9	8.41	85.23
9	9	81	9 - 10.90 = 1.9	3.61	32.49
10	13	130	10 - 10.90 = 10.1	0.81	10.53
11	16	176	11 - 10.90 = 10.1	0.01	0.16
12	4	48	12 - 10.90 = +1.1	1.21	4.84
13	7	91	13 - 10.90 = +2.1	4.41	30.87
14	8	112	14 - 10.90 = +3.1	9.61	76.88
	$N = \Sigma f =$ 62	$\Sigma fn =$ 676			$fd^2 =$ 211.42

Arthematic mean $(\bar{x}) = \frac{\Sigma fn}{N} = \frac{676}{62} = 10.90$

Standard deviation $(\sigma) = \sqrt{\frac{\Sigma fd^2}{N}}$

$= \sqrt{\frac{211.42}{62}}$

$\sqrt{3.04}$

$\sigma = 1.84$

B) Short cut Method :-

If arithmetic mean has a fractional value then the standard deviation is calculated by short cut method using the following formula :-

$$\sigma = \sqrt{\frac{\Sigma fd^2}{N} - \left(\frac{\Sigma fd}{N}\right)^2}$$

where :-

$d = X - A$

$A =$ Assumed mean

$N = \Sigma f.$

Example :- calculate standard deviation for the following data by assumed mean Method.

Haemoglobin (Hb)

values (gms) (x)	1	8	9	10	11	12	13	14
No of patient (F)	2	3	9	13	16	4	7	8

Solution :-

Hb (gms) (x)	No. of patients (f)	Deviation from Assumed mean d : (x-A)(A=11)	Deviation multiplied by frequency (fd)	fdxd (fd^2)
7	2	7-14 = -4	-8	32
8	3	8-11 = -3	-9	27
9	9	9-11 = -2	-18	36
10	13	10-11 = -1	-13	13
11	16	11-11 = 0	0	0
12	4	12-11 = +1	+4	4
13	7	13-11 = +2	+14	28
14	8	14-11 = +3	+24	72
	N = Σf = 62		Σfd = -48	Σfd^2 =
			+42 = 6	212

Assumed mean (A) - value has highest frequency

A = 11

Formula: Arthematic Mean $(\bar{x}) = A + \frac{\Sigma fd}{N}$

$= 11 - \frac{6}{62}$ $= 11 - 0.096$

$\bar{x} = 10.90$

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Standard deviation $= \sqrt{\frac{\Sigma f d^2}{N} - \left(\frac{\Sigma f d}{N}\right)^2}$

$= \sqrt{\frac{212}{62} - \left(\frac{-6}{62}\right)^2}$

$= \sqrt{3.41 - (0.096)^2}$

$= \sqrt{3.41 - 0.009}$

$= \sqrt{3.401}$

$= 1.84$

III Calculation of Standard Deviation (S.D) in continuous Series: In this firstly find the mid-value of given classes. Calculation can be simplified if we divide the deviations of the midvalues by class interval. In continous Series S.D is calculated by following method.

A) DIREC METHOD:

Formula: Stand deviation $(\sigma) = \sqrt{\frac{\Sigma f d^2}{N}}$

B) SHORT WTMENTHOD:

$SD: (\sigma) = \sqrt{\frac{\Sigma f d^2}{N} - \left(\frac{\Sigma f d}{N}\right)^2} \times i$

STANDARD ERROR

Standard error is the standard deviation of this statistical distribution. Standard error statics measures how accurate and Precise the sample is as an estimate of Population Parameter. S.E is estimated from the following formulas -

1) Standard error of mean (S.E.X) SE.M

$$S.E.X = \frac{\sigma}{\sqrt{N}}$$

2) Standard error of median (S.E.M) :-

$$S.E.m = 1.2533 S.E.X$$

(or)

$$S.E.M = 1.2533 \frac{\sigma}{\sqrt{N}}$$

3) Standard error of standard

$$S.E.S = 0.7071 \frac{\sigma}{\sqrt{N}}$$

Teacher's Signature.....

DATE

MEAN

Arithmetic mean in the most common and easily understood measure of central tendency we can define mean as the value obtained by dividing the sum of measurements with the data set and is denoted by the symbol $\bar{n}$.

The arithmetic means are computed for three types of series:

i) Individual Data series
ii) Discrete Data series
iii) Continuous Data series

$$M = \bar{X} = \frac{\Sigma X}{N} = \frac{I}{N}$$

$$M = \bar{X} = \frac{\Sigma f_i x_i}{N} = \frac{I}{N}$$

Example :- From the following frequency distribution Calculate Arithmetic mean:

Marks [out of 50)	0-10	10-20	20-30	30-40	40-50
No. of Students	10	12	20	18	10

Solution: Calculation of Arithmetic mean by direct and short-cut methods.

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I	II	III	IV	V	VI
				$A = \Sigma mv/y$	
Marks	Mean value			dx	
	x	f	fn	$A = 25$	$f dx$
0-10	5	10	50	-20	-2000
10-20	15	12	180	-10	-120
20-30	25	20	500	0	0
30-40	35	18	630	+10	+180
40-50	45	10	450	+20	+200
Total	125	$N = 70$	$\Sigma fn = 1810$		-320 + 380 = +60
					$= \Sigma f dx$

mean is 25.86 marks.

DATE .....

MODE

The mode of a distribution is the value of the point around which the items tend to be most heavily concentrated.

mode is defined as the value of the variable which occoces most frequency in a distribution.

The mode in a distribution is that item around which there is a maximum concentration

1) By Inspection
2) By classifying the unclassified values

Example:- Calculate the mode from the following frequency data

weight of testis in mice (mg)	10	11	12	13	14	15	16
number of mice (f):	7	5	8	2	3	10	4

Solution

By Grouping METHOD

weight of testi in mice (x)	number of mice frequency I	II	III	IV	V	VI
10	7	12	18	2		

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11	5			13	20		15			
12	8		10						13	
13	2			5	15					
14	3						17			
15	10		13	14						
16	4				11				15	
17	1		5	7						
18	6						16			
19	9		15							

Construction of Analysis table

Column	10	11	12	13	14	15	16	17	18	19
I						I			I	I
II									I	I
III						I	I			
IV	I	I	I							
V				I	I	I				
VI					I	I	I			
Total	I	I	I	I	I	4	3	1	1	1

MEDIAN

Median may be defined as the value of that item which divides the series into two equal parts, one half containing values less than it.

If there is an odd number of scores, the values of the median is simply chosen equal to the middle score.

Thus, if n be odd, Median = value of $\left[\frac{n+1}{2}\right]$ th item

If there is an even number of scores, the value of the median will usually be chosen as a point halfway between the two middle scores If it is even then Median = Mean value of $\left[\frac{n}{2}\right]$ th and $\left[\frac{n}{2}+1\right]$ th item.

Example: Calculate Median from the following data:

S.No. of Persons:	1	2	3	4	5	6	7	8
Weight [kg]:	15	35	43	46	48	48	49	50
S.No. of Persons:	9	10	11	12	13	14	15	16
Weight [kg]:	55	56	60	64	71	75	80	85

Solution: The above class will first be written in ascending order.

S. No.	Marks	S. No.	Marks
1	15	9	55
2	35	10	56
3	43	11	60
14	46	12	64
5	48	13	71
6	48	14	75
7	49	15	80
8	50	16	85

Median = Size of $\left(\frac{n+1}{2}\right)^{th}$ item = Size of $\left(\frac{16+1}{2}\right)^{th}$ item.

$= 8.5th$

$= \frac{\text{Size of } 8th + \text{Size of } 9th}{2}$

$= \frac{50+55}{2}$

$= 52.5kg$

Median weight are 52.5