

**SOPs: GENETICS, PTC & ATC PRACTICAL EXERCISES OF
UNDERGRADUATE STUDENTS**

***(DBT Life Sciences Star College Scheme, Ministry of Science &
Technology, Govt. of India)***



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Section A: Genetics

Experiment No.A.1: To Demonstrate Law of segregation using coloured Beads and capsules.

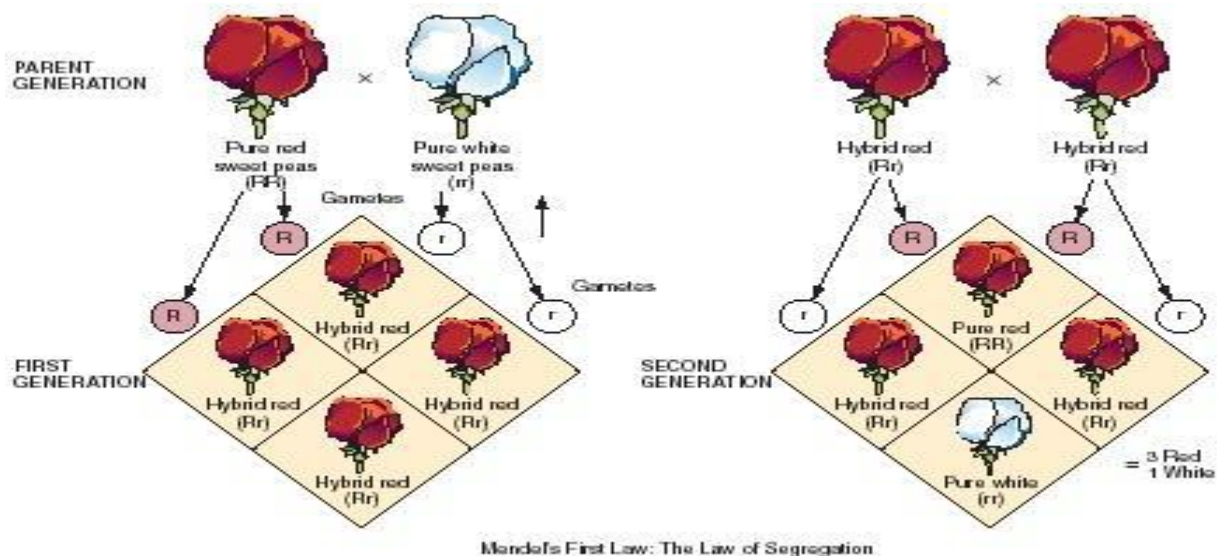
Background: Law of Segregation states that at the time of gametogenesis two genes of a character move apart from each other due to separation of homologous chromosomes so that each gamete receives one gene of a character. This law is called as law of purity of gametes because gametes are having only one gene of a particular trait so that gametes are pure for that particular character.

Material and Reagent Requirements: Piece of thermocol or white sheet, two types of coloured beads, two ink sketch pens and pins.

Methodology:

This can be demonstrated with the help of monohybrid cross. This cross involves only one character at a time. For Eg. Pure Red pea plants are crossed with pure White pea plants.

Assumption:



Experiment No.A.2: To demonstrate law of independent Assortment using coloured beads or capsules.

Background: It states that in the breeding experiments involving more than one character , gene of one character assorts independently from the gene of another character during gametogenesis i.e. gene of different characters so present on different homologous chromosomes are independent of each other regarding their segregation.

This law can be explained with the help of dihybrid cross involving two characters at a time i.e. crossing pure tall pea plants having smooth seeds with pure dwarf pea plants having smooth seeds with pure dwarf pea plants having wrinkled seeds.

Material and reagents Requirments: Piece of thermocol or white sheet, four types of coloured beads, four ink sketch pens and pins.

Methodology:

By using colorful beads and capsule various examples can be demonstrated. This can be demonstrated with the help of dihybrid cross. This cross involves two characters at a time. For E.g. Round and yellow pea plants are crossed with round and green pea plants

Parents Genotype RRYy x rryy
Parents Gametes All RY All ry

F1 Generation All RrYy
F1 Gametes, 2 of each RY rY Ry ry

Punnett Square

of gametes produced

	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRyY	RRyy	RryY	Rryy
rY	rRYY	rRYy	rrYY	rrYy
ry	rRyY	rRyy	rryY	rryy

- Round and Yellow Phenotype
- Round and Green Phenotype
- Wrinkled and Yellow Phenotype
- Wrinkled and Green Phenotype

Experiment No. A.3: Segregation demonstration in preserved material i.e. Maize cob

Background: Monocotyledonous seeds i.e. cereals contain specialized storage tissue outside the embryo which is called endosperm. Embryo is formed by union of male gamete introduced by pollen grains with egg nucleus whereas endosperm is formed by double fertilization when second male gamete fuses with 2 polar nuclei giving rise to triploid fusion nucleus. In cross fertilization plant embryo and endosperm are hybrids whereas pericarp and fruits are maternal tissues. Effect of cross fertilization is evident when new generation is raised from seeds but its effect on endosperm will be immediately visible in the seeds. The immediate effect of foreign pollen grains on visible character of endosperm is called Xenia. It was discovered by Focke in 1881. Xenia is studied in Maize, if a white endosperm variety is open pollinated in fields where there are pollinated plants of yellow endosperm also. Then the cobs produced will contain mixture of yellow and white seeds. Yellow color is dominated over white color. When plants raised from yellow seeds are self pollinated, yellow and white seeds are produced in the ratio 3:1 which is typical Mendelian ratio.

Material and Reagent Requirements: Maize cobs, needle, Pencil, copy.

Methodology:

1. Take maize cobs containing yellow and white seeds.
2. Count the number of coloured and white seeds with the help of a needle
3. Calculate the ratio of yellow and white seeds.

Observations: (Take own observation by actual counting of grains)

Observed number of yellow grains= x

Observed number of white grains= y

Observed ratio is = x:y

Expected ratio is 3:1

Results : As per observed ratio between yellow and white grains, i.e. x:y which is almost near to expected ratio 3:1 so segregation occurs in maize which is typical

mendelian monohybrid ratio. Hence law of segregation is demonstrated in preserved material.

Precautions:

1. Count maize cob seeds properly
2. Carefully count the seeds with needle .
3. Avoid plucking of seeds from cob

Experiment No. 4: Testing of blood groups and Rh factors in human beings

Background: It was discovered by Lansteiner that human blood is differentiated into four different groups and this grouping is due to presence of specific antigen on the surface of RBC. Blood group differs chemically function person to person and when to different types of blood mix then RBC come together and clumping of RBC's takes place and it is known as Agglutination. It is due to the interaction of antibody and antigens which are present on the surface of RBC and the blood plasma respectively. There are two different types of antibodies 'a' and 'b' and antigens 'A' and 'B' in the blood. Different types of blood groups contains Antigen and antibodies

Blood Group	Antigen	Antibody
A	A	B
B	B	A
AB	A,B	-
O	-	a, b

Many people have Rh factors on their surface of RBC's . This is an antigen and those who have it is called Rh+ve and those who lack are called Rh-ve naturally in the blood plasma.

Material and Reagent Requirments: Slides, sterilized needles, cotton, Alcohol, antiserum A, B, D

Methodology:

1. Take a clean slide and mark the points A,B and D on the slide
2. Sterilize the finger with alcohol and give a bold prick with the help of sterilized needle
3. Discard first drop of blood with the help of cotton.
4. Put one drop of blood on ach marked position of slie
5. Put a drop of antiserum A on mark A, antiserum B on mark B and Antiserum D on marked point D.
6. Thoroughly mix the drops of blood with the help of pricked needle.
7. Allow 2-3 minutes for agglutination to take place.

8. Observe the slide for agglutination with naked eyes .

Precautions:

1. The slide should be neat and clean
2. Sterilised needle should be used.
3. Pricking needle should be used for mixing blood with antigen
4. Always discard first drop of blood
5. Visualize agglutination properly.

Experiment No.A.5: Dermatoglyphics; Palm print taking and finger tip patterns.

Background: Dermatoglyphics is the study of epidermal ridges of thick skin on the regions like finger, toes, and palm. It was first studied by purking in 1823 and was systematized by latonin in 1892. Dermatoglyphics is well known method for personal identification. It has also become clinical, scientific interest particularly in relation to abnormalities, syndrome pattern of dermal ridges are laid down in third month of pregnancy. The pattern of ridges separated by narrow grooves present on epidermis and dermis. Many genes elaborate in determining details of those patterns (polygenic inheritance) .They remain constant for whole life of individual except when there is destruction of skin. There can be no identical finger prints not even of twins.

Pattern of palm consist of triradius which are 4 in number. Named as ABCD. Also one axial radii't' is present. An important dermal diagnostic feature of palm is the triradii. Triradii is the meeting point of three spokes with the almost parallel ridges on the palm.

Finger tip pattern; there are ridges as well as triradii which fall into categories:

1. Arches- In this pattern ridges run from 1 side of finger to other without backward turn arches made.
2. Loops- One triradius follows a loop pattern ridges transfer from one side and return on same loops are of 2 types Ulna and radius
3. Whorls- Two radius always follows a whorl .In whorls ridges make a series of circle around the central point or core may be single or double whorls may be clockwise and anticlockwise
4. Composite- It is pattern having combination of two or more pattern. It has S-shaped

Material and Reagent Requirments: Carbon paper, mustard oil, towel, plain paper.

Methodology:

1. Wash hands with soap and water and dry with clean towel.
2. Apply mustard oil on the palm,

3. Each finger and palm is applied with mustard oil
4. Then fingers and palm are rubbed on carbon paper so that ink is uniformly spread on the finger.
5. Press the palm gently on white paper keeping fingers equidistant to get proportionate palm prints with all triradii. Record the pattern of fingers too.

Precautions:

1. Hand should be washed and dried.
2. Plain white paper should be used.
3. Carbon paper ink from ink pad should be applied uniformly on palm and fingertips.
4. Hard plane surface should be used for prints.

Experiment No. A.6: Preparation and study of mitosis slides from onion root tips by squash method.

Background: Mitosis is a type of cell division in which chromosomes replicate and become equally distributed both quantitatively and qualitatively into two daughter nuclei so that daughter cell come to have the same number and type of chromosomes as were present in the parent cell. It is the most common method of division. It leads to formation of somatic cells and thus is often named as somatic cell division. Mitosis is divisible into 3 steps:-

1. Interphase
2. Karyokinesis
3. Cytokinesis

Material and Reagent Requirements: Onion bulb, needles, glass slides, coverslips, burner, test tube, compound microscope, pair of scissors, acetocarmine, stain acetic acid, glass rod, petriplates, blotting paper.

Methodology:

(A) Growing of Onion Roots:-

1. Take a healthy bulb of onion.
2. Scrap the old roots from the base of condensed stem of bulbs
3. Grow the bulb by keeping it on a beaker filled with water. Base of bulb should touch the water
4. After 5-6 days root tips are appear 2-3 cm long cut the tips from the bulb with a pair of scissor between a fixed position.
5. Now fix the root tips in glacial acetic acid and absolute alcohol in ratios 1:3 from 24 hrs. Then shift the tips in 70% alcohol from further use.

(B) Hydrolysis of root tips:-

1. Take 2-3 root tips in a test tube containing 1N HCl.
2. Keep the root tips in oven at 60° C for 10 minutes.
3. It is done to separate the cell wall from the cells.

4. Wash the root tips with water 2-3 times to remove traces of HCl.

(C) Staining and Mounting:-

1. Take a hydrolyzed root tips.
2. Put it on a clean slide. Add a drop of acetocarmine stain.
3. Wash the root tips by back of needle glass rod.
4. Warm the slide on burner, washing the slides removes the stain so put a fresh drop of stain on material.
5. Put a cover slip over it and then keep the slide under microscope.

Observations: In root tips various types of stages of mitosis are seen.

Precautions:

1. Grow the root tips 1 week before performing experiment.
2. Cut root tips only and observe all stages in that portion.
3. Properly stain and gently heat it
4. Carefully observe the stages of mitosis in root tip.

Section B: Plant Tissue Culture

Experiment No. B.1 : Preparation of stock solutions of Murashige & Skoog (1962) medium

Background: Murashige and Skoog medium is a plant growth medium used in the laboratories for cultivation of plant cell culture. MSO was invented by plant scientists Toshio Murashige and Folke K. Skoog in 1962 during Murashige's search for a new plant growth regulator. It is the most commonly used medium in plant tissue culture experiments.

Major salts (macronutrients)

- Ammonium nitrate (NH_4NO_3) **1,650 mg/l**
- Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) **440 mg/l**
- Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) **370 mg/l**
- Potassium phosphate (KH_2PO_4) **170 mg/l**
- Potassium nitrate (KNO_3) **1,900 mg/l**

Minor salts (micronutrients)

- Boric acid (H_3BO_3) **6.2 mg/l**
- Cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) **0.025 mg/l**
- Cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) **0.025 mg/l**
- Ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) **27.8 mg/l**
- Manganese sulphate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$) **22.3 mg/l**
- Potassium iodide (KI) **0.83 mg/l**
- Sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) **0.25 mg/l**
- Zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) **8.6 mg/l**
- $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ **37.2 mg/l**

Vitamins and organics

- i-Inositol **100 mg/l**
- Niacin **0.5 mg/l**

- Pyridoxine · HCl **0.5 mg/l**
- Thiamine · HCl **0.1 mg/l**
- IAA **1–30 mg/l**
- Kinetin **0.04–10 mg/l**
- Glycine (recrystallized) **2.0 mg/l**
- Agar **10 g/l**

Material and Reagent Requirments: 2 liter Erlenmeyer flask for 1 litre prep, Stirrer, Droppers, Weighing balance for weighing out chemicals, sucrose and agar. Distilled water NaOH, HCl at 1 M, Micro and major elements, Magnetic stirrer and heater.

Methodology: (for 1 liter)

1. Add about 800 ml of distilled water to the 2 liter flask. We need a 2 liter flask for 1 liter of medium to contain boil-overs that will occur during the sterilization process.
2. Add the contents. Use a dropper to mix with some water . Swirl to mix completely or put on the stirrer.
3. Add sucrose and stir to dissolve the sucrose completely.
4. Check the pH and do NOT add the agar until after we adjust the pH). The pH will be around 5-5.5. Though plants generally like acid soil, this pH is too low for the agar to gel.
5. Adjust the pH to 5.7. NOTE: the plant tissue culture medium is NOT buffered so add the base or acid in small portions.
6. Add distilled water to the 1 liter line on the Erlenmeyer flask.
7. Add the agar (7-8 g/liter). The agar will NOT dissolve.
8. Cover with 2 layers of aluminum foil and put a piece of autoclave tape on the label area of the flask. Autoclave for 15 minutes at 15 psi (standard autoclave conditions).
9. Rinse the tubes out with distilled water and autoclave with the culture medium with caps.
10. Cool to about 60° C.

11. Aseptically, pipette or pour the warm liquid medium into the sterile plastic or sterilized glass culture vessels in a hood. The gel will set in about 1 hour.
12. Store, refrigerated after the medium has completely cooled. Wrap the entire rack in plastic

Precautions:

1. If you are using disposable 50 ml centrifuge tubes or plastic Petri plates, do NOT autoclave these for they will melt.
2. Pouring and making of media should be done under proper aseptic conditions.
3. Avoid clumping of agar by constant stirring while heating.
4. Cautiously heat the media to avoid burning.

Experiment No.B.2: Selection, preparation, sterilization and inoculation of explants

Background: In Plant tissue culture, Explant is a small part of plant which contains actively dividing cell. All these cells are considered to be totipotent so that whole of the new plants can be obtained from small explants collection. Surface sterilization, inoculation is done for establishing a true tissue culture. This is transitory step and requires a greater skill and extra care various explants are collected from healthy plant and sterilization is done before inoculation.

A. Selection of Explant:

It depends upon number of factors , different types of explants can be selected from different parts of the plant for raising virus free plants, Meristem culture is preferred. It depends upon the age and also upon physiological stage of the plant.

B. Prunning:

It is preferred in early morning because at that time, transcription rate is very less. The collection of explants should be done from fresh plant.

C. Sterilization: Bacterial and fungal Contaminants in the culture donot allow the culture to be successful so contaminated culture has to be discarded. To overcome this problem is the effective sterilization of the explants

Material and Reagent Requirements: Different explants such as shoot, apex, nodal buds, leaves, flower buds, mercuric chloride, Sodium hypochlorite, CaOCl_2 , Petri plates and double distilled water.

Methodology:

1. A suitable disease free plant is selected as per the requirements.
2. Take the explants and wash it in water or teepol water.
3. Fill the culture vessel containing explants completely with disinfectant solution to sterilize it.

4. Surface sterilization can be done by following method:
 - a) 1-4% solution of sodium hypochlorite
 - b) 7% saturated solution of calcium hypochlorite
 - c) 1% bromine water
 - d) 1% silver nitrate solution
5. Take out the sterilized explants in petriplates with the help of sterilised forceps
6. Explant is then cultured in media.

Precautions:

1. Aseptic conditions should be maintained.
2. Place explants in media without rupturing explants.
3. Tools should be sterilized.

Section C: Animal Tissue Culture

Experiment No. C.1 : Preparation and sterilization of glass ware.

Background: All the apparatus and liquid that comes in contact with culture or other reagents must be sterilized. The choice of the method depends upon the stability of materials at high temperature. Items that are resistant to heat e.g. metal, glass, and the mutable plastics are sterilized by dry heating sterilization by moist heat or autoclaving is also highly effective and can be applied to heat stable materials such as plastic sterilized by gamma (γ) radiation. Heat labile liquids such as are sterilized by micro pore filtration. If the glass surface is used for self propagation it must not only be cleaved but also carry correct charge. Caustic alkaline detergent make the surface of glass unsuitable for cell attachment require subsequent neutralization with dilute HCl / H_2SO_4 . But neutral detergent do not alter are used in tissue culture. Glass pipettes should be de plugged each time they are used. Glass pipettes must be washed carefully in pipettes washer.

Material and reagent Requirements :- Detergent, disinfectant, bottle brush, stainless steel, basket, aluminum foil, oven.

Methodology: (A) collection and washing of glass ware

- (1) Immediately after use coolest the glassware into detergent containing disinfects glass ware should be soaked in the detergent.
- (2) Overnight thoroughly rinsed anything added is corroded in detergents should be cleaned directly by hand without soaking i.e steel aluminum and copper.
- (3) Brush the glass ware by hand or with bottle or brush or rinse thoroughly .in four completely change of tap water followed by 3 change of ionized water.
- (4) After rising thoroughly insert water in stain less steel basket and dry upside down.
- (5) Cap the bottles with aluminum fort and then cool and store it

(2.) Sterilization of glass ware;-

- (a) Label glassware with sterile tape.
- (b) Place the glass ware in oven at 160°C
- (c) Make sure that the whole apparatus used for sterilization wrapped in a covering that will allow the steam to penetrate fiber can be used for wrapping but will be impermeable to dust.
- (d) Semi permeable transport nylon fiber can be used for wrapping and should be covered with tape and paper before packing.
- (e) Close and set oven at 160°C and allow it to cool down.
- (f) Switch off the oven after 1 hour and allow it to cool down.
- (g) Use sterilized glassware within 24 to 48 hour.

Precautions-:

- (1) The sterilizing agent sodium hypochlorite should be included in water to prevent only microbial growth in water
- (2) Before drying glassware make sure it has been to roughly rinsed tap water followed by distilled water.
- (3) Dry glass ware in inverted position.
- (4) Always sterilize glassware by dry heat to minimize the risk depositing toxic residue from sterilization.
- (5) Keep organic matter out of oven.

EXPERIMENT No. C.2: Sterilization of laboratory

Background : Contamination by micro-organism remain a major in tissue culture, bacteria, mycoplasma and fungal spores may be introduced, via, atmosphere operation many other sources. Aseptic techniques in animals cell culture laboratory aim to exclude containing by establishing and the culture can be widespread among to culture can be widespread among several culture correct b/w micro –organism in the environmental outside the culture and the pure in contaminated culture within the flask or dish.

Methodology:

ELEMENT OF ASEPTIC ANIMALS CULTURE LABORATORY:-

- (1) **QUIET AREA :-** IN the absence of laminar flow air cabinet a separate sterile room should be used for sterile work with laminar flow an area should be selected i.e. : free from air current from doors ,window etc activity should be equipment other that connected with tissue culture.
- (2) **WORKSPACE:** its essential to keep work surface clean and tidy start with completely clear surface with alcohol .bring the surface only those items are required for particular procdure.map off up any spoilage immediately with when you have finished a swab the surface again.
- (3) **PERSONAL HYGIENE:-** hands should be washed to reduce the bacterial count on the skin .surgically gloves may be warn and stepped frequently talking should be avoided while working in laminar flow.
- (4) **STERILE HANDLING :-** Swap the bottles before using them screw up bottles should be covered with aluminum foil to protected the neck of bottles and the screw caps after and before opening and closing the bottles .work close to the flame from being replaced .when bottles can be left before open and verticals but don't vessels and HEPA air filters . Mouth pipetting should be avoided should be avoided as it may cause mycoplasma infection.

- (5) LAMINAR AIR FLOW: - major advantage of working in laminar air flow is that working experiment is protected from dust and contamination is the constant flow of filtered air.

TYPES OF LAMINAR AIR FLOW:

- (1) HORIZONTAL FLOW: - the air flow is parallel to the surface.
- (2) VERTICAL FLOW: THE AIR FLOW down from the top of the roof on the work surface. Routine maintenance checks of primary filters are required .laminar flow should be sterilized by 5% phenol , disinfected in 70%

INCUBATOR:-Humidifying incubators are major source of contamination .they should be cleaned at regular intervals with non toxic detergent fungicides which is such as 1% CuSO_4 may be placed at the bottom of incubator to restarted fungal growth . Some incubators have the micro pore filtration and laminar air flow to inhibit the circulation of micro-organism.

BOXED CULTURE: It is advantage to enclose culture media dishes, plates, flasks, in plastic boxes. The boxes should be wrapped before use and allowed to dry in sterile air.

EXPERIMENT No. C. 3: PREPARATION OF HANK BLANCED SALT SOLUTION

Background: - Balanced salt solution is composed in organic salts and may include sodium bicarbonate and in some cases glucose. the composition of common balanced salt solution is given in table.bss form basis of many complex media e.g. eagle's minimal essential media is prepared with hank balanced salt solution (HBSS). HBSS is used as diluents for conc. of antibiotics and vitamins to make complex media. Balanced salt solution is also used for working or direction for short incubation to about 4 hrs. Choice of HBSS is dependent upon its CO₂ and bicarbonate conc. and use of solution for this diggeration or mono layer dispression for this purpose Ca²⁺, Mg²⁺ions are usually emitted from Hbss but also depends upon whether the solution will be used for suspension culture .Hbss used since cell aggregation and attachment hank balanced salt solution should be autoclaved below ph 6.5 to prevent Ca²⁺, Mg²⁺ions phosphates from precipitates and should be sterilized just before.

Material and reagent Requirements: inorganic salt, sterilization ultra pure water, HCl and water, Laminar hood pH meter, auto pipettes, weighing balance, filters assembling, filters of pore size 0.45µm, and sterilized glass ware.

Methodology:

- (1) Suspend content of commercially available or parented in organic salt in 950 ml of tissue. Culture grade water at room temperature with constant gentle shaking and stirring until all powder and do not heat the water
- (2) Add the required amount of water sodium bicarbonate to 1l of medium of sodium carbonate bicarbonate to medium and stir until dissolved
- (3) Adjust the ph to 0.2 and 0.3 until below the desired pH using 1N HCl and 1N NaOH. Since pH rises during culturing
- (4) Make up final volume 200ml with tissue culture grade water
- (5) Sterilize the medium immediately by filtering through a sterilized membranous filter with possibility of 2.3mm
- (6) Aseptically add sterilize medium into the sterilize container
- (7) Sterilize bss is prepared and store it to 2°C to 6°C

Precautions:

- (1) Preparations of conc. medium is not recommended since free amino acid and salt complex have low solution and solubility preparation in concentrated medium
- (2) pH and sodium bicarbonate conc. in medium are critical factors affecting cell growth this influence by amount of medium and volume of culture vessels i.e. surface to volume ratio.

EXPERIMENT No.C.4: PREPARATION OF MINIMAL ESSENTIAL GROWTH MEDIUM

BACKGROUND : It is a complex media contains nucleosides, tricarboxylic acid cycle intermediate and lipids and minerals the essential amino acid are requirements by most cell as a source containing a large no of amino acid including non essential supplement with cysteine metabolize by most cells as a source of energy and carbon

VITAMINS: it contains only water soluble vitamins biotin is presence in most of the complex media

SALTS: the salts are those of sodium potassium, magnesium calcium chloride sulphate, phosphorus and bicarbonate these are the major components contributing to the medium and sodium potassium .

Intracellular charge in case of to minimize cell aggregation and attachment sodium bicarbonate acts as a buffer.

GLUCOSE: ITS IS SOURCE OF ENERGY. It is metabolized principally by glucose to form pyruvate . Accumulation of lactic acid in the medium is evident in embryonic and transformed cells.

ORGANIC SUPPLEMENTS: - it includes protein, peptides, nucleosides, citric acid cycle intermediates, pyruvates and lipids.

HORMONES and GROWTH FACTORS: These are added to serum free media

ANTIBIOTICS: These are added to reduce contamination

METHODOLOGY:

- 1.Add all the chemical formulations as required and check whether it is complete or incomplete medium. Add other formulations required in incomplete medium
- 2.Take medium to laminar air flow hood with any other supplements that are required.
- 3.Unwrap the bottles and Swab with 70% alcohol.
- 4..Transfer the appropriate volume of each addition to the stock bottles to make correct dilution.

5. For 100 ml media requirements is Glutamine 200mM- 1ml, antibiotics -0.5 ml, Sera 10%- 10 ml. Use different pipettes' for each dilution.
6. Move each stock to the opposite side of hood when it is added and remove all the reagents once all are added.
7. Recap the bottle, label the bottle with addition
8. Store the media at 4° C or use directly.

Precautions:

1. During the preparation , complete solution care must be taken to ensure that all the constituents are dissolved and don't get filtered out during sterilization
2. Concentrated media are often prepared at low pH between 3.5- 5.0 to keep all the constituents in solution
3. Glutamine is added separately as it is unstable and it should be stored at frozen stage.