A Practical Handbook of Life Sciences

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By

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PREFACE

This book has been designed according to student's need for mastering realistic factors of biotechnology prescribed through numerous universities in India. A laboratory guide presents a mix of conventional methodologies with greater current tactics to satisfy the pedagogical wishes of all college students analyzing biotechnology.

This book consists of a huge range of various experimental tactics, imparting commands with the ability to lay out a path that meets their specific educational approach. This book could be similarly asset to establishments wherein existence technological know-how guides are offered. We experience that this book will lessen the time required for causes at the start of every laboratory consultation and consequently shop greater time, if you want to be to be had for sporting out the experiment.

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Writing a book is harder than we thought and more rewarding than we could have ever imagined. At the beginning, we would like to express our gratitude to 'The GOD'. It is because of The God Almighty that we exist in this world. God has gifted us (as Human Beings) a brain to hypothesize, analyze, courage to dream and motivation to achieve our dreams.

Dr. Nidhi Srivastava would like to thank her dad, mom, her husband and sons for their support, love and help. I (Dr. Gauri) would like to express my indebtedness to my parents and in-laws because I do exist in the present state due to their great efforts to raise and to groom me. My brother Mr. Raghav Singhal has also supported me, as and when I needed him. It is not that only family members who have touched me during the development of this book, but my friends Dr. Manvika Karnatak and my husband Mr. Pankaj Tayal Gupta enlivened my life with sweet memories, helping me in my hard times. None of this would have been possible without them. They stood by me during every struggle and all my successes.

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Dr. Gauri Singhal
Dr. Nidhi Srivastava

LAB EQUIPMENT

1) Analytical balance-

An analytical balance is a type of electronic balance to measure the small amount of any chemical in the sub-milligram range. It has a hollow chamber with a measuring pan enclosed with transparent glass doors so that air current or dust does not affect the measuring or operation of balance. The analytical scale measures the force that needed to counter the amount of any chemical in place of its actual mass and the calibration (taring) is made to compensate the gravitational changes. An electromagnet is also used to generate the force for sample countering and result through measuring the force needed to get balance. Such measuring devices are called electromagnetic force restoration sensors.

2) Autoclave-

Autoclave is used to sterilize the various supplies such as chemicals, glassware, media reagents etc. in a pressure chamber (15 psi) at high temperature (121 °C) for about 15-20 minutes. An autoclave is widely used in the sterilization process especially in the experimental work of medicine, microbiology, and tissue culturing. Apart from experimental work, autoclaves are also used in body piercing, tattooing, dentistry, funeral homes and prosthetics fabrications. Autoclaves vary in their size, capacity and functions depending on the work to be performed. Generally, large size autoclaves are used for the sterilization of laboratory glassware and equipment, surgical instruments and biomedical waste.

3) Centrifuge-

A centrifuge is a type of instrument that is used to rotate the sample around a fixed axis by applying a strong force perpendicular to the axis of spin i.e. in an outwards direction. It can be described as rapidly rotating containers present inside a chamber and a centrifugal force is being applied to these containers having samples. The centrifuge works on the principle of sedimentation. In sedimentation, the denser particles or molecules move in an outward direction while lighter particles are displaced and move towards the center due to centripetal acceleration. In laboratory centrifuge, the sample is placed inside the centrifuge tube and due to radial acceleration denser particles settle down to the bottom of the tube while lighter particles rise to the top.

A centrifuge machine can be of multiple types depending on the rotor design and intended use.

3.1 According to the rotor design

- **Fixed angle centrifuges** hold the sample container at a fixed angle relative to the central axis.
- In **swinging bucket (swinging head) centrifuges**, sample containers are attached to the central rotor with a hinge in contrast to fixed angle centrifuges. As the centrifuge is spun, hinges allows the sample containers to swing in outward direction.
- A **continuous tubular centrifuge** does not contain any individual sample container and have only one tube that is used for the handling of large amount of samples.

3.2 According to intended use

- **Laboratory centrifuges** used for the general laboratory purposes with overlapping capabilities. This includes super-speed centrifuges, clinical centrifuges and preparative ultracentrifuges.
- The sedimentation analyses of macromolecules are performed using **analytical ultracentrifuge** that uses the Theodor Svedberg principle.
- The presence of total red blood cells by volume percentage in whole blood is measured through **h**aematocrit **centrifuges.**
- **Gas centrifuges** such as Zippe-type centrifuges are used to separate the isotopes in gas phase.

4) Incubator-

Incubator is an instrument that used to maintain and grow the plant cell culture, animal cell culture and microbial cell culture by optimizing the culture conditions such as humidity, temperature, gas content (CO_2, O_2) etc. Incubators are very important instrument for the lots of experimental work especially in the field of microbiology, cell biology and molecular biology to culture both prokaryotic and eukaryotic cells. In a simplest incubator, adjustable heaters are attached to an insulated box that maintain the temperature up to 60 - 65°C and some incubators have slightly high temperature range i.e. up to 100°C. The most common temperature for bacterial and mammalian cells is around 37°C and for other organisms such as yeast cells, the optimum temperature is around 30°C.

Some specific incubators can also lower the temperature or control the CO_2 levels and humidity as it is important to cultivate mammalian cells where relative humidity is more than 80% to avoid evaporation. The slightly acidic pH is maintained by the controlling CO_2 level at 5%. There are two major types of incubators i.e. BOD and COD incubator.

4.1 BOD Incubator

Biological oxygen demand or BOD incubators also called low temperature incubators are majorly used in many research labs, pharmaceutical labs and hospitals. It has forced chilled air circulation by durable coaxial blower to get homogeneous temperature throughout the chamber and thus having more advantages over general incubator. A BOD incubator is ideal for the vaccine preservation, testing of life cycle and shelf life, and the study of synthesizing organisms, cell culture process and many more.

4.2 COD Incubator

Chemical oxygen demand or COD incubators are also called gassed incubators and are used to create as natural an atmosphere as possible to develop cell and tissue cultures. In a COD incubator, air flows through a sterile filter and is distributed evenly throughout the internal chamber to ensure uniform supply of CO_2 for optimum growth and maintenance of cell cultures. COD incubators are ideal for the medical research and pharmaceutical industry to cultivate cell cultures in a germ free environment.

4.3 Shaking Incubator

Incubators with shakers also known as environmental shakers are usually used for the cell cultures that need aeration and solubility. Shaking incubators use the stable temperature conditions and agitating orbital of varying speed with adjustable stroke length to affect the cell culture growth. These conditions provide the flexibility in speed and orbit to achieve better results in each experiment. Shaking incubators are equipped with microprocessor controls for speed and temperature adjustment, independent alarm and a universal shaking platform. These incubators are mostly used in the field of microbiology and cell biology.

5) Laminar flow cabinet-

A laminar flow cabinet or laminar flow closet also known as a tissue culture hood is a closed bench structure that is designed to prevent contamination from working samples. In a laminar flow cabinet, the air is passed through the HEPA filters and blown towards the user in a smooth flow. The laminar cabinet is generally made up of stainless steel without any gaps or joints to prevent bacterial or ant microbial spore entry. A UV-C germicidal lamp is also present inside the laminar cabinet to sterilize the interior and its content. It is recommended to switch off the UV lamp during working to limit the UV exposure to users that can cause cancer and cataracts.

6) Microscope-

A microscope is used to check or observe very tiny objects that are not visible to the naked eye. The technique of examining such tiny objects under the microscope is called microscopy. According to the light source and functioning, microscopes are of different types. The first invented and most common microscope is the optical microscope in which a source of light is used to examine the object. Other common microscopes used in science are electron microscopes further subdivided into scanning electron microscope and transmission electron microscope. Apart from an electron microscope, compound microscope, scanning probe microscope, ultra-microscope, and fluorescent microscope are also used to investigate the objects.

7) pH meter-

The pH meter is an electronic device that is used to measure the pH or hydrogen ion concentration or hydrogen ion activity in an aqueous solution. The pH indicates the acidity or basicity of a solution. A pH meter works by its probe that senses the amount of hydrogen or hydroxide ion concentration in any liquid or semi-solid solution that shows on the display of the meter. This sensing probe is a type of glass electrode that is connected to the electric meter and acts as a main part of the pH meter. The end bulb of any pH meter should not be touched by the hands and only be cleaned with the help of tissue paper gently.

8) Electrophoresis unit-

An electrophoretic unit is used to separate the charged molecules in an electric field. Generally, an electrophoretic unit is consist of a buffer chamber, electric wires, a glass plate for gel casting, combs for wells preparation, and a voltage source. An electrophoretic unit is used in all laboratories to separate DNA, RNA, and protein samples to check their applications in diverse fields. This unit is of different types according to the separation of molecules as horizontal unit is used for the separation of DNA molecules and a vertical unit is used for the separation of protein molecules. Protein molecules based on charge and mass are also get separated through a 2D electrophoretic unit that is a completely automated system.

9) Laboratory oven-

Laboratory ovens are used to provide uniform temperature throughout the chamber that performs various functions such as glassware or sample drying, annealing, polyimide baking, sterilization, and other related functions. Generally, a laboratory oven ranges from 0.9 to 1 cubic foot in size with temperature up to 340^0C. Other than research, laboratory ovens are also used in the technology, healthcare, and transportation industries.

10) Laboratory water bath-

A laboratory water bath is a type of container that is filled with heated water. The water bath is generally used for the incubation of a sample that needs constant temperature for a longer duration. For temperature control, all water baths have some digital or similar interface to set the desired temperature conditions. The water bath is used to heat the flammable reagents that cannot be heated using an open flame. Laboratory water baths have a higher temperature limit of about 100^0C. Samples that need heating temperature above 100^0C, can be heated using an oil bath, sand bath, or silicone bath.

11) Magnetic stirrer-

A magnetic stirrer is an instrument that mixes or stirs the liquid sample by employing a magnetic field using a magnetic bead. This instrument is generally used in biology and chemistry to mix biological samples and make uniform solutions that did not dissolve under normal conditions.

12) PCR machine-

Polymerase chain reaction or PCR is also called a thermocycler or DNA amplifier. As the name suggests, PCR or DNA amplifier is used to amplify the DNA segments. The machine possesses a thermal block with several holes. These holes are used to hold the PCR tubes containing the reaction mixture.

PCR machines play the function of replicating the DNA sections by heating the reaction mixture. During a typical PCR cycle, the temperature first rises to 95^0C which melts the double-stranded DNA to single-stranded. After DNA melting, as the temperature gets lower, the primers bind with them using different reaction reagents such as free nucleotides and DNA Taq polymerase enzyme. At the end of the first cycle, two strands of partially double-stranded DNA molecules have been formed. Thousands of copies of DNA molecules of the particular sequence have been formed after repeating this process again and again.

13) Spectrophotometry-

It is a technique by which we can measure the amount or concentration of a sample or quantify it. Spectroscopy is a dimension of the reflection or transmission properties of a sample as a function of wavelength. In spectroscopy, the sample holder can be of transparent or opaque glass.

14) Vortex mixer-

The vortex mixer is an instrument that has been used in all laboratories to mix small amount of liquid samples. An electric motor is placed inside the instrument that is attached to a cup-shaped rubber piece. This rubber piece rotates in a circular motion as the motor runs, and these motions are transmitted to the sample inside the tube or container. Through this process, the sample gets vortexed. Some vortex mixers have settings for variable speed that can be set according to the sample.

LABORATORY GLASSWARE

A variety of glass equipment is generally used for scientific experiments and other functions in the laboratory. Glassware in the laboratory is generally not as commonly used due to its cost and breakable nature. However, certain experiments in the laboratory cannot be done in plastic-ware because of chemical reactivity. Under these conditions, glass wares are used as they are chemically inert, heat-resistant, and transparent. There are different types of glasses that are used in experiments according to their applicability. In reagent bottles, borosilicate glasses are used that are heat resistant. Cuvettes are typically made of quartz glass, which is transparent, withstands high temperatures, and is used in spectrophotometric spectrum analysis. Dark brown or actinic glasses, which block the reach of UV and infrared radiation on chemicals, are used in the storage of light-sensitive chemicals. Various types of glass-wear that are commonly used in laboratories are discussed below.

1) Beaker-

A simple cylindrical-shaped container with a flat bottom used for mixing or heating reagents is known as a beaker. Most of the beakers have a beak or pout-like structure at the opening to help pour the reagents. On the basis of functions and volumes, beakers are available in a wide range of sizes, from one millilitre to several liters.

2) Burette-

A burette is a cylindrical, long tube used in analytical chemistry to measure or dispense the amount of chemical solutions. On the basis of volume transfer, burettes are of different types, such as volumetric burettes, which are used to deliver a measured amount of liquid solution. Another burette that is similar to a syringe is used to deliver liquid with more precision. Similar to a burette, a pipette is also used to deliver the liquid reagent. However, the burette differs from the pipette in that the amount or quantity of liquid reagent transferred is variable.

3) Graduated cylinder-

A graduated or measuring cylinder is a narrow cylindrical-shaped container that is used to measure the volume of any reagent. It has a marking on this surface that represents the amount of any reagents that have to be measured and is more accurate than other beakers and flasks.

4) Test tube-

Test tubes, or culture tubes, are glass containers of finger-like length that are open at the top and have a U-shaped bottom. Some large test tubes that are specifically used for boiling purposes are called "boiling tubes." Generally, test tubes come in a variable width and length. Typically, the length of a laboratory test tube is 50–200 mm and the width is 10–20 mm. Some test tubes come with a glass stopper or a screw cap.

PLASTIC WARES

1) Spreaders-

Plastic spreaders are made of pre-sterilized polypropylene to reduce the need for any other sterilization such as flaming or autoclaving. A Petri plate spreader comes as an L-shaped structure that is disposable and used for the even spread of bacterial or cell cultures on the entire Petri plate surface.

2) Centrifuge tubes-

Centrifuge tubes are made of plastic and are made with precision to exactly fit in the rotor cavity. Their size varies according to the capacity and type of rotor, ranging from 50 ml to a few microliters. Plastic centrifuge tubes are less costly and more durable than glass. In the micro-centrifuge, disposable eppendorf tubes with a capacity of 0.5 to 2 ml are commonly used. They are made up of flexible transparent plastics, semi-conical in shape, marked on the surface with sealing caps.

3) Petri plates-

A Petri plate or dish, also known as a cell culture dish, was named so after the German scientist Julius Richard Petri. He uses shallow cylindrical glass to culture the bacterial cells and calls them Petri plates. New Petri dishes come with a ring or slot-like structure on their lids and bases to avoid sliding off of one another. Petri dishes are made up of glass that is reusable and disposable.

4) Pipette-

A common laboratory tool that is used in biology, chemistry, or medicinal fields to measure and transport a certain amount of liquid is known as a pipette. Pipettes come with different precision, accuracy, and modes of function, from simple manual pipettes to more complex electronic pipettes.

5) Spatula-

It is a modified spoon-like structure that has a broad and flat blade that is used to mix the different components in any mixture. A spatula is made of stainless steel and is used to transfer, scrape or apply chemicals in laboratory experiments. Some spatulas are resistant to heat, solvents, acids, and bases, making them ideal for a variety of laboratory applications.

6) Wash bottle-

In a wash bottle, a nozzle is present with a squeeze bottle that is covered with a lid and is used to rinse different plastic or glass-ware. While applying pressure to the bottle, the liquid present inside the bottle is forced to move out of the nozzle as a narrow stream.

SAFETY LEVELS

While working in any laboratory, there is a risk of contamination or spillage of chemicals. To avoid this problem, laboratories are designed according to the experimental work to be done, from a low-risk zone to the highest risk zone. These zones are known as biosafety zones or levels. The biosafety levels are described according to the equipment, design features, containment facilities, construction of labs, experiments, and procedures required for working with certain microbial or chemical agents. These biosafety levels are classified as biosafety level 1 (basic, lowest risk), level 2 (basic, low risk), level 3 (containment, medium risk) and level 4 (containment, highest risk).

The biosafety level given to a certain work has been decided by the laboratory personnel based on the risk assessment rather than automatic assignment.

Risk assessment is the backbone of biosafety practices. The risk assessment is performed by the special laboratory personnel that are familiar with the characteristics of specific microbes, their contamination levels, required equipment, protocols, and procedures.

Table 1: Biosafety levels with their laboratory practices and types

Biosafety Level	Laboratory Practices	Safety Equipements	Laboratory Type
Level 1: Basic	Good Microbial Techniques	Not Required	Basic Teaching and Research
Level 2: Basic	Good Microbial Techniques with Personal Protective Equipments	Open bench with Biosafety Cabinet	Research labs in health and diagnostic services
Level 3: Containment	PPE Kit, controlled access of individuals, directional airflow	Biosafety cabinets with primary devices for all activities	Research labs in special diagnostic services
Level 4: Highest Containment	Level 3 practices with airlock entry, special waste disposal	Class III biosafety cabinets, double ended autoclave, filtered air	Dangerous Pathogen units

The most useful method for analyzing the risk of specific microbes is to list their risk groups. There are various references that are used to analyse the risk group of microbes, such as:

1. Possible consequences of microbial exposure
2. Pathogenic nature of microbial agent and its infectious dose
3. Primary pathway of microbial infection
4. Secondary pathways of microbial infection
5. Microbial resistant nature in environment
6. Suitable host for microbial infection
7. Availability of information after clinical laboratory reports.
8. Planning of laboratory activities such as sterilization, centrifugation, sonication etc.

9. Alterations in genetic nature of microbes to collect the information regarding varying host range, host sensitivity to microbe and their effective treatment system.

1. Biosafety levels 1 and 2

In every laboratory, an operational or safety manual is used to identify the potential hazards and to eliminate those hazards further. Good microbiological techniques (GMT) are the basis of this laboratory safety. In addition to suitable handling and operation of microbes, specialized laboratory equipment is essential for laboratory safety. Some important concepts in biosafety levels are as followed:

1. The warning symbol of biohazard should be displayed at the laboratory doors where microbes of risk group 2 or higher are handled.
2. The doors of laboratory should be closed all the time.
3. Only laboratory personnel are allowed to enter the working area of laboratory.
4. Restricted access to animal house.
5. Only experimental animal should be entered in the laboratory.

1.1 Personal protection-

1. Laboratory gowns should be worn all the time in the laboratory.
2. Suitable gloves should be worn during all experimental procedures that involve direct or indirect contact with body fluids

and infectious microbes. After usage, gloves should be discarded under aseptic conditions and hands should be washed.

3. Laboratory personnel should wash their hands after handling infectious materials and before leaving the laboratory.
4. Safety devices such as glasses and face shields should be worn to protect the eyes and face from any chemical splash and radiation.
5. Laboratory devices and clothing should not be worn outside the laboratory.
6. While working in a laboratory, only closed-toe footwear should be worn.
7. Eating and drinking are prohibited in the laboratory.

1.2 Guidelines for laboratory workers to handle microorganisms at Biosafety Level 1

The microbes handled at biosafety level 1 showed little or no evidence of infection to humans or animals. However, all the workers working in these laboratories should undergo a pre-employment health check-up to avoid any conditions. Quick reporting of any laboratory accident with infection is necessary, and all staff members should be aware of the importance of maintaining good microbiological techniques.

1.3 Guidelines for laboratory workers to handle microorganisms at Biosafety Level 2

A health checkup of employees is necessary to work at biosafety level 2 laboratories. Laboratory management should keep a record of every disease or infection and the absence of an employee.

1.4 Handling and disposal of contaminated materials

In laboratories of biosafety level 1 and 2, the procedure for identification and separation of infectious materials from other materials should be implemented. For this, various national and international regulations have been followed, such as:

1. Non-infectious materials should be reused and disposed of as general waste.

2. Infectious materials such as knives, needles, scalpels, and broken glasses should be collected in puncture-proof containers.
3. Reusable infectious materials should be autoclaved and washed.
4. Non-reusable material is autoclaved and disposed of.
5. Some infectious materials are for direct incineration.

2. Biosafety level 3

The biosafety level 3 laboratories are designed to work with microbes of risk group 2 that spread through aerosols and microbes of risk group 3. Biosafety level 3 laboratories require more stern operations and safety than level 2 laboratories. It is necessary to register the laboratories with national health authorities that fall into this category. The major modifications in biosafety level 3 laboratories are:

- Health and medical supervision
- Laboratory design and facilities
- Code of practice

2.1 Health and medical surveillance

The basic health and medical supervision that was followed in biosafety level 1 and 2 laboratories is also followed in level 3 laboratories, with a few changes, such as:

- It is mandatory to medically examine all the personnel who work in level 3 laboratories and maintain records of physical and medical history.
- After successful clinical valuation, a medical contact card is given to the employees with a picture, name, and other details of the cardholder that are compulsory to carry while entering the laboratory.

2.2 Laboratory design and facilities

The designs and facilities of biosafety level 3 laboratories are the same as level 1 and 2 laboratories with some modifications that are described as follows:

- The laboratory should be separated from other uncontrolled movement areas. Separation can also be achieved by adding an extra door to the entry gate of the laboratory. To stop the entry of unwanted contamination, a shower and clean clothing should be necessary.
- All the surfaces of laboratories, such as floors, walls, and ceilings, should be resistant to water. Openings of such surfaces should be sealed to prevent the spread of contamination.
- To stop the spread of contamination through the air, an air ducting system should be used.
- A separate hand washing station should be installed near each exit door to wash the hands.
- To maintain the direction of airflow, a controlled ventilation system with a monitoring system should be installed in laboratories to check the airflow.
- For decontamination, an autoclave should be used in containment laboratories. The infectious waste should be sealed in unbreakable and leak-proof containers and then transported according to the appropriate regulations.
- All the designs for facilities and operational procedures should be documented in biosafety level 3 laboratories.

2.3 Code of practice-

The codes of practice for level 3 laboratories are the same as those for biosafety levels 1 and 2 except for some modifications. Such as:

- The display of the biohazard symbol at the main entrance of the laboratory is necessary with the laboratory personnel's name and any specific conditions that have to be followed in the laboratory, such as immunization.

- Personal protective equipment (PPE kit) like head covers, scrub suits, wrap-around or solid front gowns, dedicated shoes, and shoe covers should be worn before entering the laboratory. These clothes should be decontaminated before washing.

- Any operation related to infectious material should be done in a biosafety cabinet.

3. Biosafety level 4-

Biosafety level 4 laboratories are considered the highest containment laboratories that work with risk group 4 microbes under the regulations of national or international health authorities. For the designing and operational activities of such laboratories, high-output consultation is required.

3.1 Laboratory design and facilities

The features of biosafety level 3, with added modifications, are applied to the level 4 laboratories. The main features are as follows:

- ***Primary containment***

  The effective primary control system can be one or a combination of the following laboratories:

  Class III cabinet laboratory

  A minimum of a two-door pathway is required before entering level 4 laboratories, and a Class III cabinet provides primary control for such functions. In addition, the laboratory personnel shower in inner and outer changing rooms before entering the labs. All the materials are double-autoclaved or fumigated for the removal of contamination.

Suit laboratory

A PPE kit is required to work with contagious microbes and materials in biosafety level 4 laboratories with class III safety cabinets. While entering the lab, a decontamination shower should be used after wearing the PPE kit and before leaving the laboratory.

- ***Controlled access***

  The maximum containment laboratory should be located in a separate space or within a secure building. The entry and exit of such labs should be through an airlock system.

- ***Controlled air system***

  Inside biosafety level 4 laboratories, negative pressure should be maintained and there should be HEPA filters attached to the entry and exit of the air gate.

- ***Decontamination of effluents***

  All the wastewater collected from level 4 decontamination showers, chambers, or laboratories should be neutralized to normal pH and decontaminated using heat treatment before discarding.

- ***Sterilization of waste and materials***

  In a level 4 biosafety laboratory, all the waste materials should be double autoclaved before leaving the containment area. The materials that cannot be autoclaved should be sterilized using steam treatment.

3.2 Code of practice-

The code of practice for level 4 laboratories is the same as level 3 except for some modifications, such as:

- There should be two people working in the laboratory, especially in biosafety level 4 facilities. No individual should work alone in these laboratories.

- Before entering and leaving the laboratories, personnel should change their clothes completely.

- Laboratory personnel should be trained in emergency extraction operations.

- There must be routine and emergency contact between laboratory personnel working inside the lab and support personnel working outside the lab.

WORK PLAN

While working in laboratories, simply performing the experiments is not enough. The reports should be maintained in a clear, concise, and accurate manner. The orderly outline of the work plan is given below:

1. Aim of the experiment
2. Introduction
    a) Objective
    b) Theory
3. Experimental details
    a) Requirement of reagents and equipment
    b) Procedure
4. Calculations
    a) Record of new data
    b) Method of calculations
    c) Final data in the form of tables, graphs or figure
5. Result and discussion
    a) Conclusion
    b) Significance of experimental data

BASICS OF LABORATORY SOLUTIONS

In order to understand the calculations, one has to understand the basic difference between solute, solvent, and solution.

- **Solute**: The reagent that has to dissolve. It is usually a solid chemical. An example: sugar is a solute in a sugar solution.
- **Solvent**: The dissolving reagent that is usually liquid in form. Example: water in a sugar solution.
- **Solution**: The mixture of solute and solvent is known as a solution and is clear in nature. Example: sugar solution.

Calculations and preparation of solutions:

1. Molarity (M)

Molarity is the number of molecular solutes dissolved in one liter of solution. To calculate the number of moles of any substance, divide the given weight of that substance by its molar mass.

$$M = \frac{moles\ of\ solute\ (mol)}{volume\ of\ solution\ (L)}$$

Example: what is the molarity of 25.5 grams of NaCl dissolved in 100 ml of water?

Solution: First calculate the moles of NaCl:

$$1\ mole\ of\ NaCl = \frac{given\ weight\ of\ NaCl\ (25.5\ gm)}{molecular\ mass\ of\ NaCl\ (58.5 g/mol)}$$

= 0.436 mol

Secondly, convert the 100 ml of water to liters,