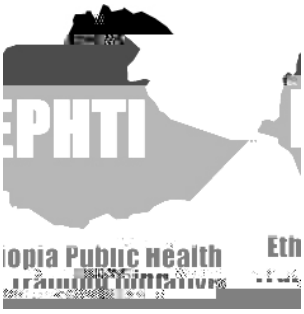
LECTURE NOTES

For Medical Laboratory Technology Students

Introduction to Medical Laboratory Technology



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PREFACE

There is acute shortage of references and / or textbooks in higher teaching institutions especially in newly opened institutions engaged in training of various health professionals in the country. Hence, some of the strategies that are used to circumvent these problems are developing of lecture notes on various subjects. Therefore, this lecture is developed to fill the existing gap and strengthen the teaching -learning processes.

This lecture note is primarily prepared for Medical Laboratory Technology students pursuing their studies at bachelorrate

trainees. Nevertheless, constructive comments and suggestions from readers are welcome so as to further strengthen this lecture note.

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LIST OF ABBREVIATIONS

1.	CSF:	Cerebrospinal fluid
2.	DNA:	Deoxy Ribos Nucleic Acid
3.	G:	Gravitational force
4.	Gm:	Gram
5.	Kg:	Kilogram
6.	Lab:	Laboratory
7.	M:	Molar solution
8.	Ml:	Milli liter
9.	MLT:	Medical Laboratory Technology
10.	Mv:	Milli volt
11.	Nm:	Nano meter
12.	PTB:	Pulmonary tuberculosis
13.	RPM:	Revolution Per Minute.
14.	RCF:	Relative Centrifugal Force
15.	UV:	Ultra Violet

INTRODUCTION

In the era of modern technology, health care delivery system involves so many different personnel and specialties that the caregiver must have an understanding and working knowledge of other professional endeavors, including the role of diagnostic evaluation. Basically, laboratory and diagnostic tests are tools by and of themselves, they are not therapeutic. In conjunction with a pertinent history and physical examination, these tests can confirm a diagnosis or provide valuable information about a patient status and response to therapy. In addition to these, laboratory findings are essential for epidemiological surveillance and research purposes.

If the entire network of a laboratory service is to be effectively utilized and contribute to health care and disease prevention, every member of its work force need to:

- Understand the role of the laboratory and its contribution to the nation's health service;
- Appreciate the need to involve all members in the provision of health service;
- Follow professional ethics and code of conduct;
- Experience job satisfaction and have professional loyalty.

Medical laboratory science is a complex field embracing a

Hematology, Clinical Chemistry, Urinalysis, Immunology, Serology, Histopathology, Immunohematology and Molecular biology and others.

Introduction to Medical Laboratory Technology is a basic course that equips the student with the most essential knowledge and skill pertaining to medical laboratories such as:

- Importance of laboratory services;
- Role of medical laboratory technologist;
- Use of laboratory wares, instruments and sterilization techniques;
- Prevention and control of laboratory accidents and;
- Institution of quality control system.

Moreover, this course is extremely important for the student as it paves the ways to easily understand various professional courses such as Hematology, Bacteriology, Urinalysis, Parasitology, and others. Hence, great emphasis should be given to this subject matter so as to train qualified, competent and task oriented medical laboratory technologists.

History of Medical Laboratory Science

It is difficult to exactly say when and where medial laboratory science was started. However, some early historical references have shown that there was examination of body fluid around the era of Hippocrates. The most important event

that contributes for the development of the profession was the discovery of microscope by a German scientist Antony Van Leoun Hook. Previously one cannot talk about the field of medical laboratory science without also talking abut the medical specialty of pathology. Early laboratory practioners were physicians, pathologists or both. But sooner medical laboratory profession was developed into a separate discipline having g its own educational requirements and standards.

In Ethiopia, the Italians were the first to establish health laboratory during The Second World War. Immediately after independence, a British Scientists took over health laboratory activity in Addis Ababa. They were organized the laboratory under the name of Imperial Medical Research Institute. After short period of time, they handed over the organization to the French Team on contractual basis. Then, the team developed the first well organized the laboratory under the name Institute Pasteur d' Ethiopie. Between 1955 and 1964, they established facilities for the production of vaccines and some diagnostic activities. Developing of rabies vaccine was the main research area for the team.

The name of the institute was changed into Central Laboratory and Research Institute.Finally; Ethiopian professionals took over the responsibility. Mean while, laboratory technician training programme was launched at

Salmonella typhi and others. Such laboratory should be clean, provide enough space, have adequate sanitary facilities and equipped with autoclave.

to another. These laboratories are usually a separate building with strictly controlled access.

1.3 Laboratory organization

Organization: - is a system, an orderly structure, putting things together into a working order, and making arrangements for undertakings that involve cooperations. The emphasis is on arrangements that enable peoples working together and accomplishing common objectives in an efficient, planned and economic manner. In a single medical laboratory at least there are two interlocking components of organizations. These are laboratory head and other staff having their own duties and responsibilities.

1.4

community. Such investigations usually include bacterial diseases, parasitic diseases and other causes of illness.

- Assist health care worker in deciding the severity of a patient's conditions.
- Collect and refer specimens for testing to the district laboratory.
- Notify the district hospital at an early stage of any laboratory results of public health importance and send specimens for confirmatory tests.
- Screen pregnant women for anemia, proteinuria, malaria, and refer serum for antibody testing.
- Promote health cares and assists in community health education
- Keep records, which can be used by health authorities in health planning and for epidemiological purposes.
- Keep an inventory of stocks and order supplies.
- Send an informative monthly report to the district hospital laboratory.

1.4.2 District hospital laboratory

Duties:

In addition to the works stated above, these laboratories have an important role in supervising the work of the peripheral

community based laboratories, testing referred specimens, and performing a range of tests compatible with the work of district hospital

Main activities are to:

- Perform a range of tests relevant to the medical, surgical, and public health activities of the district hospital.
- Support the work of the community-based laboratories by

  testing referred specimens, providing reagents, controls, standards, specimen containers, and other essential laboratory supplies. And also visit each primary health care laboratory in their catchments area to inspect and discuss the investigations being performed and, comment on their quality assurance system, record keeping, safety procedures, as well as the status of equipment maintenance.
- Refer specimens to the regional laboratory for test (s) that cannot be performed in district laboratory.
- Notify the regional laboratory of any result of public health importance and to send specimens for confirmatory tests.
- Participate in the external quality assurance programme organized by the regional laboratory.
- Prepare and send periodical reports to the regional laboratory.

1.4.3 Regional hospital laboratory

Duties:

In addition to the duties done at the two above lower levels, the regional laboratory assists and supervises the district laboratories. It analyses referred specimens and performs a range of specialized and other tests as required by the work of the regional hospital.

Main activities are to:

- Operate a regional blood transfusion center;
- Prepare reagents, controls, standard solutions and others as found necessary;
- Investigate epidemics and perform tests of public health importance in the region;
- Supervise and support the work of district laboratories;
- Send specimens that require special investigation to the central and public health laboratory;
- Prepare periodical reports and send to the central and public health laboratory.

1.4.4 Central and public health laboratory

The central and public health laboratory is responsible for

planning, advising and overall coordinating of medical laboratory services in the region.

Main activities are to:

- Formulate a professional code of conduct to medical laboratory personnel.
- Perform a range of special tests not normally undertaken in the regional laboratories such as viral, histopathological, cytological, immunological, forensic and genetic investigations.
- Carry out appropriate research of importance in order to mitigate public health problems.
- Evaluate new technologies and standardize techniques.
- Purchase supplies and equipments for the national laboratory service and organize an efficient system of requisition, distribution, and maintenance of equipment.
- Communicate and collaborate with International Organizations in promoting laboratory standards.
- Organize laboratory-teaching seminars and prepare training manuals for the different laboratory-training programmes.
- Support the work of the regional hospital laboratories.
- Organize refreshment training and seminars/ workshops for district and primary health care

laboratory personnel.

- Prepare training manuals for the different laboratory-training programmes.
- Participate in the prompt laboratory investigation of epidemics and outbreaks of serious illness among communities.

1. 5 Role of medical laboratory services

The medical laboratory services play a pivotal role in the promotion, curative and preventive aspects of a nation's health delivery system.

The service gives a scientific foundation by providing accurate information to those with the responsibility for:

- Treating patients and monitoring their response to treatment,
- Monitoring the development and spread of infectious and dangerous pathogens (disease causing organisms),
- Deciding effective control measures against major prevalent disease,
- Deciding health priorities and allocating resources.

Without reliable laboratory services:

1. The source of a disease may not be identified correctly.
2 Patients are less likely to receive the best possible care.
3. Resistance to essential drugs may develop and continue

to spread.

4. Epidemic diseases may not be identified on time and with confidence.

1.6 Role of medical laboratory technologist

Some of the major roles of medical laboratory technologist are to:

- Carry out routine and advanced laboratory tests using standard laboratory methods;
- Apply problem-solving strategies to administrative, technical and research problems;
- Conduct community – based researches in collaboration with other categories of health professionals;
- Provide professional consultancy on matters related to the establishment, renovation, upgrading and reorganization of medical laboratories of intermediate levels.

1.7 Medical laboratory rules, ethics and professional code of conduct

1.7.1 Rules of medical laboratory

A. Medical Laboratory request form

Many different types of laboratories requests are received daily in a medical laboratory. The format of laboratory

requisitions may vary from one health institution to the other. In many health institutions, the test request form serves as a test result report form. An efficient laboratory will be able to monitor the laboratory request and its requisition forms from the time the specimens arrive until the time that results are released.

Laboratory request form should be made in writing and provide the following information:

- The patients identification (full name, age, sex, address);
- Inpatient or out patient identification number;
- Specific test(s) required and date requested;
- Type of specimen provided;
- Name of medical officer requesting the test and to whom the report should be sent;
- Any other information relevant to the test requested.

B. Keeping of laboratory records

The laboratory must keep a record of all results and it should contain:

- Patient's identification (name, age, sex, full address),
- Type of the specimen (s),
- Type of test(s) done,
- Date and result(s) of the test (s).

A record of a test results must be kept by the laboratory as carbon copies, work sheets, or recording test results in registers. Whichever system is used, it must enable patients' results to be found quickly. Records of tests and results are required in the laboratory to issue copies to patients who have lost their results. Moreover, they are also required when preparing duty reports and estimating the workload of the laboratory.

C.

included in reports.

1.7.2 Professional code of conduct and ethics

The code includes those attitudes and practices which characterize a responsible medical laboratory technician and which are necessary to ensure that a person works up to the level of a recognized standard. The people receiving the service expect such a situation. Above all, a code of Professional conduct can upkeep our motivation and remind us that the medical laboratory profession is primarily dedicated to the service of the sick and the promotion of good health care.

Major codes of professional conduct are enumerated below:

1. Place the well - being and service of the sick above your own interests.
2. Be loyal to your medical laboratory profession by maintaining high standards of work and by improving your professional skills and knowledge.
3. Work scientifically and with complete honesty.,
4. Do not misuse your professional skills or knowledge for personal gain.
5 Never take any thing from your place of work that does not belong to you.
6. Do not disclose to a patient or any unauthorized person

the results of your investigation.

7. Treat your results and your patient's information with strict confidentiality.
8. Respect colleagues and work in harmony.
9. Be sympathetic and considerate to the sick and their relatives.
10. Promote health care and the prevention and control of disease.
11. Follow safety precautions and know how to apply first aid. (See chapter five)
12. Do not consume alcohol or any other abusive substances during working hours or when on emergency standby.
13. Use equipment and laboratory wares correctly and with care.
14. Do not waste reagents or other laboratory supplies.

1.8 Laboratory policies

Laboratory policies are those decisions, which are taken in consultation with other medical staff to enable a laboratory to operate reliably and effectively in harmony with other departments.

These polices usually cover:

A. Laboratory hour and emergency work

As far as possible there should be definite laboratory working hours. In peripheral laboratories, it is often more difficult to

Immuno Deficiency Virus (HIV), HBV, etc

7. If a specimen is to be sent by airmail to a referral laboratories, it must be packed with due regard to the correct postal regulations.

   These include:

   A Specimen (s) must be sent by airmail.

   B. Must be labeled clearly with permanent marker.

   C. It must be in sealed containers.

   D. The sealed containers must be placed in plastic bag (s).

D. Workload capacity of a laboratory

Workload capacity should be matched to the number of staff and their level of training, the size of the laboratory and the availability of laboratory facilities. Ideally, microscopic work (which is universal to all level of laboratories) per day should not exceed a total of four hours (example, a total of about 24 stool microscopy per day).

N.B: When the amount of work requested is beyond the capabilities of a laboratory, testing of specimens become unreliable and safety measure tend to be ignored. On the other hand, too little work can also lead to unreliable test results due to lack of concentration.

1.9 Solutions used in medical laboratory

Solution is a homogeneous mixture of two or more

substances. Solute is the dissolved substance, whereas solvent is a substance in which solutes dissolves to make the mixture. There are different types of solutions used in medical laboratory procedures. These include reagent solution, staining solution, standard solution and buffer solution.

1.9.1 Reagent Solutions

Any solution that is used in conjunction with a given sample and expected to produce a measurable or noticeable change is called a reagent solution.

Necessary care, including the followings should be taken in preparing a given reagent solution:

- Chemical selection;
- Following instruction of preparation;
- Using of accurate measurements of substances (ingredients);
- Using of appropriate type of glass or plastic wares.

1. 9.2 Staining solutions

Staining solutions are solutions that contain colored dyes. These solutions can contain basic, acidic or neutral dyes. Different stains are used in medical laboratories to give an artificial color for the substances to be identified from a given biological specimen (whole blood, body fluids, urine, etc.). The substances may be identified by their characteristic

reaction with the staining solutions.

Different types of blood cells, bacteria, parasites, and tissues together with their cellular elements can be stained by using

highest purity and can be used directly for the exact measurement of substances of unknown concentration in a given solution. These solutions include sodium chloride, sodium bicarbonate, potassium iodide, etc.

Primary standard solution should be made of substances that are:

- Free of impurities,
- Stable on keeping in solid state and in solution,
- Able to be accurately weighed or measured to give a solution of exactly known concentration,
- Not hygroscopic (does not absorb moisture) and vaporize at 20^{O}c.

2. Secondary standard solutions

Secondary standard solutions are solutions of lower purity and their concentrations are determined by comparison to primary standard solutions. Secondary standard solutions are used for analytical procedures after their concentration is already determined. Some examples of these solutions are nitric acid, hydrochloric acid, sulfuric acid, etc.

In the preparation of secondary standard solutions, the following points should be taken into consideration:

- Using analytical balance for weighing;
- Dissolving the weighted substance in the prescribed volume of solvent;
- Determining the exact concentration by comparison

against a primary standard solution;

- Diluting stock secondary standard solutions using exact measurements.

1.9.4 Buffer solutions

A buffer is a solution of a weak acid or base and one of its respective salts. Buffers are able to resist changes in the p^H. For example, if small amount of hydrochloric acid is added to a buffer solution, the hydrogen ion concentration (H^+) does not increase very much since it combines with the weak base of the buffer resulting in only slight decrease in p^H values. Further more, if a weak base is added to a buffer, the released hydroxyl ion (OH^-) will not result in a significant change in p^H. Buffers are used especially in Medical Chemistry when the p^H needs to be carefully controlled for the diagnostic procedures, such as in measuring enzyme activities.

1.10 Expressing concentration of solutions

Concentration of solutions should be accurately expressed for the appropriate use in the desired procedures. The units may generally be expressed in physical units, chemical units and proper name.

1.10.1 Physical Units

1. Weight of solute per unit volume of solution (weight per

of sulfuric acid (H_2SO_4) is 98. Therefore, one mole of H_2SO_4 contains 98 gm of H_2SO_4 per liter of solution.

A normal solution is a solution that contains one-gram equivalent weight of the solute in one liter of solution. The equivalent weight of H_2SO_4 is 98 divided for 2 (valancy of H_2SO_4), which is 49. Therefore, one normal solution of H_2SO_4 contains 49 gram of H_2SO_4 per liter of solution.

To convert the given concentration to the form of molarity, the following steps should be applied.

1. Calculate the density of that solution from the specific gravity.
2. Find the amount of substance per liter of solution (multiply density with percentage by weight).
3. Calculate the molar concentration using the following formula:

Molarity = $\frac{\text{Number of mole of solute}}{\text{Volume of solution in liter}}$

= $\frac{\text{Amount of substance (weight)}}{\text{Molar weight x volume of solution in liter}}$

Molarity (M) is amount of substance per unit mass of solvents and it is commonly expressed as mole/ Kg.

If the information about a solution in a given bottle is in the

form of percentage (weight by volume, that is w / v %), the concentration can be changed to molar solution using the following formula:

$$\textbf{Molarity (Mol / liter)} = \frac{\textbf{Gm \% (w / v) x 10}}{\textbf{Molar mass}}$$

Example: Convert 4% (w /v) NaOH into mol/liter of solution?

$$M = \frac{4\% \times 10}{40 \text{ (molar mass of NaOH)}}$$

$$\textbf{M} = \textbf{\textit{1M solution}}$$

$$\textbf{Normality} = \frac{\text{Number of gram equivalents of solute}}{\text{Volume of solution in liter}}$$

$$= \frac{\text{Amount of substance}}{\text{Equivalent weight x volume of solution in liter}}$$

$$\textbf{Equivalent weight} = \frac{\text{Molecular weight}}{\text{Valancy}}$$

1.10.3 Proper name

There are few instances where a solution is described by proper name as far as its concentration is concerned. Example; Benedict's solution (copper sulfate hydrated, sodium citrate, sodium carbonate and distilled water).

1.11 Dilution of solution

Dilution is a process by which the concentration or activity of a given solution is decreased by the addition of solvent. A dilution represents the ratio of concentrated or stock material of the total final volume of a solution.

1.11.1 Simple dilution

A general process of preparing less concentrated solutions from a solution of greater concentration.

1.11.2 Serial dilutions

A serial dilution may be defined as multiple progressive dilutions ranging from more concentrated solutions to less concentrated solutions. A serial dilution is initially made in the same manner as a simple dilution. Subsequent dilutions will then be made from each preceding dilution. Therefore it is a sequential set of dilutions in mathematical sequence.

Dilution is made to prepare:

- A working solution from the stock
- Measurable concentration of a sample (for reporting the actual concentrations of body-fluid constitutes) --- etc.

In the performance of dilution, the following equation is used to determine the volume (V_2) needed to dilute a given volume (V_1) of solution of a known concentration (C_1) to the desired

lesser concentration (C_2).

$$C_1xV_1=C_2V_2$$

Likewise, this equation also is used to calculate the concentration of the diluted solution when a given solution is added to the starting solution.

Example. To make 45 ml of 30% Solution from 70% solution.

C_2 = 30%

V_2 = 45ml

C_1 = 70%

$$V_1 = \frac{30 \times 45}{70} = \textbf{19.3 ml}$$

Therefore, 19.3 ml of 70% solution must be diluted with 25.7 ml of distilled water to obtain 45ml of a 30% solution.

Diluting body fluids and calculating dilutions.

In the laboratory, it is frequently necessary to dilute body fluids.

Diluting body fluids

To prepare a dilution of a body fluid.

Example:

1. To make 8ml of a 1 in 20 dilution of blood.

   Volume of blood Required = $\frac{8}{20}$ **= <u>0.4ml</u>**

Therefore, to prepare 8 ml of a 1 in 20 dilution, add 0.4 ml of blood to 7.6 ml of the diluting fluid.

2. To make 4ml of a 1 in 2 dilution of serum in physiological saline.

Volume of serum = $\frac{4}{2}$ = **2.0ml**

Required

Therefore, to prepare 4ml of a 1 in 2 dilution, add 2ml of serum to 2 ml of physiological saline.

B. Calculating the dilution of a body fluid.

- To calculate the dilution of a body fluid.

Examples:

1. Calculate the dilution of blood when using 50 micro liter (µl) of blood and a 50 µl of diluting fluid. Total volume of body fluid and diluting fluid.

   50 + 50 = **100**

1.12 Review Questions

1. Define laboratory and state the different classification of medical laboratories.
2. Describe the role of medical laboratory services.
3. State laboratory rules, ethics and professional code of conduct.
4. Explain how to collect, handle and transfer laboratory specimens.
5. Explain the use of solutions in medical laboratory.

CHAPTER TWO
LABORATORY WARES

Learning objectives

At the end of this chapter, the student will be able to:

1. State the different laboratory wares.
2. Describe the use of laboratory wares.
3. Explain the general cleaning and care of laboratory wares.

2.1 Laboratory Glass Wares

Laboratory glassware and plastic wares are widely used in medical laboratories. Glasswares are usually manufactured from boro-silicate glass.

Boro - silicate glass is a material with the following defined characteristics:

- Resistant to the action of chemical with the exception of hydrofluoric and phosphoric acid,
- Made to withstand mechanical breakage,
- Made to withstand sudden change of temperature.

Glassware produced from the soda lime type of glass does not fit the above requirements and is easily broken by mechanical stress produced by a sudden change of

temperature. Hardened glasses, such as Pyrex, monax, and firmasil have low soda-line content and are manufactured especially to resist thermal shock (high temperature). The walls of these vessels are generally thicker than those made from soda lime. The high proportion of boro - silicate increases the chemical durability of the glasswares.

Precautions

1. All glasswares must be handled carefully.
2. Breakage can some times be dangerous and may result in the loss of valuable and irreplaceable materials.
3. Flasks and beakers should be placed on a gauze mat when they are heated over a Bunsen flame. Gauze mat is made from asbestos and its function is to distribute the heat evenly.
4. Test tubes exposed to a naked flame should be made of heat resistant glasses.
5. If liquids are to be heated in a bath or boiling water, the glass contents should be heat resistant.

   N.B: Sudden cooling of hot glass should be avoided.
6. When diluting concentrated acids, thin walled glassware should be used since the heat evolved by the procedure often cracks thick glasswares. Examples:- hydrochloric and sulfuric acid.
7. Heat- expansion is liable to cr

not be tightly clamped.

8. Containers and their corresponding ground glass stoppers should be numbered in order to ensure direct matching when stoppers are replaced.
9. Because of the danger of chemical and bacteriological contamination, pipettes should never be left lying on the bench.

2.1.1 Volumetric Wares

Volumetric wares are apparatus used for the measurement of liquid volume. They can be made from either glass or plastic wares such as pipettes, volumetric flasks, cylinders and burettes.

2.1.2 Pipettes

There are several types each having its own advantages and limitations. Pipettes are designated as class “A” or “B” according to their accuracy. Class “A” pipettes are the most accurate and the tolerance limits are well defined that is, + 0.01, $\pm$ 0.02 and 0.04 ml for 2, 25, and 50 ml pipettes respectively.

Class “B” pipettes are less accurate but quite satisfactory for most general laboratory purposes. Significant errors will result if the temperature of the liquid pipetted is widely different from

the temperature of calibration. The usual temperature of calibration is 20^{o}C and this is marked on the pipette.

2.1.2.1 Volumetric pipettes

Volumetric pipettes are calibrated to deliver a constant volume of liquid. The most commonly used sizes are 1, 5, and 10ml capacities. Less frequently used sizes are those which deliver 6, 8,12, and so on ml. They have a bulb mid - way between the mouthpiece and the tip. The main purpose of the bulb is to decrease the surface area per unit volume and to diminish the possible error resulting from water film. The Volume (capacity) and calibration temperature of the pipettes are clearly written on the bulb.

They should be used when a high degree of accuracy is desired. The pipette is first rinsed several times with a little of the solution to be used, then filled to just above the mark. Then the liquid is allowed to fall to the mark and the tip is carefully wiped with filter paper. The contents are allowed to drain in to the appropriate vessel. A certain amount of liquid will remain at the tip and this must not be blown out.

N.B: The reliability of the calibration of the volumetric pipette decreases with an increase in size and, therefore, special micropipettes have been develop for chemical micro-analysis.

2.1.2.2 Graduated or measuring pipettes

Graduated pipettes consist of a glass tube of uniform bore with marks evenly spaced along the length. The interval between the calibration marks depends up on the size of the pipette.

Two types calibration for delivery are available.

These are:

A. One is calibrated Two types Tws4m15 Mohr). Tc.0808 Tw[Gradu598 0 One Bs c1.20l)]Tocsr ha

Beakers have capacities from 5 to 5,000 ml. They are usually made up of heat resistant glass and are available in different shapes. The type most commonly used is the squat form, which is cylindrical and has a spout.

There is also a tall form, usually with out a spout. Beakers are often supplied for heating or boiling of solutions.

2.1.6 Cylinders

Cylinders are supplied in 10 to 2,000 ml capacities. Some are made of heat resistant glass or plastic and some are fitted with ground- glass stoppers Measurement of liquids can be made quickly with these vessels, but a high degree of accuracy is impossible because of the wide bore of the cylinders.

2.1.7 Test tube

Test tubes are made of hardened glass or plastic materials that can withstand actions of chemicals, thermal shock and centrifugal strains.

They are used to hold samples and solutions, during medical laborat d≥s/o roce dupes. Thsle icluadesiampl grouno holowl centrifuet

2.1.8 Reagent bottles

Reagent bottles are used to store different types of laboratory reagents.

They are made from glass or plastics. Depending on their use, they are available in various sizes.

2.1.9 Petridishes

Petridishes are flat glass or plastic containers, which have a number of uses in the medical laboratory. They are used predominantly for the cultivation of organisms on solid media. They are made with diameters of 5 to 14 centimeter. To isolate, identify and study the characteristics of microorganisms it is essential to grow them on artificial media, and in routine bacteriology the most important requirement of a culture medium is its ability to allow detectable growth from a minute inoculum within the shortest period of incubation.

2.1.10 Funnels

There are two types of funnels that are widely used in a medical laboratory. These are filter funnel and separating funnel.

2.1.10.1 Filter Funnels

Filter funnels are used for pouring liquids into narrow mouthed

containers, and for supporting filter papers during filtration. They can be made from glass or plastic materials.

2.1.10.2 Separating funnels

Separating funnels are used for separating immiscible liquids of different densities. Example, ether and water.

2.1.11 Pestle and mortar

Pestle and mortar are used for grinding solids, for example, calculi and large crystals of chemicals. Those of unglazed portion have porous surfaces, and those of heavy glass are made with roughened surfaces. After each use always clean the pestle and mortar thoroughly. This is because chemicals may be driven into the unglazed surfaces during grinding, resulting in contamination when the apparatus is next used.

2.1.12 Laboratory cuvettes (absorption cells)

Cuvettes can be glass cuvettes or plastic cuvettes. Glass

immediately. If the cuvettes turn to cloudy it should not be used for any analytical procedures. Any scratch or white spot on glass cuvettes cannot be washed out with any solvent and therefore, disturbs absorbance of a given solution. Therefore, such cuvettes should be discarded. Glass cuvettes are the choice for photometry.

Absorption cells must be absolutely clean. Optical surfaces should not be touched, as grease smudges are difficult to remove. As soon as possible after each use, absorption cells should be rinsed and soaked in distilled water. When cleaning cells, a mild detergent should be used. Stubborn contaminants can be removed by soaking the cells in diluted sulfuric acid. Absorption cells should never be allowed to soak in hot concentrated acids, alkalis, or other agents that may etch the optical surfaces. When drying cuvettes, high temperatures, and unclean air should be avoided. A low to medium temperature often not to exceed 100^0C or vacuum or a combination of the two can be used to rapidly dry cuvettes.

2. 1.13 Cleaning of glasswares

It is clear that volumetric glasswares and glass apparatus must be absolutely clean, otherwise volumes measured will be inaccurate and chemical reactions are affected adversely.
One gross method generally used to test for cleanness is to fill the vessel with distilled water and then empty it and

examine the walls to see whether they are covered by a continuous thin film of water. Imperfect wetting or the presence of discrete of droplets water indicates that vessel is not sufficiently clean.

A wide variety of methods have been suggested for the cleaning of most glassware. Chromic-sulfuric acid mixture is the cleaning agent in common usage. It is imperative that glassware cleaning should be as mild as possible and should be appropriate to the type of contamination present.

Fats and grease are the most frequent causes of severe contamination present and it is advisable to dissolve these contaminants by a liquid solvent (water- miscible organic solvent) followed by water washing. The most widely used oxidant is a solution of sodium dichromate in concentrated sulfuric acid. Because of its oxidizing power, the solution, particularly when hot, removes grease and fats quickly and completely.

Cleaning solution, as a mixture, is not a general solvent for cleaning all apparatus but only for cleaning boro-silicate glasswares, including volumetric wares. Glass- ware is generally in contact with the mixture for 1 to 24 hours, depending up on the amount of grease or liquid present. After removal of the acid and draining, the glass ware should be washed out at least four times with tap water and then rinsed

three times with distilled water

N.B: New galss wares should also be washed and socked in 1% HCL since they are slightly alkaline while they are manufactured.

2.1.14 Cleaning of pipettes

Pipettes should be placed in a vertical position with the tips up in a jar of cleaning solution in order to avoid the breakage of their tips. A pad of glass wool is placed at he bottom of the jar to prevent breakage.

After soaking for several hours, the tips are drained and rinsed with tap water until all traces of cleaning solution are removed. The pipettes are then soaked in distilled water for at least an hour. Filing with water, allowing the pipette to empty, and observing whether drops formed on the side within the graduated portion make a gross test for cleanness. Formation of drops indicates greasy surfaces after the final distilled water rinse the pipettes are dried in an oven at not more than 110 ^{o}c.

Most laboratories that use large numbers of pipettes daily use a convenient automatic pipette washer. These devices are made of metal or polyethylene and can be connected directly to hot and cold water supplies. Polyethylene baskets and jars

may be used for soaking and rinsing pipettes in chromic acid cleaning solution.

2. 1.15 Cleaning of flasks, beakers, cylinders and other glass wares

Pour warm cleaning solution into each vessel and stopper or cover carefully. Each vessel should be manipulated so that all portions of the wall are repeatedly brought into contact with the solution. This procedure should be followed for at least five minutes. The cleaning solution can be poured from one vessel to another and then returned to its original container.

The vessels should then be rinsed repeatedly with tap water four times and finally rinsed three times with distilled water. It

2.2.1 Cleaning of plastic wares

After each use Laboratory plastic wares should be immediately soaked in water or if contaminated, soaked overnight in a suitable disinfectant such as 0.5% w/v sodium hypochlorite or bleach. Most plastic ware is best clean in a warm detergent solution, followed by at least two rinses in clean water, and ideally a final rinse in distilled water. The articles should then be left to drain and dry naturally or dried in a hot air oven, set at a temperature the plastic can withstand. A brush or harsh abrasive cleaner should not be used on plastic ware. Stains or precipitates best removed using dilute nitric acid or 3% v/v acid alcohol.

2.3 Review Questions

1. Explain the composition and uses of medical laboratory wares.
2. Describe the types and uses of medical laboratory funnels.
3. Discuss about the cleaning of different medical laboratory wares.
4. Explain the function of pestle and mortar in medical laboratory.

CHAPTER THREE
LABORATORY INSTRUMENTS

Learning Objectives

After reading this chapter, the student will be able to:

1. Identify the types and uses of laboratory balances.
2. Explain the advantages of laboratory refrigerators.
3. Describe the importance of ovens, water baths and incubators.
4. State the use of photometers and desiccators.
5. Identify the types and uses of microscopes.
6. State the basic components centrifuge

3.1 Balances

Balances are essential laboratory instruments that are widely used for weighing of various substances (powders, crystals and others) in the laboratory. For instance, to prepare reagents, stains and culture media, balances are required to weigh accurately and precisely within the needed range.

They should be kept scrupulously clean and located in an area away from heavy traffic, large pieces of electrical equipment, and open windows. To minimize any vibration, as interference that may happen, a slab of marble is placed

under the balance.

Balances in medical laboratory may be:

A. Rough balances (mechanical balances)
B. Analytical balances

3.1.1 Rough balances

Rough balances are several types. Some of them use sliding scale, some have a single or double pan (s) and others utilize dial - operated fractions. They are used for weighing substances, which do not call for extreme accuracy. While operating, they do not require mains electricity or battery power and are currently less expensive than analytical balances of the similar sensitivity.

Some rough balances weigh accurately to 0.1 gm of a substance.

Two - pan balance is a rough balance, which has two copper pans supported by shafts.

It is used:

- To weigh large amounts (up to several kilo grams)
- When a high degree of accuracy is not required.
  Example: 20.5 gm, 36. 5 gm, etc. The sensitivity of a two-pan balance is 0.5 gm.

The sensitivity of a balance is the smallest weigh that moves

the pointer over one division of the scale. For instance, if the sensitivity of balance is 1 mg, this means that a weight of at least 1.0 mg is needed to move the pointer over one scale. For routine laboratory purposes the sensitivity of a balance can be considered to be the smallest weigh that it will measure accurately. Usually the larger the amount of substance to go into a reagent, the least accuracy is required.

3.1. 2 Analytical balances

Nowadays analytical and electronic balances (single pan balances that use an electron magnetic force instead of weights) are the most popularly used balances in medical laboratories to provide a precision and accuracy for reagent and standard preparation. Analytical balance is a highly sensitive instrument. It may have two pans suspended from a cross beam, inside a glass case. It requires mains electricity or battery
(D.C) supplied power.

These balances are used:

1. To weigh small quantities usually in mili gram(mg) range
2. When great accuracy is required
   Example, 2.750mg, 0.330 mg, 5.860mg, etc
   Its sensitivity is 0.5 mg to 1 mg depending on the model.

N.B: The accuracy of a balance should be checked regularly

as recommended by the manufacturer.

3.1. 3 Use and care of balances

A balance is a delicate instrument that requires practical instruction in its correct use.

The following should be applied when using a balance:

1. Read carefully the manufacturer's instructions.
2. Always handle a balance with care.
3. Position the balance on a firm bench away from vibration, draughts and direct sunlight.
4. Before starting to weigh, zero the balance as directed by the manufacturer. If using a beam balance, check the position of the beam.
5. Weigh the chemicals at room temperature in a weighing scoop or small beaker. Never put the chemicals directly

11. Keep the balance clean, being particularly careful not to let dirt accumulate near the pivots and bearings.

3.2 Centrifuges

Centrifuge is equipment that is used to separate solid matter from a liquid suspension by means of centrifugal force. They sediment particles (cells, bacteria, casts, parasites, etc.) suspended in fluid by exerting a force greater than that of gravity. The suspended materials are deposited in the order of their weight.

There are many types of centrifuges, but the basic principle is the same, that is, the all use centrifugal force. When a body is rotated in circular movement at speed, centrifugal force is created that drives the body away from the center of the circular movement. The greater the outward pull due to rotation, that is centrifugal force, the more rapid and effective is the sedimentation. As a result, heavier elements are thrown to the bottom of the tube followed by lighter particles.

Centrifugal force increases with the speed of rotation that is the revolution of the rotor per minute and the radius of rotation. The actual sedimentation achieved at a given speed depends therefore, on the radius of the centrifuge. Most techniques requiring centrifugation will usually specify the required relative centrifugal force (RCF) expressed in gravity.

For example, an RCF of 2000 x G refers to a force 2000 times the force of gravity. Most centrifuge manufacturers specify both the RPM and G.

RCF (g) = $1.12x10^{-5}xr$ (in cm) $(rpm)^2$

Where;

RCF = relative centrifugal force.

r = radius from the shaft to the tip of the centrifuge tube.

rpm = Revolution per minute.

g = Gravitational force.

3.2.1 Basic components of centrifuges

1. **Central Shaft: -** It is a part that rotates when spinning is effected manually.
2. **Head: -** It is a part that holds the bucket and connected directly to the central shaft or spindle.
3. **Bucket or tube: -** Are portions that hold test tubes containing a given sample to be spined.

3.2.2 Classifications of centrifuges

3.2.2.1 Hand centrifuges

These centrifuges are:

- Operated by hand or water pressure and they are most commonly used in small laboratory for routine purposes,
- Used for preparation of urinary sediments and to

concentrate parasites from the given specimen and it is not advisable to use them to separate serum from whole blood.

- They are fixed type.

3.2.2.2 Electrical Centrifuges

Electrical centrifuges are those centrifuges that are operated by electrical power and produce high centrifugal force. They are used in most medical laboratories.

Based on their tube angle rotation, there are two types.

A. **Swing out head: -** This is the most frequently used type and the head is designed to swing the tubes to the horizontal position during centrifugation process.

B. Fixed head: - They have different angles. They are useful for certain laboratory techniques. Example, for agglutination tests in blood grouping using test tube method. There are some special types of centrifuge such as micro hematocrit centrifuge. It is a special type of centrifuge with a slotted plate in it. It is used for determination of packed cell volume or hematocrit values.

3. 2. 3 Kinds of centrifuges

1. Micro-centrifuges or serofuges.

2. Placing a centrifuge on a firm level bench out of direct sunlight, towards the back of the bench.
3. Whenever possible using plastic tubes made from polystyrene or autoclavable.
4. Always closing the centrifuge top before turning it on.
5. Always balancing the tubes that are being centrifuged. Tubes of the same weight should be placed directly opposite to each other. Tubes should also be of the same size and should also contain the same amount of liquid.
6. Increasing spinning speed gradually is important. That is if you are required to spine a mixture at 3, 000 rpm, first put the dial on 1,000 rpm. Give the centrifuge a chance to come up to that speed, then turn up the dial a little further until it reaches the desired 3,000 rpm. Five minutes are the usual time required to centrifuge most substances.
7. Never open the centrifuge while it is still spinning. Never try to slow it down with your hand. Most centrifuges have a brake, which will cause the centrifuge to stop faster.

3.3 Refrigerators

Refrigerators are physical means of preserving various laboratory specimens. They suppress the growth of bacteria and maintain the specimens with little alteration.

In addition to this, they are also used in the medical laboratory to preserve some reagents such as:

- Pregnancy tests kits,
- Rapid plasma reagin (RPR) test kits,
- Blood grouping anti sera and others which are kept in the refrigerators to prevent their deterioration which may happen if they stay at a room temperature.

Culture media are also preserved in refrigerators to avoid bacterial contamination from the environment. For routine uses, refrigerators are commonly set at a temperature of **2 to 8 ^{0} C**. There are also other deep freeze refrigerators with different ranges of temperature for example 0^{0}C to -70^{0}C, which are mostly utilized for research purposes.

N.B: When whole blood is preserved in refrigerators, it is essential that the temperature is maintained at **2 to 8 ^{0} c** to avoid damage of red blood cells.

3.4 Ovens

Hot - air ovens are instruments that are used for drying of chemicals and glasswares. They are also used for the sterilization of various glasswares and metal instruments. They consist of double walls that are made of copper or steel. They are heated by circulation of hot air from gas burners between the metal walls or by electrical mains. There is a thermometer on the top of the ovens and generally an automatic device (thermostat) is fitted to regulate the temperature.

3.5 Water Bath

A water bath is an instrument where water is heated and the set temperature is maintained at a constant level. It is used to incubate liquid substances. When only a few samples in tubes require incubating, it is more convenient and less expensive to use a dry heat block (dry bath incubator).

Chemical tests react best at a specific temperature. Many tests react at room temperature (18 to 22 ^{O}c) and others require a specific temperature as body temperature (35 to 37 ^{0}c). Such procedural requirements are met by using water bath. When the reactants in tubes are placed in a water bath, the water surrounding the tubes warms the substances in side the tube and it takes the same temperature as the water.

Use and Care of a Water bath

1. Read the manufacturer's instructions carefully.
2. Fill the bath and maintain its level with distilled water if unavailable with boiled water, preferably boiled and filtered rainwater. This is necessary to minimize salts depositing on the heater.
3. To minimize the growth of microorganisms in the water, add a bactericidal agent such as merthiolate at a dilution of 1 in 1000 to the water.

4. Before incubating samples check that the temperature of the water is correct using thermometer.
5. Ensure that the level of the water is above the level of whatever is being incubated.
6. Use the lid to prevent loss of heat from the bath and to minimize particles from entering the water. When removing the lid after incubation, take care to avoid any water entering uncapped tubes. Whenever possible, use capped tubes.
7. Clean the water bath regularly, taking care not to damage the heating unit. If there is a build up of scale on heater and sides of the bath, this can be removed by using lemon juice.
8. Unplug the bath from the wall socket when not using it, when there is an electric storm, and when cleaning the bath and carrying out any maintenance work.
9. Every three to six months, check the bath for correction.

Note: If you are using a boiling water bath and ovens, be sure you use heat resistant glass or plastic wares.

3.6 Incubator

Incubation at controlled temperature is required for bacteriological cultures, blood transfusion, Serology, Hematology and Medical Chemistry tests. For bacteriological cultures, an incubator is required whereas for other tests a dry

heat block or a water bath may be used. For the incubator, the air inside is kept at a specific temperature (usually at 37^{0}_{c}). When tubes are kept inside the incubator, they take the temperature of the incubator.

The appropriate temperature is obtained by means of temperature regulator and is maintained by a thermostat. This permits a more accurate temperature control.

Use and Care of Incubator

-Transmission: - refers to the situations where some portions

Concentration of test (C_t) = Absorbance of test (A_t) X Concentration of
Absorbance Standard (A_s) of standard (C_s)

or $C_t = \frac{A_t}{A_s} X C_s$

In colorimetric tests, the path is kept constant by using optically matched cuvettes usually of 10 mm light path distance or tubes of known light path distance. In selecting the correct band of wavelength to use, both the maximum absorbance and selectivity of the wavelengths for a particular substance need to be considered.

For the **Beer's - Lambert's law** to hold true, both the solution being tested and the instrument used to measure the absorbance must meet certain requirements.

These include:

A. Solution Requirements

The solution must be the same through out and the molecules of which it is composed must not associate or dissociate at the time absorbance is being measured. The substance being measured in the solution should not react with the solvent. Reagent blanks must be used to correct for any absorption of light by solvents. A reagent blank solution contains all the reagents and chemicals used in the chemical development of

the color but lack the substance being assayed.

B. Instrument Requirement

The instrument used in colorimetric tests must show satisfactory accuracy, sensitivity and reproducibility at the different wavelengths used. The cuvettes used in the instrument must be optically matched, free from scratches, clean.

Measuring instruments

Different types of instruments are produced for measurement of substances in a given colored solution, including colorimeter, spectrophotometer, absorptiometer, spectrometer and flame photometer. Some of the biochemical methods provide solutions of colored compounds while others are involved in a chemical reaction to yield colored solutions for the quantitative measurement of substances. Elementary colorimeter was used previously for the analytical purpose, but it is now totally superseded by the modern ones. Elementary colorimeters are prone to errors that may result due to differences in the individual ability to visually identify colors. So it is replaced by the modern photoelectric

B. Spectro photometer or absorption spectrometer.

A. Absorptiometer

It is called absorptiometer because it is the amount of absorbed light, which is, measured not merely color development. It provides a wider band of wavelength to determine the complementary diffracting radiation.

The components of this instrument include:

- Light source;
- Filter cells (cuvettes);
- Photosensitive detector system and;
- Galvanometer to measure the out put of photo sensitive element.

Theory of Absorptiometry

On passing white light through a colored solution, some part of the white light will be absorbed while the others are transmitted depending on their frequencies (wavelengths). For analytical purposes, we are interested in the extent of absorption of light energy by solutions of the same compound in known and unknown concentrations under identical conditions, which can be used to determine the unknown concentration.

B. Spectrophotometer

Spectrophotometer is an instrument, which measures light

absorbance at various wavelengths by producing a monochromatic light using a diffraction grating or glass prism. Light is passed through a monochromator to provide selection of the desired wavelength out of the spectrum to be used for the measurement. Slits are used to isolate a narrow beam of light and improve its chromaticity.

The light is then passed through the cuvette, where a portion of the radiant energy is absorbed depending on the nature of the substances in a solution. Any light not absorbed is transmitted to a detector, which converts light energy to electrical energy. A monochromator is a system of isolating radiant energy of a desired wavelength and excluding that of other wavelengths.

Spectral isolation can be accomplished by various means including the use of filters, prisms and diffraction grating. Method of producing the monochromatic light is different in spectrophotometers and absorptiometer. Filter photometer (absorptiometer) uses filter for wavelength isolation while a spectrophotometer isolates the light by a prism or diffraction grating system. The color intended to be measured should be due to the substance under investigation but not due to any of the reagents used. This is controlled by using reasent blank.

Flame photometry or flame emission spectroscopy

Flame photometry is a spectral method in which excitation is

caused by spraying a solution of the sample in a hot flame. A characteristic radiation is emitted in a flame by individual

chemicals stay for long period of time out of dessicators, they sometimes absorb water. When we are weighing chemicals

Major components of p^H meter are:

- Glass bulb electrode;
- Reference (calomel) electrode and;
- Potentio meter (sensitive meter) which measures the

following conditions:

- Continuous use;
- Protein solution that can poison the glass membrane;
- Dehydrating agents;
- Change of temperature and;
- Scratching or fracturing of the glass membrane.

When not in use, electrodes should be immersed in distilled water. New electrodes can be generated by immersing in 0.1 Molar solution of hydrochloric acid over night. Washing of the electrodes with distilled water before and after use is very important.

3.9.2 Standard short-range p

under the procedure;

- The color of the unknown solution is compared with the color of series of buffers;
- The p^H of the unknown solution is considered as the same with the p^H of buffer, which gave us similar color with the solution to be determined.

3.9.4 Precautions while using buffers

1. Calibrate with buffers having p^H values that bracket the p^H of the sample. For example, if the expected p^H

N.B: Calibration is to mean that the reading in the display on a measuring instrument is checked against a standard; any deviation that exists between the true value and the value displayed in the reading is determined.

3.10 Instruments for purifying water

The quality of water used in the laboratory is very crucial. Its use in reagent and solution preparation, reconstitution of lyophilized materials and dilution of samples demands specific requirements for its purity. All water used in medical laboratory should be free from substances that could interfere with the tests being performed.

In medical laboratory work, water of an appropriate quality and quantity is required for the preparation of:

- Standard solutions, buffers and controls;
- Various laboratory stains;
- Reagents used in Clinical Chemistry, Immunology, contrater N Tc-.erf1(Hciforeq

activities carried out in Immunology, Urinalysis, Hematology, Microbiology and other clinical test areas, Type II Reagent Water can be used when the presence of bacteria is tolerated. Type III Reagent Water can be used as a water source for preparation of Type I and Type II Water and for washing and rinsing of laboratory wares.

Depending on the requirements, available facilities and quality of the laboratory's water supply, the following instruments can be used to obtain water of adequate purity and quality.

3.10.1 Water distilling apparatus (Still)

Water distilling apparatus is an instrument that is used to purify impure water by a process known as distillation. Distillation is a process by which impure water is boiled and the steam produced is condensed on a cold surface (condenser) to give chemically pure distilled water that is water from which non-volatile organic and inorganic materials are removed. Distillation does not remove dissolved ionized gases such as ammonia, carbon dioxide, and chlorine.

Distilled water should be clear, colorless and odorless. Distilled water is sometimes found to be contaminated with non-volatile impurities that have been carried by steam in the form of spray. Example, sodium, potassium, calcium, carbonate ions, sulfate ions, etc.

3.10.2 Gravity water filter

Filtration is defined as the passage of a liquid through a filter and accomplished via gravity, pressure, or vacuum. Filtrate is the liquid that has passed through the filter. The purpose of filtration is to remove particulate matter from the liquid. When using a gravity water filter fitted with and reusable ceramic candle filter of 0.9 micro meter porosity, most bacteria, parasitic microorganisms and suspended particles can be removed from the water but not dissolved salts.

3.10.3 Deionizer

Deionizer is an apparatus used to produce ion free water.

Deionizartion is a process in which chemically impure water is passed through anion and cation exchange resins to produce ion free water. Deionized water has low electrical conductivity, near neutral p^H and is free from water-soluble salts but is not sterile. Cations, which may be present in the water such as calcium, magnesium and sodium, are exchanged by the cation resin, which in turn releases hydrogen ions. Anion impurities such as sulfate, bicarbonate, silicate, nitrate and chloride are exchanged by the anion resin, which in turn releases hydroxyl ions. Finally, the hydrogen ions combine with the hydroxyl ions to give ion - free water.

N.B: Deionizer resin can cause irritation if it is allowed to enter the eye or skin. It is therefore, advisable to wear plastic

gloves and protective eye goggles when filling the plastic tube.

3.11 Microscope

Microscope is an important device that enables us to visualize minute objects (animate and inanimate) that cannot be seen by our naked eye.

3.11.1 Major parts of microscope

A. Frame work of the microscope

This includes:

- **An arm (stand): -** The basic frame of the microscope to which the base, body and stage are attached.
- **A stage: -** the table of the microscope where the slide or specimen is placed.
- **A foot, or base: -** is the rectangular part up on which the whole instruments rest.

B. Focusing system

This encompasses:

- **Coarse and fine focusing adjustments**

  - **Course adjustment:-** The course focusing adjustment is controlled by a pair of large knobs positioned one on each side of the body. Rotations of these knobs move the tube with its lenses, or in some microscope the stage, up or

down fairly rapidly.

- **Fine adjustment:** - While low power objectives can be focused by the course adjustment, high power objectives require a fine adjustment.

- **Condenser adjustments:-** The condenser is focused usually by rotating a knob to one side of it. This moves the condenser up or down. The condenser aperture is adjusted by the iris diaphragm, which is found just below the condenser. The principal purpose of the condenser is to condense the light required for visualization.

C. Magnification system

This comprises:

- **Objectives:** - Objectives are components that magnify the image of the specimen to form the primary image. For most routine laboratory work, 10x, 40x, and 100x (oil immersion) objectives are adequate.
- **Eyepiece**

Eyepiece is the upper optical component that further magnifies the primary image and brings the light rays to a focus at the eye point. It consists of two lenses mounted at the correct distance. It is available in a range of magnifications usually of 4x, 6x, 7x, 10x, 15x and sometimes as high as 20x.

N.B: Based on their number of eyepiece, microscopes can be classified as monocular and binocular microscopes.

D. Illumination system

- **Condenser and iris**

- Condenser is a large lens with an iris diaphragm.
- The condenser lens receives a beam from the light source and passes it into the objective.
- The iris is a mechanical device mounted underneath the
- Condenser and controls the amount of light entering the condenser.

- **Mirror**
  - Mirror is situated below the condenser and iris.
  - It reflects the beam of light from the light source up wards through the iris into the condenser. The mirror is used to reflect ray or electrical light. Some microscopes have a built in light source.

- **Sources of illumination**

Day Light - A Microscope must not be used in direct sun light.
- Ordinary daylight may be sufficient for some work.
- Daylight, however, is scarcely enough for oil immersion work.

Electric light

An ordinary 60-watt pearl electric bulb placed about 18 inches from the microscope is sufficient for most routine work. Quartz halogen (quartz iodine) and other high intensity lamps are available and are very good light sources because they give excellent white illumination and do not blacken like ordinary tungsten lamps. Many microscopes are now provided with correctly aligned built-in sources of illumination, which use tungsten or quartz halogen lamps operating on 6,8or 12 volts through variable transforms.

- **Filters**

Light filters are used in the microscope to:

- Reduce the intensity of light;
- Increase contrast and resolution;
- Adjust the color balance of the light to give the best visual effect;
- Provide monochromic light;
- Absorb light;
- Transmit light of selected wavelength; and
- Protect the eye from injury caused by ultra-violet light.

3.11.2 Working principle of the microscope

A microscope is a magnifying instrument. The magnified

image of the object (specimen) is first produced by a lens close to the object called the objective. This collects light from the specimen and forms the primary image. A second lens near the eye called the eyepiece (ocular) enlarges the primary image converting it into one that can enter the pupil of the eye. The magnification of the objective multiplied by that of the eyepiece, gives the total magnification of the image seen in the microscope.

See the following example:

Objective Magnification	**Eyepiece** Magnification	**Total** Magnification
10X	10X	100X
40X	10X	400X
100X	10X	1000X

3.11.3 Resolving power of the microscope

It may be defined as the ability to level closely adjacent structural details as being actually separate and distinct. The increase in magnifying power is always linked to an increase in resolving power. The higher the resolving power of an objective, the closer can be the fine lines or small dots in the specimen which the objective can separate in the image. The resolving power of an objective is dependent on what is known as the numerical aperture (NA) of the objective.

The numerical aperture is a designation of the amount of light

entering the objective from the microscope field, i.e. the cone of light collected by the front lens of the objective (an index or measurement of the resolving power). It is dependent on the diameter of the lens and the focal length of the lens.

The following are the usual numerical apertures of commonly used objectives.

- 10 X objective ---------- NA 0.25
- 40 X objective ---------- NA 0.65
- 100 X (immersion oil) objective ------- NA 1.25

3.11.4 Working principle of an oil immersion objective

When a beam of light passes from air into glass it is bent and when it passes back from glass to air it is bent back again to its original direction. This has effect on oil immersion objective and affects the NA of the objective and consequently its resolving power. The bending effect on the objective can be avoided by replacing the air between the specimen and the lens with oil, which has the same optical properties as glass, i.e. immersion oil. By collecting extra oblique light, the oil provides better resolution and a brighter image.

3.11.5 Routine use of the microscope

A microscope must always be used with gentleness; care and the following should be noted.

1. Place the microscope on a firm bench so that it does not vibrate.
    - Make sure that it is not be exposed to direct sun light.
    - The user must be seated at the correct height for the convenient use of the microscope.
2. Select the appropriate source of light.
3. Place the specimen on the stage, making sure that the underside of the slide is completely dry.
4. Select the objective to be used.
    - It is better to begin examination with 10x objective.
    - The 10x objective can be used for adjusting the illumination and for searching the specimen before using a high power lens.
5. Bring the objective as close as possible to the slide preparation and while viewing in the eye piece slowly move the objective up ward with the coarse adjustment until the image comes into view and is sharply focused.
6. Adjust the light source until the illumination of image is at its brightest.
7. Focus the condenser.
   To do this, open fully the iris of the condenser. Using the condenser adjustment knob, focus the condenser on the details of the light source.
8. Adjust the aperture (opening) of the condenser iris according to the specimen being examined.
    -

oil to the specimen and swing the 100x oil immersion objective into place, then open the iris fully to fill the objective with light. Example, stained blood smear, acid-fast stain, etc.

3.11.6 Types of microscope

A. Compound (simple) microscope (routinely used in medical laboratories)
B. Phase contrast microscope
C. Dark field microscope
D. Fluorescence microscope

A. Compound (simple) microscope

Compound microscope is a light microscope, which is routinely used in medical laboratories of hospitals and/or health centers.

B. Phase contrast microscopy

Transparent microorganisms suspended in a fluid may be difficult and sometimes impossible to see. One method of making them more P1D72 is to use what is called phase contrast.

Value of phase contrast

Phase contrast is particularly useful for examining:

- Unstained bacteria, e.g. cholera vibrios in specimens and cultures;
- Amoebae in faecal preparations;
- Trypanosomes in blood, cer

- Promastigotes of leishmanial parasites in culture fluid;
- Trichomonas species in direct smears and cultures;
- Urine sediments.

C. Dark field microscope

It is an instrument used for lighting microorganisms suspended in fluid, enabling their structure and motility to be seen more clearly. It makes some living organisms visible, which cannot be seen by ordinary transmitted lighting.

Á Some microorganisms such as Traponema palladium stain poorly or not at all by routine staining techniques, and are not sufficiently refractile to be seen in unstained preparations. Such microorganisms, however, can be detected in wet preparation by dark - field microscopy, also referred to as dark ground illumination.

Á Using dark - field microscopy, motile microorganisms can be seen brightly illuminated against a black background.

To obtain dark - field, a system must be used which prevents light, from passing directly into the objective but allows enough light to enter the outer edge of the condenser to

Problems associated with dark field microscopy

Difficulties in using dark field microscopy may arise from:

- Imperfect focusing or centering of a dark ground condenser;
- Using a lamp that is not sufficiently bright;
- Using a slide that is not completely clean;
- A specimen which is too dense;
- A bubble in the immersion oil or insufficient oil contact; between the specimen and immersion oil objective or between the specimen and dark ground condenser.

D. Fluorescence microscope

PRINCIPLE

In Fluorescence microscopy, ultra - violet light, which has a very short wavelength and is not visible to the eye, is used to illuminate organisms, cells, or particles, which have been previously stained with fluorescing dyes called fluorochromes. These dyes are able to transform the invisible short wavelength ultra - violet light in to longer wavelength visible light. The fluorescent stained organisms, cells, or particles can be seen glowing (fluorescing) against a dark background.

This microscopy is widely used in the immuno - diagnosis of important bacteriological and parasitic diseases.

Values of fluorescence microscopy

Important applications of fluorescence microscopy include:

1. Examination of sputum and cerebrospinal fluid for acid-fast bacilli using an auramine staining technique.
2. Examination of acridine orange stained specimens for:
    - *Trichomonas vaginalis*;
    - Flagellates;
    - *Entamoeba histolytica* cysts;
    - Intracellular gonococci and meningococci and
    - Other parasites and bacteria.
3. Immunodiagnosis by indirect and direct fluorescent antibody tests.

3.11.7 Care, cleaning, and repair of the microscope

1. Care and cleaning

A microscope is a delicate instrument both mechanically and optically. Therefore, the following important points should be taken into considerations.

1. Always carry a microscope using both hands.
2. When not in use, a microscope should be protected from dust, moisture, direct sunlight and put in

microscope case.

3. Keep it standing in place ready for use, but protected by light cover.
4. In humid climate it is necessary to cover the microscope in a plastic bag with a drying agent (silica gel) over night to avoid molds growing on the lenses.
5. At the end of each day's work, the surface lenses of the objectives, eyepieces, and condenser should be cleaned using lens tissue.

N.B: Never clean the lens of the objectives and eyepiece with alcohol.

2. Repair of the microscope

Except for obvious and simple measures, if a microscope becomes damaged optically or mechanically, it is better to send it or the damaged part to a

Steps in PCR technique:

1. Denaturation of double stranded DNA into single stranded DNA.
2. Annealing or primer binding.
3. Final extension or DNA synthesis.

3.13 Flow cytometery

Flow cytometery is an instrument used to measure and quantify cells that are suspended in fluid medium. Example, determination of gametocytes (white blood cell types) from whole blood samples.

3.14 Automated analyzers

Automated analyzers are instruments that are used in medical laboratories to process a large number of laboratory tests quickly (i.e. hundreds or even thousands of tests can be done within an hour).

The methods avoid the use of manual methods such as:

1. Measuring and adding reagents;
2. Mixing samples and reagents;
3. Calibrating the assay;
4. Recording, analyzing and storing sample data.

3.15 Review Questions

1. Explain the functions of balance and centrifuge in medical laboratory.
2. Discuss why refrigerators and ovens are important in the laboratory.
3. Explain the use of desiccator and P^H meter.
4. Describe the different parts of simple microscope.
5. Write names of instruments that are used for purifying of water in medical laboratory.
6. Mention advanced instrument and / or machines used in medical laboratory.

CHAPTER FOUR

STERILIZATION AND DISINFECTION

Learning Objectives:

At the end of this chapter, the student will be able to:

1. Define sterilization and disinfection.
2. Identify the various ways of sterilization techniques.
3. Explain why materials are sterilized in medical laboratory.
4. Describe how to disinfect and decontaminate laboratory waste.

4.1 Sterilization

Sterilization is the process of destruction or elimination of all forms of microorganisms by phy

will be sucked in to them, causing contamination, if they are removed before the oven is cold. This is due to the contraction of hot air as it cools. This method is used only for glass or metal articles such as test tubes, petridishes, all glass syringes, and instruments.

4.1.1.2 Flaming

Metal spatula, glass slides, and cover slips may be sterilized by passing them through a Bunsen flame, with out letting them become red hot. Alternatively they may be dipped in metylated spirit, and the alcohol burned off. This procedure should be repeated two or three times.

be st 4.1.1.3 Boil t lettzed

for 1-2 hours. The addition of 2% sodium carbonate increases the disinfecting power of boiling water for 1-2 hours. Spores, which resist boiling water for 10 hours, have been killed within 30 minutes by the addition of sodium carbonate. Sodium carbonate also prevents the rusting of metal instruments.

N.B: This method is unsuitable if instruments are to be stored in a sterile condition.

4.1. 2.2 Steam under pressure (autoclave)

Autoclave is an instrument that operates by creating high temperature under steam pressure. Autoclaving is the most common, effective, reliable and practical method of sterilizing laboratory materials.

The principle of autoclave is that steam is kept at a pressure of 15 pound (lb) per square inch to give a temperature of 121^{0}_{C}, which will kill spores with in 15 minutes. At this particular temperature, pressure and time, all forms of lives are destroyed.

Steam is more penetrating than hot air, and will give up its latent heat on striking a colder object; there by raising the temperature of the object rapidly It is used to sterilize syringes, needles, glasswares, culture media, etc.

For most purposes, the following cycles will ensure

gravity displacement autoclave is not available. They are loaded from the top and heated by gas or electricity. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat is reduced. The pressure and temperature rise until the safety valve operates at a preset level, which is the start of holding time. At the end of the cycle, the heat is turned off and the temperature allowed to fall to 80^0C or below before the lid is opened.

4.1.2.2.2 Precautions in the use of autoclaves

The following guidelines can help to minimize risks while working with autoclaves.

1. 1.Proper use and care of autoclaves.
2. 2.Regular inspection of the chamber, door seals and gauges.
3. 3.The steam should be saturated and free from chemicals that could contaminate the items being sterilized.
4. Materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration.
5. The chamber of the autoclave should be loosely packed so that steam will reach the load evenly.
6. Slow exhaust setting should be used when

Some of the commonly used chemicals are hypochlorite solution (‘berekina’), phenolic derivatives and ethylene oxide.

Disinfection and decontamination of laboratory wastes

4.2.1 Disinfection

Disinfection is the process of destruction of pathogenic or potentially pathogenic microorganisms by chemical means. All specimens, cultures and other material that have been examined in a laboratory must be made non-infectious before being discarded or leaving the laboratory. This is necessary to protect all those health professionals working in the laboratory and the members of the general public who may handle the waste materials before its final disposal. Ideally all material should be sterilized. Unfortunately it is not always possible to achieve this. But the most recommended method is the destruction of the vegetative forms of all pathogenic organisms. Laboratory waste, which includes articles that will be reused, may be disinfected by chemical or physical means.

4.2.2 Decontamination using chemical disinfectants

Decontamination is the process of decreasing the virulence (ability to cause disease) of microorganisms by using different chemical agents.

Some of the chemicals that are most suited for this purpose

are indicated below.

A. Phenolic compounds

Phenolic compounds are a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict them their use. They are active against vegetative bacteria and lipid containing viruses, and when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against non-lipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (example, triclosan and chloroxylene) are among the more commonly used antiseptics. They are used at 2 - 5% volume/volume (v/v) concentration according to the

its activity.

C. Alcohol

Ethanol and isopropanol, at 70 -80% volume / volume (v/v) concentration in water, are useful for skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small surgical instruments. They are active against vegetative bacteria, fungi and lipid containing viruses, but not against spores. Their activity is improved by mixing them with formalin or hypochlorite. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.

D. Formaldehyde

Formaldehyde is a gas that kills all microorganisms and spores at temperatures above 20^0C. It is relatively slow acting chemical and needs a relative humidity level of about 70%. Five percent (5%) formaldehyde is widely used for decontamination and disinfection of enclosed volumes such as safety cabinets and laboratory rooms.

E. Glutaraldehyde

Like formaldehyde, glutaraldehyde is also active against vegetative bacteria, spores fungi and lipid and non- lipid containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores.

formaldehyde or boiling formaline. This is a highly dangerous process and that requires well-trained personnel. All windows, doors and others in the room should be sealed with masking tape or similar materials before the gas is generated. Fumigation should be carried out at an ambient temperature of at least 21^0

efficient.

N.B: The disposal of laboratory and medical waste is governed by various regional, national and international regulations.

4.3 Review Questions

1. Define sterilization and explain its purposes.
2. Discuss the various methods through which sterilization can be achieved.
3. Describe the most recommended method(s) of sterilization techniques.
4. Define disinfection and explain its advantages.
5. Mention some of the chemicals that are mostly used in the medical laboratory for disinfection and decontamination purposes.

CHAPTER FIVE
LABORATORY ACCIDENTS AND SAFETY

Learning Objectives

After completion of this chapter, the student will be able to:

1. Identify the different medical laboratory accidents.
1. Explain the possible factors contributing to medical laboratory accidents.
3. Carry out first aid for laboratory accidents.
4. Describe safe use and storage of chemicals and reagents.
5. Explain the importance of planning for safety and general precautions to avoid medical laboratory accidents.

5.1 Laboratory hazards and accidents

5.1.1 Chemical hazards

These apply to all who use chemicals in their work. It is wise to regard all chemicals as toxic unless you know other wise. The main dangers to the person are associated with toxicity, chemical burns and dermatitis. Chemical related risk of explosion and fire are also possible. Toxic symptoms may follow ingestion, inhalation or skin absorption. If eating,

drinking and smoking are prohibited in the laboratory, the risk is usually low. Benzene vapors are fire risk and inhalation may lead to chronic poisoning. Swallowing of strong acid or alkali causes corrosive poisoning. Strong acids or alkalis can also cause acid burns or alkali burns.

Some chemicals are carcinogenic and are regarded as the potential causes of tumors of the urinary tract. Example, 0rto-toludine.

Oxidizing agents, when they come in contact with organic matter or other readily oxidizable compounds frequently causes explosions.

The followings are examples for highly poisonous substances.

A. Solids

- Antimony
- Berilium
- Iodine
- Cyanides
- Phenol
- Oxalic acids

B. Liquids

- Nesselers reagent.
- Benzene
- Bromine
- Fluorine compound

C. Gases

- Hydrogen cyanide
- Hydrogen sulfide
- Carbon-mono oxide

B. Fire from sprit lamps, Bunsen burners, lighted tapers (e.g. when heating. Ziehl Nelsen stain, or from faulty electrical equipment or over loaded circuits.

C. Corrosive chemicals being spilt on the skin or ingested when mouth pipette.

3. Cuts

May be caused by:

A. Breakage.

B. Using glassware that is cracked or has damaged edges

C. Walking on glass chipping

4. Harmful effects of toxic chemicals

Can be caused by:

A. Inhaling fumes from toxic chemicals.

B. Ingesting toxic chemicals by mouth - pipetting

C. Skin contact with toxic chemicals

5. Injury from Explosions

These can be caused by:

A. Explosion of leaking gas.

B. Leaking gas exploding.

6. Electric shock

Can be caused by:

A. Faulty electrical circuit

B. In correct installation of equipment

C. Touching exposed live wires.

7. Fire

A significant fire risk exists in laboratories due to frequent use of matches and open flames in close proximity to highly flammable chemicals and reagents such as acetone, diethyl ether, methanol, methylated spirit, acid alcohol and stains that are alcohol based.

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Fire fighting equipment for laboratories should include:

- Buckets of water to extinguish paper and wood fire. Water; however, must never be used to extinguish an electrical fire or one caused by a flammable chemical;
- Buckets of sand or dry soil to smother flames and contain and extinguish a free flowing liquid fire;
- Fire blankets made from heavy cotton twill treated

an outpatient or blood donor collapses in the laboratory.

First Aid Equipment

An adequately equipped first aid box should be kept in the laboratory, in a place that is known and accessible to all members of staff. The box should be clearly identified by a white cross on a green background. It should be preferably made up of metal or plastic to prevent it being destroyed by pests and to protect the contests from dust and dirt. The contents should be inspected regularly.

Recommended contents of laboratory first aid box include

- Clear instruction on how to apply emergency treatment of cuts, bleeding, heat burns, chemical burns, chemical injury to the eye, swallowing of acids, alkalis and other poisonous chemicals, treatment fainting, electric shock, and how to perform emergency resuscitation.

N.B. The instructions should be read well in advance so that laboratory staff are very familiar with it and not waste time in reading and understanding them during an actual accident situation.

- Sterile un medicated dressing to cover wound;
- Absorbent cotton wool;
- Triangular and roll bandages;

- Sterile adhesive water proof dressing in a variety of sizes;
- Sterile eye pads;
- Roll of adhesive tape;
- Scissors;
- Sodium bicarbonate powder;
- Boric acid powder;
- 5% acetic acid;
- Magnesium hydroxide suspension.

Emergency treatment of cuts and bleeding

If the cut is small:

- Wash with soap and water;
- Apply pressure with a piece of cotton wool;
- Disinfect the area with a skin antiseptic such as tincture of iodine;
- Cover with a waterproof dressing;

  If the cut has been caused by contaminated glassware:
- Encourage bleeding for two minutes;
- Seek medical attention.

Emergency treatment of burns

Heat burns:

- Immediately immerse the burnt area into cold water or

- Immediately turn off the electricity from the mains;
- If the person has collapsed, send immediately for medical help and if the person is not breathing give artificial respiration until unit of assistance arrives.

5.4 Safe use and storage of chemicals and reagents

Even in the smallest laboratory, dangerous chemicals are used directly or incorporated into stains and reagents. Hence the correct handling and storage of hazardous chemicals is essential to prevent injury and damage. In addition to this, to reduce accidents caused by chemicals, labeling is very important.

5.4.1 Flammable chemicals

These include ether, xylene, toluene, methanol, ethanol, other alcohol, glacial acetic acid, acetone, and acetic anhydride. Alcoholic Romanovsky stains and acid alcohol solutions are also highly flammable.

Storage:

Flammable chemicals should be stored in a fire proof metal box at ground level, preferably in and out side cool and locked store. If a metal box is not available, at least a container well lined with tin foil should be used.

N.B: Only small quantities of flammable solvents should be

kept on lab, benches and **shelves.**

Safe Use:

Before opening a bottle containing a flammable solvent, check that there is no open flame such as that from a Bunsen burner. Do not light match near flammable chemicals.

N.B: Never heat a flammable liquid over a Bunsen burner or lighted gas.

5.4.2 Corrosive chemicals

Corrosive chemicals include strong acids such as concentrated sulfuric acid, nitric acid, glacial acetic acid, trichloroacetic acid, ortho - phosphoric acid, and caustic 1a30.02.fkali

Storage:

Highly toxic chemicals such as potassium cyanide must be kept in a locked cupboard. Stock solutions or solids of harmful and irritating chemicals should be stored safely in cap board, not on an open shelf.

Safe use:

Handle toxic, harmful and irritating chemicals with great care by wearing protective gloves. Always lock away highly toxic chemicals immediately after use. Keep the lab, well ventilated while the chemicals are being used.

N.B: Never mouth pipette any chemicals, instead use automatic pipette or dispenser or pipette filler.

5.4.4 Oxidizing chemicals

These chemicals include chlorates, perchlorates, strong peroxides, potassium dichromate, and chromic acid.

Storage:

Oxidizing chemicals must be stored away from organic materials and reducing agents. They can produce much heat when in contact with other chemical, especially flammable chemicals.

Safe use:

Handle oxidizing chemicals with great care. Most are dangerous to skin and eyes and when in contact with reducing agents.

5.4.5 Explosive chemicals

Heat, flame, or friction can cause explosive chemicals to explode. An example of explosive chemical is picric acid, which must be stored under water. If picric acid is allowed to dry, it can become explosive. This can occur if the chemical is left to dry in pipes without being flushed away with adequate amount of water.

5.4.6 Carcinogens

A chemical that can cause cancer by ingestion, inhalation, or by skin contact is known as a carcinogen. Chemicals with proven carcinogenic properties include benzene, 0rtho - tolidine, alpha and beta- naphthylamine, nitrosamines and selenite. The risk in handling of these chemicals is proportional to the length and frequency of the exposure and the concentration of the chemical.

Storage:

Carcinogens should be kept in closed containers and labeled as ‘carcinogenic, handle with special precautions’.

Safe use:

Always wear protective plastic or rubber gloves, and face mask when handling carcinogenic chemicals. Carcinogens must not be allowed to come in contact with the skin because some carcinogens can be absorbed through the skin such as beta - naphtylamine.

N.B: See safety symbols given in the appendix

5.5 Planning for safety

A laboratory should be planned not only for efficient work but also designed with a view to eliminate accidents.

The following are among the features of a safety designed or planned and organized laboratory.

- Adequate floor, bench and storage space for staff to work safely;
- Ample light is essential, especially in the examination areas of the laboratory;
- A sufficient supply of wall electric points to avoid the use of adapters;
- Overcrowding must be avoided;
- Good ventilation is essential with adequate provision of fume cupboards;
- There should be a system for marking ‘’**high risk’’**

specimens.

- Discard containers that contain infectious microorganisms after each use;
- The floor should be well constructed with a surface that is non-slippery, impermeable to liquids and resistant to those chemicals used in the laboratory;
- ain infectious microorganisms

not create any hazard for those working near by;

- The chances of an accident occurring in the laboratory are much reduced if:

  - Á Every one works in a tidy fashion;
  - Á Every one works with out rush;
  - Á Benches are clean;
  - Á Reagents returned to the shelves after each use;
  - Á

4. Use pipettes that are plugged with non- absorbent cotton wool or with a rubber tube attachment.
5. Never heat the bottom of a test tube. The liquid inside might sputter. Always heat the middle portion of the test tube, shaking gently. The mouthpart of the test tube should be facing away from the worker and other person.

6. Only boro - silicate glass wares can be heated over a Bunsen flame.
7. Only small quantities of inflammable liquids should be kept in the lab.

   Caution: Ether ignites at a distance of several meters from a flame. Hence, never place a bottle of ether on a workbench where there is an open flame (Bunsen burner, sprite lamp, etc.)
8. When lighting a butane gas (gas burner) always light the match and hold it to the burner before turning on the gas tap. Turn off the main valves of all bottles of butane gas every evening.
9. Eating, drinking and smoking in the laboratory should be strictly forbidden.
10. Students and junior staff must be aware of the dangers of unauthorized experiments.
11. Safe disposal of specimens and contaminated materials is highly essential.
12. Safe use and storage of different lab chemicals is

mandatory.

13. Safe use of electrical equipment is of paramount importance.
14. Adequately equipped first aid box should be available in the laboratory
15. Dangerous laboratory chemicals and specimens should be labeled clearly.
16. Laboratory equipments should be sterilized as required;
17. Using of Biological Safety Cabinets when appropriate. Biological

Safety Cabinets are designed to protect the laboratory personnel, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens.

N.B: It is extremely important to use **gloves** as a personal means of protection from various infectious agents while working in medical laboratories.

5.7 Review Questions

1. Describe the types and possible causes of laboratory accidents.
2. Explain the importance of first aid for laboratory accidents.
3. Discuss safe use and storage of chemicals and reagents.
4. Discuss the merit of planning for safety in minimizing laboratory accidents.

CHAPTER SIX
QUALITY ASSURANCE

Learning Objectives

After completion of this chapter, the student will be able to:

1. Explain quality assurance program.
2. Discuss about the types and causes of errors in medical laboratory.
3. Describe the steps in quality assurance program.
4. Discuss the difference between internal and external quality control programme.

All laboratory personnel must be aware of how the quality of their work affects the medical diagnosis and treatment of patients. Laboratory tests s

together the various activities in the laboratory that are designed to detect, control and hopefully prevent the occurrence of errors. In other words, quality assurance is the overall program that ensures the final results reported by the laboratory are correct as much as possible. It is not a single activity nor the responsibility of a single individual but must be

6.1.1 Unpredictable errors

The unpredictable errors could be systematic shift, systematic trend and wild errors.

1. Systematic shift

When six or more consecutive daily values distribute themselves on one side of the mean value line, but on maintaining a constant level, the chart is said to have taken a shift. There could be an upward or dawn ward shift. Deteriorated standard, new standard prepared at a lower concentration or reagents which are shifted to a new level of sensitivity could all result in an upward shift. Dawn ward shifts are as a rule caused by conditions that are opposite to those causing upward shifts.

2. Systematic Trend

It refers to an increasing or decreasing test results for the control in a quality control chart over a period of six consecutive days. They may be caused by the deterioration of one or more reagents, changes in standard solutions, incomplete protein precipitation, etc.

The distinguishing feature between a shift and a trend is that the values in a shift do not continue to rise, but instead the distribution of these values is away from the mean on one side or the other.

3. Wild Error

Temporarily adjustable deterioration in test conditions caused by a single ‘wild’ event (sudden event). Example, use of chipped pipette, wrong dilution or improper technique.

6.1.2. Predictable errors

These are irregular random errors within a predictable range and caused by intrinsic properties of the test method due to chances. Such errors follow normal frequency distribution. Example, use of wrong wavelength, touching cover glass with objectives of microscope, improperly calibrated reagent dispensers. In predictable errors, the results differ from the correct result by varying amount (it can be due to carelessness).

Quality assurance program has three steps.

These are:

1. Pre analytical step
2. Analytical step (quality control)
3. Post analytical step

1. Pre analytical step

It includes precautions that should be done before performing different laboratory tests. Some of the activities are patient identification, preparation of patients, collection and labeling of specimen, preparation of standard operating procedures and selection of appropriate method for the particular test. During method selection, besides other factors like its cost

and availability, the precision, accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of the procedural method should be considered.

Precision:

Precision of measurements are concerned with the agreements between replicate analysis or it is the expression of the variability of analysis. It is completely independent of accuracy or truth and a method can be precise as determined by repeat analysis but the result can be inaccurate.

Accuracy:

Accuracy is concerned with the relationship of a set of results to the true value. This relationship is most conveniently measured by relating the mean of the replicate analysis to the true value. For effective diagnosis and management of patients, a method with high precision and accuracy must be used.

N.B: Precision dose not mean accuracy, because measurements may be highly precise but inaccurate due to a faulty instruments or techniques.

Sensitivity:

Sensitivity can be defined in two ways. The first one is the ability of a diagnostic test to detect very small amounts of the analyte. The other is the ability of a test to detect truly infected

sputum is highly specific for diagnosing pulmonary tuberculosis, because it gives only a few false positive results.

Positive predictive value (PPV):

It can be defined as the percentage of positive results that are true positives when a test is performed on a defined population containing both healthy and diseased persons. It depends not only on the specificity and sensitivity of the test, but also particularly on the prevalence of the disease in the population tested.

$$PPV = \frac{\text{True Positive}}{\text{True positive + False Positive}} \times 100$$

Negative predictive value (NPV):

Negative predictive value is the frequency of non- infected individuals among all persons with negative results. This also depends on the prevalence of a given disease.

$$\textbf{NPV} = \frac{\textbf{True negative}}{\textbf{True negative + False Negative}} \times \textbf{100}$$

N.B: A method used for diagnosis and follow up should not only be accurate and precise but also sensitive and specific and, should have high positive and high negative predictive values.

2. Analytical step (quality control)

Quality assurance refers to those measures that must be included during performing of the test. Its primary concern is control of errors in the performance and verification of test results.

Analytical methods are usually monitored by analyzing control materials. This control material that will check the quality are usually run simultaneously and under the same condition as unknown specimen. There are two types of quality control programs. These are Internal quality control and external quality control.

Internal quality control (IQC)

These are controls included with the test kit or prepared in the laboratory and tested with patient samples. They are essential for quality control measures for each run and are intended for use only with the lot number of corresponding test kit. An internal quality control programme should be practical, realistic and economical.

An internal quality control programme should not attempt to evaluate every procedure, reagent and culture medium on every working day. It should evaluate each procedure, reagent and culture medium according to a practical

schedule, based on the importance of each item to the quality of a test as a whole. Internal quality control should involve continuous monitoring of test quality and comprehensive checking of all steps, from collection of the specimen to issue of the report.

External quality control (EQC)

External quality control evaluates a laboratory analytical accuracy by comparing its results with the results on the sample or similar samples analyzed in a referral laboratory. The external control samples sent to the laboratory must be given to the technician along with other samples without them being identified as external quality control samples.

The purposes of external quality control programme are:

- To provide assurance to both health professionals and the general public that laboratory diagnosis is of good quality,
- To assess and compare the reliability of laboratory performance on a national scale,
- To identify common errors,
- To encourage the use of uniform procedures,
- To encourage the use of standard reagents,
- To take administrative measures against substandard laboratories,
- To stimulate the implementation of internal quality control programme.

3. Post analytical step

This refers to correct transcription, reporting, recording and storage of specimens, if necessary; for further investigations. Thus, quality assurance can be summarized as the right result at the right time, on the right specimen, from the right patient, with interpretation based on correct reference data.

Finally, quality assurance is the most important part of any laboratory and each department or section should develop and practice its own quality assurance programmme as a routine activity.

6.2 Review Questions

1. Define quality assurance program and explain its advantages.
2. Describe the different steps in quality assurance program.
3. Define precision, accuracy, sensitivity and specificity and discuss their importance.
4. Explain the difference between internal and external quality control programmes.

GLOSSARY

1. **Accuracy: -** is the measure of the nearness of a result to the absolute or true value.
2. **Aerosols: -** airborne droplets.
3. **Antiseptic: -** A substance that inhibits the growth and development of microorganisms without necessarily killing them.

   Antiseptics are usually applied to body surface.
4. **Chemical germicide: -** A chemical or a mixture of chemicals used to kill microorganisms.
5. **Decontamination: -** Any process for removing and/ or killing microorganisms.
6. **Disinfectant: -** A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
7. **Disinfection: -** refers to the destruction of pathogenic or potentially pathogenic microorganisms by physical or chemical means ; but not necessarily spores.
8. **Ethics: -** Are moral principles that govern or influence a person's behavior.
9. **Immiscible liquids: -** are those liquids that cannot dissolve or mix together.
10. **Oven: -** is a compartment or receptacle for heating or

drying by means of heat.

11. **Pathogens: -** are microorganisms that can cause diseases.
12. **Precision: -** is the degree of agreement between repeated measurements.
13. **Sensitivity: -** the ability of a diagnostic test to detect very small amounts of the analyte.
14. **Specificity**: - It is the ability of a method to identify all samples which do not contain the substance being detected (identify non - infected individuals correctly).
15. **Sporocide: -** A chemical or mixture of chemicals used to kill microorganisms and spores.
16. **Spout: -** is a material, which is used to pour liquid to the narrow containers.

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ANNEX

LABORATORY OPERATIONS MANUAL AND REAGENT PREPARATION

Each laboratory should have an operations manual, covering the following:

1. Cleaning of the working space;
2. Personal hygiene;
3. Separation of working areas from eating and smoking areas,
4. Safety precautions;
5. Handling and disposal of infected materials;
6. Appropriate immunization;
7. Care of equipments;
8. Collection of specimens;
9. Registration of specimens,
10. Elimination of unsuitable specimens;
11. Processing of specimens;
12. Recording of results;
13. Reporting of results.

Operation of Rough Balance

1. Place apiece of paper on the right hand pan and another of equal size on the left hand pan.
2. The balance must now be adjusted so that the pointer rests exactly at the mid point

This preparation is best carried out in a sink.

Store in a glass- stoppered bottle or covered glass jar. Clear supernatant solution should be decanted from the bottle each time it used.

The solution may be used repeatedly until the reddish color of the dichromate has been replaced by the green color of the chromate ion.

N.B: Do not allow this cleaning solution to come into contact with your clothing. This is because it will burn the skin severely and destroy clothing.

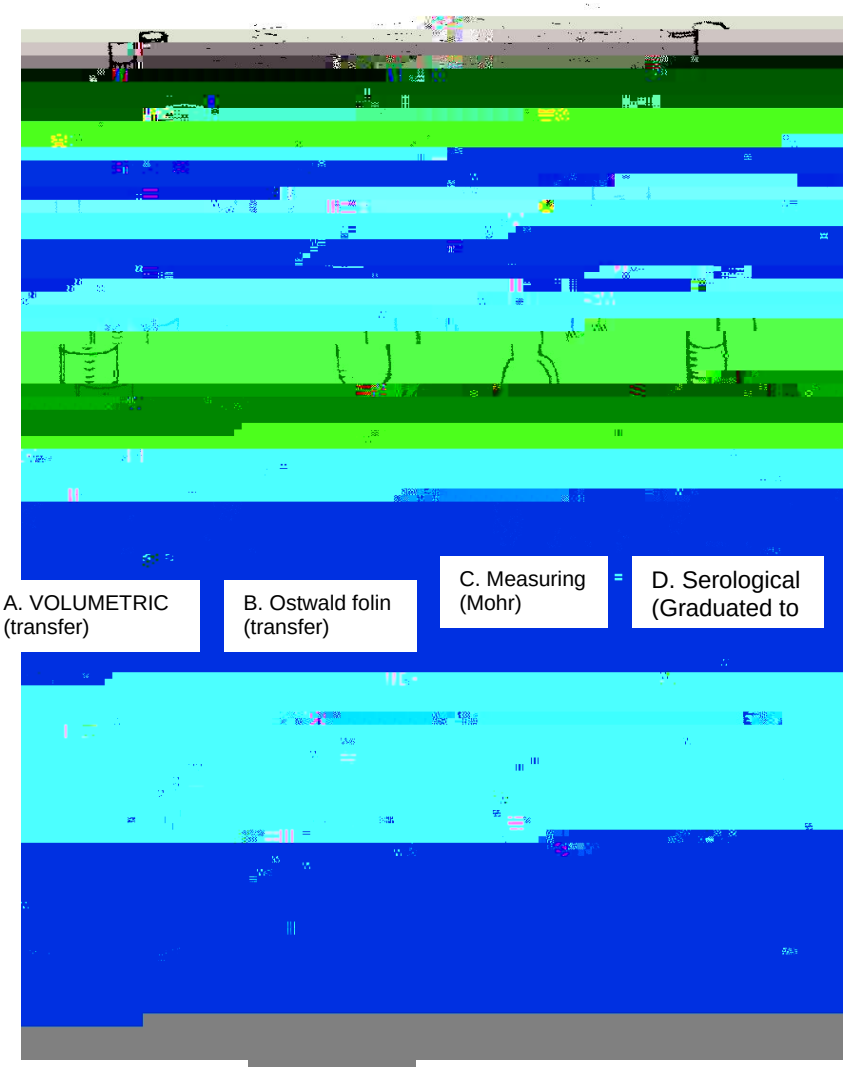
Cleaning of p^H Probe

p^H sensing glass bulb can be cleaned by one of the following methods :

1. If Inorganic substances are deposited on the glass

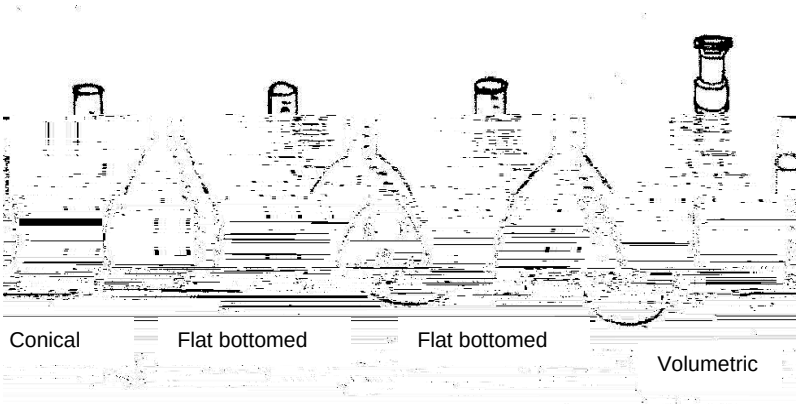
solution for 24 hours.

Laboratory wares

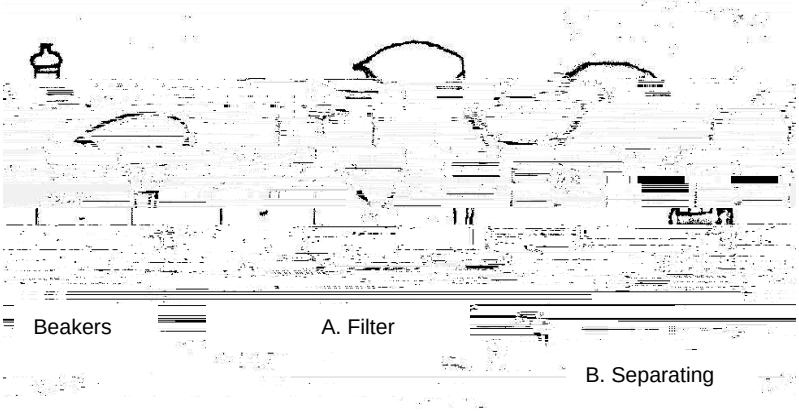


Measuring Cylinder

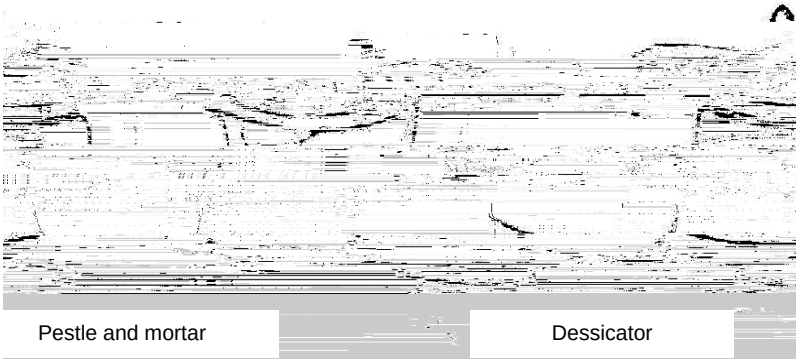
Burret with glass stop cork



Conical Flat bottomed Flat bottomed Volumetric

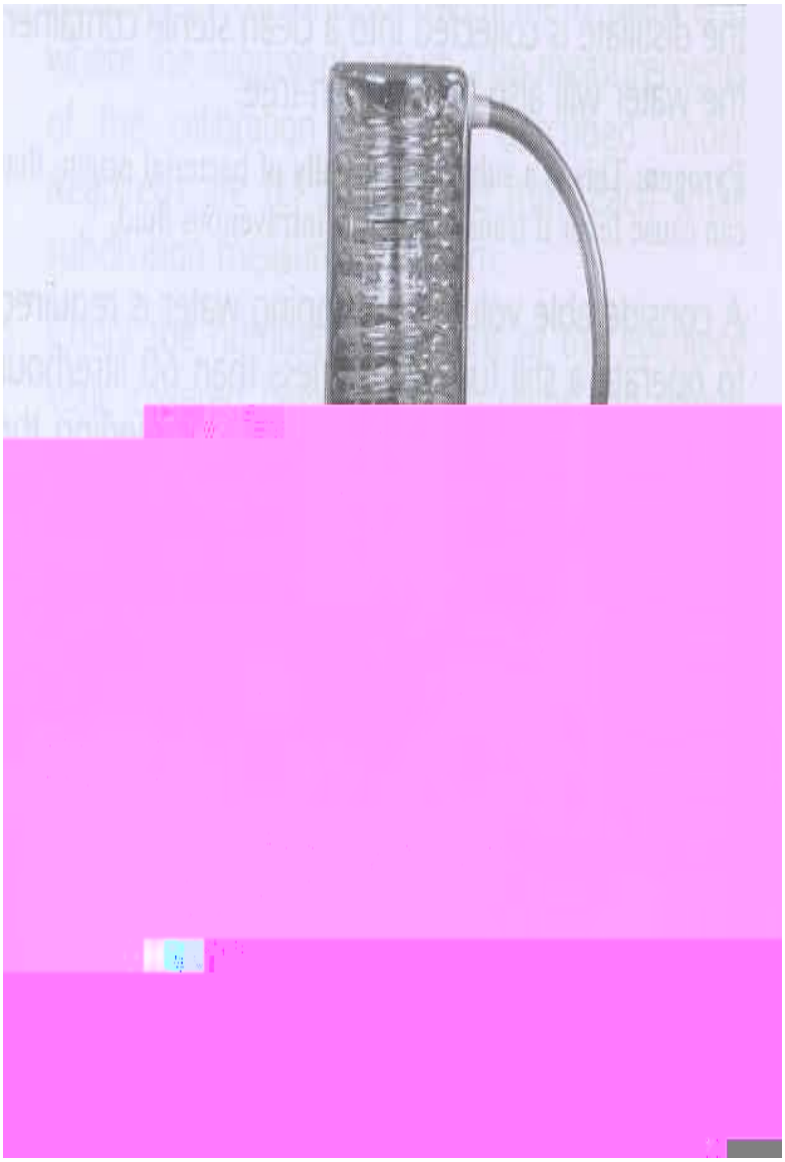


Beakers A. Filter B. Separating

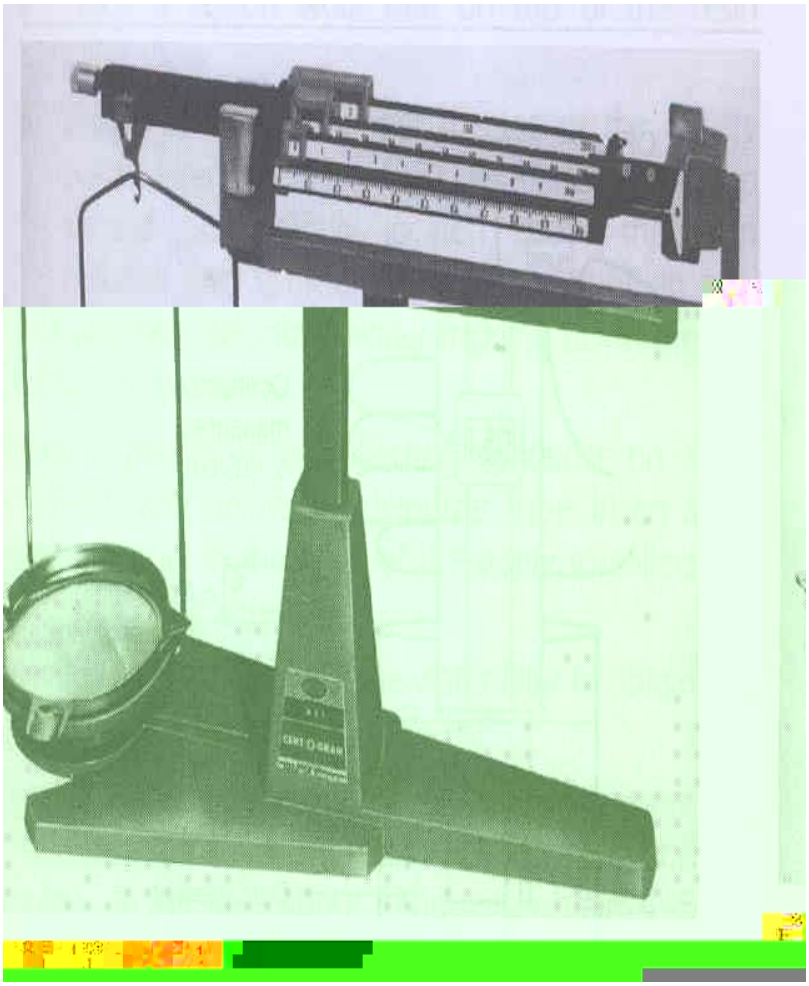


Pestle and mortar Dessicator

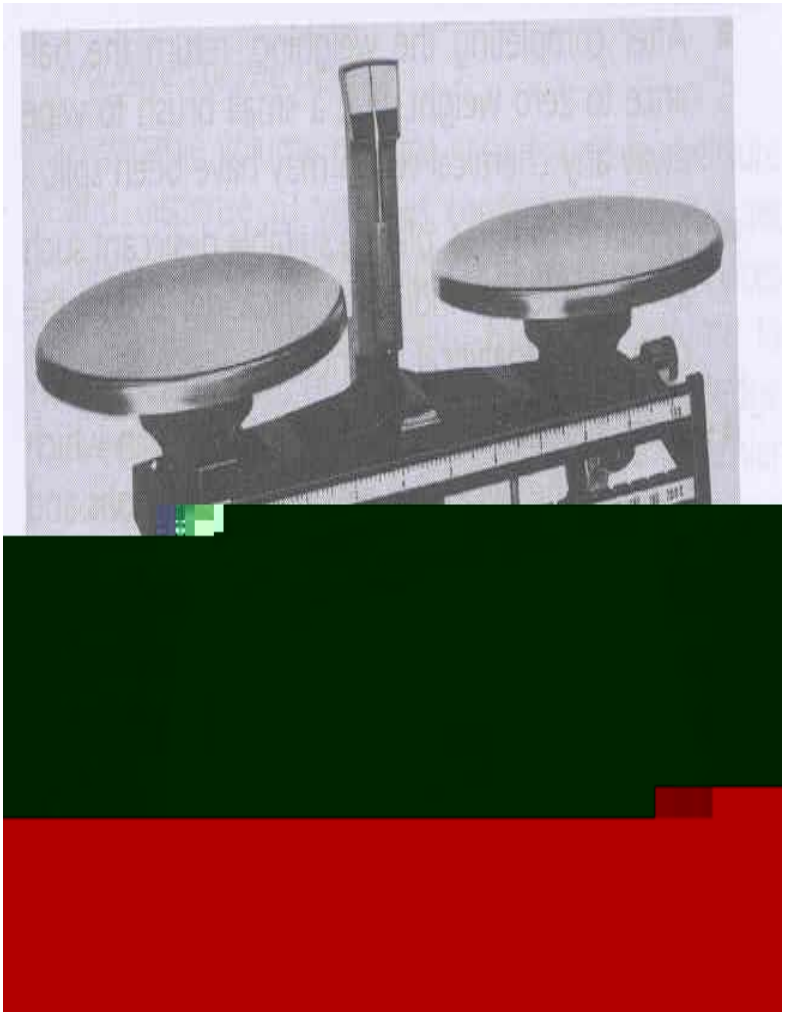
Laboratory equipment

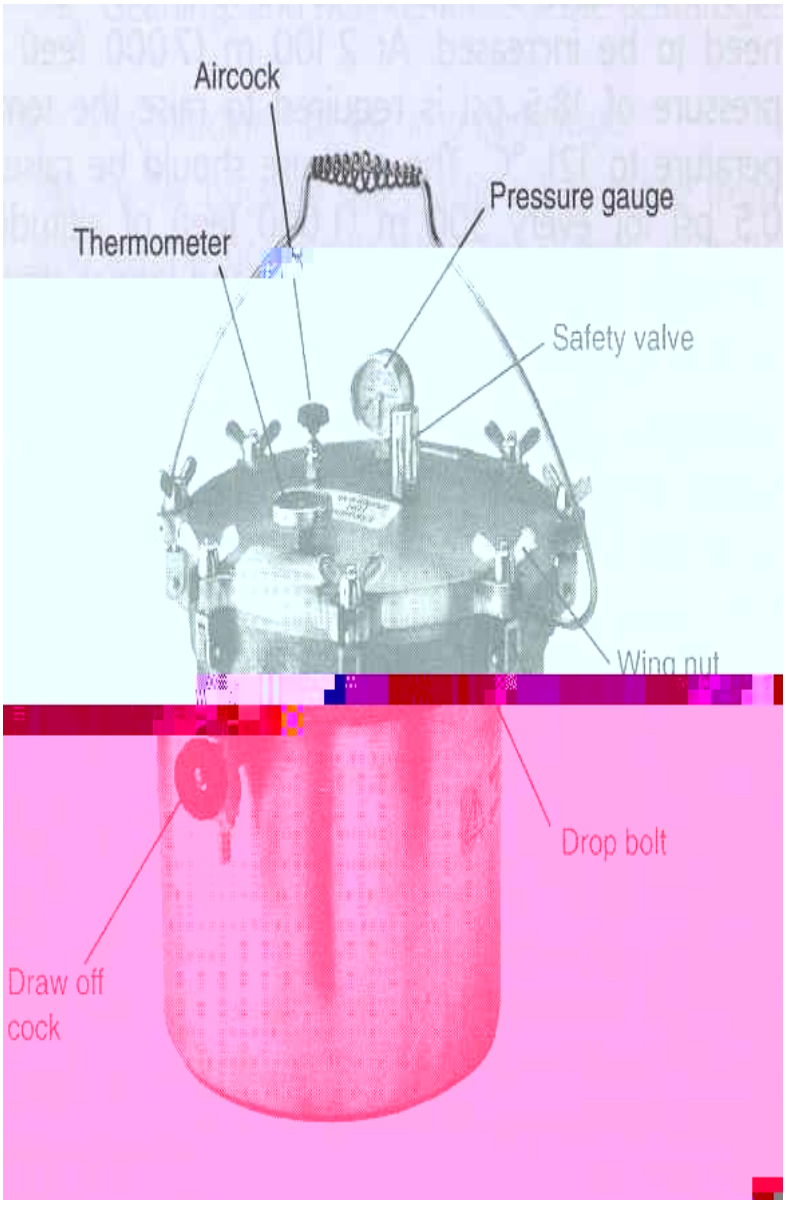


Water distiller

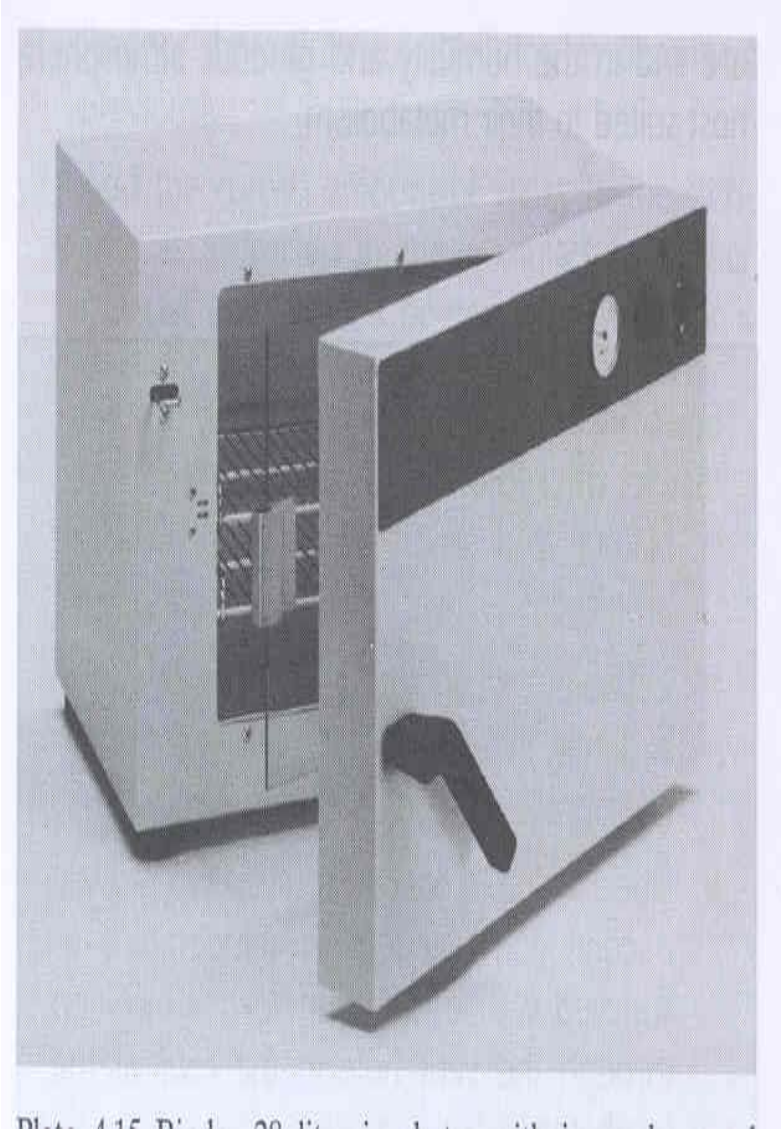


Mechanical beam balance

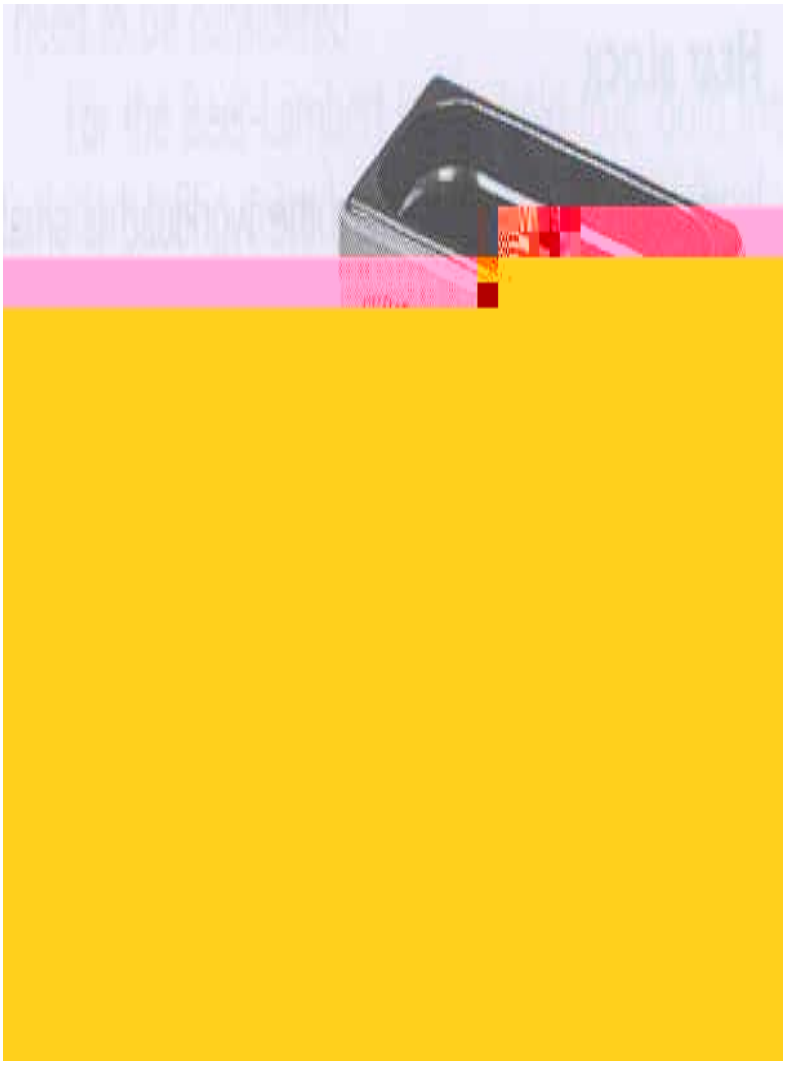



Aircock
Pressure gauge
Thermometer
Safety valve
Wing nut
Drop bolt
Draw off
cock

Autoclave



Incubator



Safety signs and symbols

Safety signs and symbols