LECTURE NOTES

For Medical Laboratory Technology Students

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Introduction to Medical Laboratory Technology

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In collaboration with the Ethiopia Public Health Training Initiative, The Carter Center,
the Ethiopia Ministry of Health, and the Ethiopia Ministry of Education

December 2006

Funded under USAID Cooperative Agreement No. 663-A-00-00-0358-00.

Produced in collaboration with the Ethiopia Public Health Training Initiative, The Carter Center, the Ethiopia Ministry of Health, and the Ethiopia Ministry of Education.

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 level in various higher teaching institutions. It can also be helpful for those graduates who are in service. In the development of this lecture note, materials have been gathered and adapted from different standard books.

This lecture note is divided into seven chapters covering major and relevant topics of the subject matter. Within each chapter, important topics are identified and discussed in simple language so as to facilitate rapid reading and understanding of important concepts. Each chapter is also followed by review questions that can enable the reader to use them as self-assessment tools.

The author strongly believes that this teaching material will play a pivotal role in promoting the teaching – learning process through delivery of pertinent information to the

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

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ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and thanks to The Carter Center (Ethiopian Public Health Training Imitative) for the financial input, written materials and other valuable logistic supports that were extremely important for the development of this lecture note.

My special thanks also goes to professor Denis Carlson, Senior Consultant, The Carter Center, for his relentless effort, devotion and invaluable contribution for the initiation and laying ground in the preparation of this lecture note.

I would like to extend my special appreciation and thanks to Professor Joyce Murrey, for her immense contribution in the development of this lecture note in various aspects.

I also wish to extend my special thanks to the staff of Haramaya University; Faculty of Health Sciences, University of Gondar; Department of Medical Laboratory Technology, Addis Ababa University; School of Medical Laboratory Technology, Jimma University; School of Medical Laboratory Technology, Hawass University; Department of Medical Laboratory Technology and Defense University College for their reviewing and valuable professional comments.

My grateful acknowledgment is also made to national reviewers;
Dr. Seyoum Taticheff and Ato Ibrahim Ali for their tremendous professional comments and suggestion in the preparation of this lecture note.

I am also indebted to all teaching staff of Haramaya University, Faculty of Health Sciences, Department of Medical Laboratory Technology not only for numerous ideas and suggestions, but for their arguments too. Their professional support and encouragement are highly appreciated. Finally, I am also very grateful to W/t Tigist Negga and W/t Aschalech Temesgene for typing the final manuscript of 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 number of different disciplines such as Microbiology,

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 Gondar Public Health College, The Minilik II Hospital and Jimma Hospital.

CHAPTER ONE
LABORATORY

Learning Objectives

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

1. Define laboratory.
2. Identify and enumerate the different kinds of medical laboratories.
3. Explain the role of medical laboratory services.
4. State the laboratory rules, ethics, professional code of conduct and polices.
5. Describe and practice collection, handling and shipment of medical laboratory specimens.
6. Identify solutions used in medical laboratories.

1.1 General overview

Laboratory is a place that is equipped with different instruments, equipments and chemicals (reagents) etc., for performing experimental works, research activities and investigative procedures. Medical laboratory is one part of the laboratory that is equipped with various biomedical instruments, equipments, materials and reagents (chemicals) for performing different laboratory investigative activities by

using biological specimens (whole blood, serum, plasma, urine, stool, etc).

1. 2 Classification of medical laboratories

The world Health Organization (WHO) lists four kinds of levels of laboratories based on their biosafety.

1. 2.1 Basic laboratory level I

Basic laboratory level I is the simplest kind and adequate for work with organisms which have low risk to the individual laboratory personnel as well as to the members of the community. Such organisms are categorized under **Risk Group I** by WHO. These organisms are unlikely to cause human diseases. Example, food spoilage bacteria, common molds and yeasts.

1.2.2 Basic laboratory level II

Basic laboratory level II is suitable for work with organisms that predispose to moderate risk to the laboratory worker and a limited risk to the members of the community. Such organisms are categorized under **Risk Group II** by WHO. They can cause serious human diseases but not serious hazards due to the availability of effective preventive measures and treatment.

Example, staphylococci, streptococci, entero bacteria except

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

1.2.3 Containment laboratory (Level III)

Containment laboratory is more advanced and it is used for work with infectious organisms that present a high risk to the laboratory personnel but a lower risk to the community. Such organisms are categorized under **Risk Group III** by WHO. Example, Tubercle bacilli, *Salmonella typhi,* HIV, Yersina and others. The principle is to remove from the basic laboratory those organisms and activities which are particularly hazardous. They are easily transmitted through airborne, ingestion of contaminated food or water and paranterally.
Such laboratory should be a separate room with controlled access by authorized staff. It should also be fitted with microbial safety cabinet.

1. 2. 4 Maximum containment laboratory

Maximum containment laboratory is intended for work with viruses, which predispose to a high risk for both laboratory personnel and the community. Such organisms are categorized under **Risk Group IV** by WHO. Example, *Small pox*, Ebola, Lassa fever and others. Most of these organisms cause serious disease and readily transmitted from on person

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 *S*tructure of medical laboratory services

A laboratory service network consists of:

1.4.1 Community based primary health care laboratory

Duties

▪To support primary health care in investigating, controlling and preventing major diseases in the country.

▪ Promoting health care by integrated health education

Main activities are to:

- Investigate by referral or testing on site, important diseases and health problems affecting the local

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. Delivery of laboratory results

Laboratory staff should provide as much relevant information as possible to assist those requesting tests. Standardization in the presentation of reports and use of units is important because it helps in the interpretation and comparison of results, contributes to the efficiency of a laboratory service, and is of great value when patients are referred from one health unit or hospital to another. Reports should be clearly and neatly written, particularly figures.

Therefore, to ensure the validity and accuracy of test results, the following points should be taken into consideration.

1. Experienced member of medical laboratory professional must check all laboratory results before dispatching them to respective departments or units.
2. Any unexpected result should be investigated and repeated if necessary.
3. If possible, reference values (normal ranges) should be

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 maintain working hours. This is because of large outpatient flow and the emergency nature of much of the work. Out side of normal working hours, each laboratory should organize a system for testing urgent specimens. Only those investigations that are essential for the immediate care and assessment of a patient should be requested urgently.

B. Range of tests to be performed and those to be referred to higher level

- Range of test to be performed depend on:
  - The number of staff available;
  - The availability of material resources;
  - The types of health institutions (hospital or health center).
- Referral of specimens (when necessary).
  Example, specimens for HIV detection and water samples for bacteriological analysis.

C. Collection of laboratory specimen

This includes collection, handling and shipment of medical specimens. Many different types of specimens are received daily in a laboratory and it is necessary to observe certain details to ensure that appropriate specimens are collected.

1. The specimen containers should be clearly labeled with the patients name, identification number, date of collection and time of collection.
2. A fully completed, request form should accompany each specimen with the detail mentioned above.

The quality of specimens has an effect on the reliability of tests that are performed. For example, a clotted blood sample has a little value for white cell count. Therefore, such sample must be rejected from being used.

Specimens received in a laboratory should fulfill the following requirements.

1. The specimen containers must be clean and leak proof and also sterile when necessary.
2. Special collecting trays or boxes must be used and they must able to with stand repeated autoclaving or disinfection.
3. All specimens must be carried up right with fitted plugs.
4. Request forms should be kept separate from the specimens to prevent contamination.
5. Enough amount of specimen should be collected to perform the intended laboratory test (s).
6. Specimens that are suspected or containing dangerous pathogens, must be labeled as "**Dangerous or highly infectious**" and handled with special precautions.

Example: Specimens containing Bacillus anthrax, Human

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 appropriate types of stains (differential stains) such as Giemsa stain, Wright stain, Gram stain, Leishman stain, Acid Fast Stain, etc. Simple stains are used to reveal the morphology (shape, size and content) of an organism(s) and single dye is utilized for the procedure.

1.9.3 Standard solutions

These are solutions in which the concentration of a given chemical is precisely known and are used to determine the value of an identical chemical with unknown concentration of a given solution. Chemicals that are used to prepare these solutions should be of analytical grade. Since poor standard solutions cause errors in the estimation of the intended substances, their accurate preparation is of paramount importance in order to obtain accurate and precise laboratory findings in medical laboratories.

Classification of standard solutions

1. Primary standard solution

Primary standard solution is a chemical solution that has the

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

volume or

w /v). Example, 40 gm % w / v glucose solution means, 40 gm of glucose is dissolved in 100 ml of a given solvent to give a 40 % solution.

2. Weight of solute per weight of solvent (weight per weight or w / w)

   Example, 30 gm % w / w hydrochloric acid means, each 100 gm of hydrochloric acid solution contains 30 gm % of hydrochloric acid and the rest 70 gm is the solvent (distilled water).

3. Volume of solute per volume of solvent (volume per volume or v / v)

   Example, 90 % v / v ethanol means 90 ml of absolute ethanol is mixed with 10 ml of distilled water.

1.10.2 Chemical units

Most common acids and some basic solutions like ammonium hydroxide are usually found with their concentrations expressed in specific gravity and percentage by weight of the specific solution. These two information (specific gravity and percentage by weight) should be changed to the commonly known expressions of concentration, like morality and normality.

A molar solution is a solution that contains one mole of the solute in one liter of solution. For example, the molar weight

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:

$$\textbf{Molarity} = \frac{\text{Number of mole of solute}}{\text{Volume of solution in liter}} = \frac{\text{Amount of substance (weight)}}{\text{Molar weight x volume of solution in liter}}$$

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:

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

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

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

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

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

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

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%

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

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 $= \frac{8}{20} = \mathbf{\underline{0.4ml}}$

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 (μ l) of blood and a 50 μ l of diluting fluid. Total volume of body fluid and diluting fluid.

   50 + 50 = **100 μ l**

Therefore, dilution of blood. $\frac{100}{50}$ = **2**

i.e. **1 in 2 dilutions**

1. Calculate the dilution of urine using 0.5 ml of urine and 8.5 ml of diluting fluid (physiological saline)

   Total volume of urine and diluting fluid.

   0.5 + 8.5 = **9.0 μ l**

2. Therefore, dilution of urine. $\frac{9.0}{0.5}$ = **18**

i.e. **1 in 18 dilutions**

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 crack bottles if their caps are screwed on tightly so if heat is to be applied, flasks should

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 between two marks on the stem (Mohr).
B. The other has graduation marks down to the tip (serological pipette)

These pipettes are intended for the delivery of predetermined volumes. The serological pipette must be blown out to deliver the entire Volume of the liquid and it has an etched ring (pair of rings) near the mouth end of the pipette signifying that it is a blow out pipette. Measuring pipettes are common only in 0.1, 0.2, 0.5, 1.0 5.0, and 10.0 ml sizes. The liquid is delivered by allowing it to fall from one calibration mark to another.

N.B. The classification of pipettes may not always be based on the presence or absence of a bulb and etched ring.

2.1.2.3 Micropipettes

Micropipettes are frequently used in medical chemistry, Virology, immunology and serology laboratories. This is because in these laboratories often only small quantities of materials are available for measurement. Whole blood or serum or plasma is often measured and when such viscous fluids are used these pipettes are convenient. They are found

in different capacities such as 5, 10, 25, 50, 100 and 1000 micro liter. There are also other kinds of pipettes that are used in medical laboratories. Example; Toma pipette, ESR pipette, Pasteur pipette, automatic pipettes and others.

2.1.3 Burettes

Burettes are used for measuring variable quantities of liquid that are used in volumetric titrations. They are made in capacities from 1to100 milliliters. They are long graduated tubes of uniform bore and are closed at the lower end by means of a glass stopper, which should be lightly greased for smooth rotation.

2.1.4 Flasks

There are four types of flaks having 25 to 6,000 milliliter (ml) capacities.

2.1.4.1 Conical (Erlenmeyer) flasks

Conical (Erlenmeyer) flasks are useful for titrations and also for boiling solutions when it is necessary to keep evaporation to a minimum. Some have a side arm suitable for attachment to a vacuum pump.

2.1.4.2 Flat bottomed round flasks

Flat-bottomed round flasks are convenient containers to heat liquids. A gauze mat should be interposed between the flask and flame. These flasks are widely used in the preparation of

bacteriological culture media.

2.1.4.3 Round bottomed flasks

Round bottomed flasks can with stand higher temperatures than the flat- bottomed type and they may be heated in a necked flame, or in an elector- thermal mantle. They can be used for boiling of different kinds of solutions and to make titration.

2.1.4.4 Volumetric flasks

Volumetric flasks are flat - bottomed, pear-shaped vessels with long narrow necks, and are fitted with ground glass stoppers. Most flasks are graduated to contain a certain volume, and these are marked with the letter"C". Those designed to deliver a given volume are marked with the letter "D". A horizontal line etched round the neck denotes the stated volume of water at given temperature, for example at 20 C^{o} .They are used to prepare various kind of solutions.The neck is narrow so that slight errors in reading the meniscus results in relatively small volumetric differences (minimizes volumetric differences or errors.)

2.1.5 Beakers

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 laboratory procedures. These include simple round hollow tubes conical centrifuge tubes, vaccutainer tubes and nunck tubes.

Test tubes can be with or with out rims (lips). Test tubes with out rim are satisfactory because there is less chance of chipping and eventual breakage.

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 cuvettes resist many laboratory reagents like organic solvents, whereas plastic cuvettes are affected by many reagents and become cloudy, hence affecting the absorbance of the reacting mixture and so lack accuracy & precision. Therefore plastic cuvettes whenever used should be cleaned

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 is important that the necks of volumetric flasks above the graduation mark be clean because, when solutions are diluted in the flask, drops of water may adhere to an unclean wall and may invalidate the measurement of volume.

2.2 Plastic wares

Plastic wares are usually manufactured from polymers of polyethylene, polypropylene and TEFLON. These plastics are chemically inert and unaffected by acid /alkali.

Plastic wares are durable and suitable to store alkaline solutions. However, surface bound may be leached to the solution, absorb dyes and proteins.

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 on the balance pan.
6. When adding or removing a chemical, remove the container to avoid spilling any chemical on the balance.
7. When using an analytical double pan balance, bring the pans to rest before adding or removing a chemical.
8. Always use forceps to add or remove weighs. Protect the weights from dust, moisture and fungal growth.
9. Use small brush to remove any chemical, which may have been spilt on the balance.
10. A container of self - indicating silica gel should be kept inside the analytical balance case to remove any moisture present in the atmosphere.

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.

They are used for spinning small tubes as in blood bank laboratories.

2. Medium size centrifuges.

   Are used for centrifuging of urine specimens for microscopic analysis of urinary sediments.

3. Large centrifuges

They are widely applied in bacteriology and medical chemistry laboratories. A centrifuge may have a preset speed or more often there is a knob by which the laboratory personnel control the speed. The speed is given in revolution per minuets (rpm). Small models are designed to centrifuge volumes up to 200 ml at maximum speeds of 3,000 - 4,000 rpm. Large models will centrifuge volumes up to 2,200 ml with maximum speeds of 5,000 rpm.

A centrifuge may have built in timer or may have to be timed with a watch. Some centrifuges may have a temperature gauge in order to keep the temperature constant as it spines.

3.2.4 Use and care of centrifuges

Although most centrifuges are fitted with an imbalance detector, lid interlock, and an automatic braking system, it is important for laboratory workers to know how to use a centrifuge correctly to prevent it from damage and breakages.

These include:

1. Reading the manufacturer's instructions.

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

1. Read carefully the manufacturer's instruction.
2. Make sure the incubator is positioned on a level surface and that none of the ventilation openings are blocked.
3. If the incubator does not have a temperature display, insert a thermometer in the vent hole through the roof of the incubator. Adjust the thermostat dial until the thermometer shows the correct reading, i.e., 35 - 37^Oc for the routine incubation of bacteriological cultures.
4. Before incubating cultures and tests, check the temperature of the incubator.
5. Clean the incubator regularly; making sure it is disconnected from its power supply.
6. Every three to six months check the condition of the incubator.
7. At the time of purchase, it is advisable to buy a spare thermostat and thermometer if these are of special type and are not available locally.

3.7 Colorimeter (Photometer)

Colorimeter is an instrument used to measure the concentration of a substance in a sample by comparing the amount of light it absorbs with that absorbed by a standard preparation containing a known amount of the substance being tested. In a test a colored solution of the substance being measured or a colored derivative of it is produced this is measured in a color meter colored solutions absorb light at a given wavelength in the visible spectrum. Biological samples contain many substances that can be determined quantitatively or qualitatively.

Visible light spectrum

When a beam of light passes through a colored solution, it interacts with matters in the solution and the result may be refraction, reflection, absorption and transmission among others.

- **Refraction: -** is defined as sudden change in the direction of the beam when the light passes from one medium to another with a different physical density.
- **Reflection: -** is a condition where the beam returns back towards its source. Example mirror.
- **Absorption: -** is a situation where some components of the light (colors) are retained or absorbed.

-Transmission: - refers to the situations where some portions of the light permitted to pass through a given medium.

Radiation is characterized by waves on which basis the electromagnetic radiation spectrum could be divided in many regions including gamma rays, x-rays, ultra violet rays, visible, infrared, microwaves and radio waves. Of the above, the visible region is the radiant energy to which the human eye responds s and their wavelength varies between 400 and 700 nm.

Wavelength of about 700 nm are seen by the eyes as red colors while those of progressively shorter wavelengths give in descending order to orange, yellow, green, blue, and finally violet colors which is produced in the short wavelength of 400 nm.

Beer's and Lambert's Law

Most colorimetric analytical tests are based on the Beer's - a Lambert's law which states that under the correct conditions the absorbance of a solution when measured at the appropriate wavelength is directly proportional to its concentration and the length of the light path through the solution. Using a standard, this law can be applied to measuring the concentration of a substance in unknown (test) solution by using the formula:

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 instrument, which measures the intensity of the transmitted or absorbed light not merely, color. Photoelectric instruments used in colorimetry are:

A. Absorptio - meter or filter absorption spectrophotometer or filter photometer.

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 elements and the emission intensity is proportional to the concentration of the element introduced into the flame. Each element emits a radiant power with a specific wavelength. So using different filters, elements in a mixture can be analyzed at different wavelength.

Flame photometry is used for the determination of electrolytes in a given solution. It is most commonly used for the quantitative analysis of sodium and potassium ions in body fluids. Solutions that contain Sodium and potassium ions when placed in a Bunsen burner produce characteristic colors and the brightness of the flame varies according to the concentration of the elements in solution.

The instrument measures individual elements by correlating with the intensity of emitted radiation. Lithium releases a red, sodium a yellow, potassium a violet and magnesium a blue color in a flame when placed in an ordinary burner. The color helps for the qualitative analysis while the flame emission spectroscopy reading is needed for the quantitative analysis of the elements.

3.8 Desiccators

Desiccators are instruments, which are used for drying of chemicals or to keep other chemicals from being hydrated. As

chemicals stay for long period of time out of dessicators, they sometimes absorb water. When we are weighing chemicals where a very high degree of accuracy is needed, as sodium chloride (NaCl) used as a standard for the chloride test, the chemical must not contain water. The chemical is dried in an oven at 110 ^{O}c for 1 hour, then it is placed in a dessictor over night before weighing on the analytical balance.

The purpose of the oven is to remove the water and that of the dessicator is to store the chemical at an ambient temperature where it cannot reabsorb water. A dessicator contains substances called drying agents. These absorb the water in the air of the dessicator. The most commonly used drying agents (desiccants) are calcium chloride and concentrated sulfuric acid. The chemical that is to be dried is placed in another bottle or test tube and is put on top of the desiccants present in a securely closed dessicator.

3.9 Instruments and materials used for p^{H} determination

3. 9.1 p^{H} Meter

^{pH} meter is an instrument used to measure the p^{H} or hydrogen ion concentration of a given solution by the potential difference between two electrodes.

Major components of p^H meter are:

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

p^H is the universal accepted scale for the concentration of hydrogen ion in aqueous solution, which gives a measurement of the acidity or alkalinity of a given solution. p^H is defined as the negative logarithm of the molecular concentration of the active hydrogen ions, $\mathbf{p^H = - log^{H+}}$. The p^H of a given solution is measured by this instrument and displayed as digital or galvanometric reading by converting the milli volt (Mv) difference between the two electrodes to p^H value. If the potential difference is zero milli volts between the two electrodes, the corresponding value of p^H is 7.0.

The p^H of any solution will be in the range of 0 to 14.0. If p^H of a solution is less than 7.0, it is known as acidic, whereas, p^H value greater than 7.0 is considered as basic. A mixture with p^H value of 7.0 is a neutral solution. p^H value may be changed inversely with the change of temperature. For optimum readings it is better to use a temperature of 25^Oc.

The glass electrodes of a p^H meter may be affected by 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^H strips

Uses:

- Dip a small piece of strip into a given solution;
- Compare the color change on the strip with the standard chart or paper strip;
- Approximate values of p^H can be found from this technique.

3.9.3 Use of different buffers

- Universal indicator and series of buffers with different p^H value are used;
- The universal indicator solution is added in the different p^H solutions as well as in unknown solution

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 value is between 8.0 and 9.0 calibrate with p^H 7.0 and 10.0 values.
2. Before starting calibration, be sure that the sensor and the buffer are at the same temperature.
3. If possible, calibrate with buffers having the same temperature as the process. If the buffer and the process temperature differ by more than about 15^0C, an error as great as 0.1 p^H may result.
4. Buffers have limited shelf lives. Do not use a buffer if the expiration date has passed.
5. Store buffers at controlled room temperature.
6. Do not return used buffer to the stock bottle. Discard it immediately.
7. Protect buffers from excessive exposure to air. Atmospheric carbon dioxide lowers the p^H of alkaline buffers.

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, Hematology and Microbiology;
- Reagents used for culture media;
- Reagents used in blood transfusion work and for rinsing of cleaned glass and plastic wares, cuvettes, etc.

For preparation of standard solutions, buffers and controls, the most pure water quality that is free from bacteria (Type I Reagent Water) should be used. However, for most routine

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.
    - The wider the condenser aperture, the brighter will be the specimen and the smaller will be the details,

which can be resolved.

- The smaller the aperture, the greater will be the contrast.
- Certain specimens, example stained and mounted specimens give little glare illuminated image with fine detail.
- Other specimens, example, urine, unstained cerebrospinal fluid, and saline mounted fecal specimens give much glare and require a reduced source of light to increase contrast.

9. Examine the specimen by systematically moving the slide with the mechanical stage.

N.B: The image of the specimen will be up side down and will move in the opposite direction to the side.

10. For a higher magnification, swing the 40x objective into place.
    - Focus the 40x objective, using the fine adjustment.
    - If for any reason the image is not visible, lower the objective until it is nearly but not quite touching the specimen.
    - Then looking through the eyepiece, focus up wards with the fine adjustment until the image comes into view.
11. For the highest magnification, add a drop of immersion

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 visible 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, cerebrospinal fluid, lymph gland fluid;

- 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.

- 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.
- 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 illuminate the sample.

This can be achieved by using special dark - field condenser for use with the 100X objective.

- Light entering the objective comes from the microorganism. Microorganism scatter the light and can be seen shining brightly against the dark background

Principle

In dark field microscope, the light enters a special condenser, which has a central blacked out area or a condenser fitted with dark field stop so that the light cannot pass directly through it to enter the objective. Instead, the light is reflected to pass through the outer edge of the condenser lens at a wide angle. The only light entering the eye comes from the microorganisms themselves, with no light entering the eye directly from the light source. In this way, small microorganisms are seen brightly illuminated against a black ground, like stars in a night sky or dust in a shaft of sunlight across a darkened room.

Value of dark field microscopy

This form of microscopy is particularly useful for examining living microorganisms such as:

- Treponema pallidum (Spirochetes),
    - Borreliae species in blood,
    - Microfilariae in blood,
    - Vibrios and campylobacters in specimens and cultures.

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 reliable scientific instrument repairer or preferably to the manufacturer.

3.12 Polymerase chain reaction (PCR) machine

PCR is a machine that is widely employed in the molecular biology laboratory. It helps to detect and amplify small fragments of nucleic acid (DNA/RNA) of interest from various clinical samples.

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 physical means (including heat, radiation, and filtration) and chemical agents (acids, alkalis, heavy metals, salts, halogens, etc). The equipment or material treated then becomes **"Sterile"**.

In medical laboratories, materials and equipments are sterilized for the following main purposes.

1. In preparation for taking specimens, such as needles,

syringes, test tubes, etc.

2. To sterilize contaminated materials and equipments.
3. To prepare apparatus used for bacteriological cultures, such as petridishes, Pasteur pipettes, and others.

Methods of sterilization techniques.

A. Physical method

1. Dry heat (hot air oven, flaming and red - hot)
2. Moist heat (autoclave or steam under pressure and boiling 3. Radiation

Mechanical methods

4.1.1 Dry heat

4.1.1.1 Hot Air oven

Owing to the low penetrating power of dry heat and great resistance of bacteria to it, higher temperatures are needed with a hot- air oven than with an autoclave. A temperature of 180^0c for 30 minutes will kill most resistant spores. The material to be sterilized is placed in an oven and the temperature is raised and maintained at 180^0_c for 30 minutes.

The sterilized material should not be removed until the oven is cold. This is important particularly with pertidishes, as cold air

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.

4.1.1.3 Red - hot

Wire loops and tips of forceps may be sterilized by heating them in a Bunsen flame until they become red hot and allow the materials (instruments) to cool before using them.

4.1.2 Moist heat

4.1. 2.1 Boiling water

Moist heat may be applied by boiling water or steam. Boiling water is generally used for sterilizing instruments and syringes. These are boiled for 10 minuets in a water bath. This will kill all non-sporing organisms but certain spore forming organisms can resist the temperature of boiling water

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

sterilization of correctly loaded autoclaves correctly loaded:

- Three minute holding time at 134C^0;
- Ten minute holding time at 126 C^0;
- Fifteen minute holding at 121 C^0;
- Twenty holding time at 115 C^0.

4.1.2.2.1 Types of autoclaves

A. Gravity displacement autoclaves

In gravity displacement autoclave, steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.

B. Pre- vacuum autoclaves

These autoclave allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134C^0 and the sterilization cycle can therefore be reduced to 3 minute. They cannot be used to process liquid because of the vacuum.

C. Fuel heated pressure cooker autoclaves

Fuel heated pressure cooker autoclaves should be used if a

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

autoclaving liquids, as they may boil over when removed due to superheating.

7. Operator should wear protective gloves for protection when opening the autoclave.
8. Thermocouples should be placed at the center of each load in order to determine proper operating cycles.
9. Ensure that the relief valves of pressure cooker autoclaves do not blocked.

4.1.3 Radiation (ultra - violet ray)

UV radiation is lethal to certain microorganisms by inactivating the DNA of the organisms. It is effective and valuable in sterilization of air in a given room or place. E.g: Pulmonary tuberculosis Laboratory

4.1.4 Mechanical method

Filtration is a mechanical method for eliminating bacteria from biological fluids and from the laminar flow systems, which are used to ventilate operating rooms, laboratories and areas having immune suppressed and burn patients. Filtered air is pumped into the space at a pressure required to displace regular circulating air.

B. Chemical methods

Generally, many chemicals are lethal to microorganisms.

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 manufacturers instruction. Dilutions should be kept for more than twenty-four hours.

B. Chlorine (sodium hypo chloride)

Chlorine, a fast - acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypo chloride, which can be diluted with water to provide various concentrations of available chlorine. Products containing 100,000 parts per million (ppm) of chlorine are suitable for laboratory use. They are effective against viruses as well as bacteria. Dilutions should not be kept for more than 24 hours so as to maintain

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.

F. Iodine and iodophors

Iodine and iodophors have similar action to that of chlorine. Iodophors and tincture of iodine are good antiseptics. Iodine can be toxic. Organic iodine based products must be stored at 4 -10^{0}C to avoid the growth of potentially harmful bacteria in them.

Caution: Most of the disinfectants are toxic and irritant to skin and mucous membranes. Therefore, dilutions should be made in fume - hood or in well ventilated areas.

4.2.3 Decontamination of laboratory space

Decontamination of laboratory space, its furniture and equipment requires a combination of liquid and gaseous disinfectants. Laboratory surfaces can be decontaminated using a solution of sodium hypochlorite. A solution containing 1g/ l available chlorine may be suitable for general environmental sanitation. However, stronger solutions of chlorine (5g/l) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen per oxide make suitable substitutes for bleach solutions.

Working rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating Para-

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 21^0C and a relatively humidity of 70%. After fumigation, the room must be ventilated thoroughly before commencing routine activities in the laboratory.

4.2.4 Incineration

Incineration is useful for disposing of animal carcasses, anatomical and other laboratory waste with or without prior decontamination. Proper incineration requires an efficient means of temperature control and a secondary burning chamber. Those incinerators with two combustion chambers are suitable for dealing with infectious materials, animal carcasses and plastics. The temperature in the primary chamber should be at least 800^0C and that in the secondary chamber at least 1000^0C.

For incineration, materials should be transported to the incineration in bags or plastic. Incinerator attendants should receive proper instructions about loading and temperature control. Efficient operation of an incinerator depends on the right mix of materials in the waste being treated. It should also be noted that great efforts should be made to make incinerators more environmentally friendly and energy

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

5.1.2 Physical hazards

Hazards of glass are the biggest cause of lab, accidents. More than 30% of all laboratory causalities are cuts from broken glass. If one is not careful enough, profuse bleeding might result following a deep cut. The glass may also be contaminated with stool, pus, and bacteriological cultures resulting in severe infections. Always inspect glass apparatus from defects before use. Don't use damaged, cracked, badly scratched or chipped glasswares. Broken glasswares should be discarded in to a container reserved for this purposes. Always replace reagent bottles on the shelves and never leave bottles of acid or alkali where they may be over turned.

Always label all bottles clearly to show their contents. Moreover, mechanical hazards are common in laboratories. Laboratory personnel should be aware of the mechanical hazards of equipment like centrifuges, shakers and autoclaves. Accidents occur in the lab, mainly through ignorance, lack of foresight, and lack of care. A careless worker endangers not only himself / herself but also his / her colleagues as well.

The main hazards and accidents associated with laboratory work are as follows:

1. Infection.
2. Burns.
3. Cuts.

4. Harmful effects of toxic chemicals.
5. Injury from explosions.
6. Electric shock.
7. Fire.

1. Infection

This can be caused by:

A. Pathogens being inhaled in aerosols when snap closing specimen containers, dispensing or pipetting infectious fluids.
B. Pathogens being ingested from contaminated fingers, or in food that has been contaminated, e.g. by being stored in a medical laboratory refrigerator.
C. Mouth- pipetting of specimens and cultures, which is one of the commonest ways of ingesting pathogens.
D. Pathogens entering the skin through needle punctures, cuts, scratches insect bites sores or other open skin lesions, Hence, always handle infected needles with great care.

2. Burns

Can be caused by:

A. Flammable chemicals and stains, or by reagents catching fire easily.

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.

Fire may also caused by over heating in faulty poorly maintained electrical equipment, overloading of electrical circuits, use of adapters, or overheating of electrical motors due to inadequate ventilation. Gas tubing or electrical cables that are worn for too long are also fire risks.

It is essential for laboratory personnel to receive adequate instruction and training in fire safety and appropriate fire fighting. Injury, damage and loss by fire can be minimized when laboratory staff:

- Understand how fires are caused and spread;
- Reduce the risk of fire by following fire safety regulations at all times;
- Know what to do if there is a fire in their laboratory;
- Know how to use fire fighting equipment;
- Know how to apply emergency First Aid, for burns.

Fire Fighting Equipment

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 with a fire retardant chemical or preferably a manufactured fire blanket made from woven fibre glass;
- Dry powder chemical fire extinguisher to extinguish electrical fires caused by flammable liquids.

N.B: When ever possible, the laboratory should be fitted with a battery operated smoke detector alarm.

5.2 Factors contributing to laboratory accidents

A poorly designed laboratory and overcrowding can increase the risk of accident occurrence. Most lab, accidents are the result of bad lab. Practices like:

- Poor training;
- Lack of concentration;
- Noisy environment;
- Untidy working and not using racks to hold sample

containers;

- Allow the working bench to become cluttered;
- Carelessness and negligence;
- Over work and fatigue;
- Hot and humid climatic conditions;
- Hurrying to finish work.

N.B. Accidents are also more likely to occur when working under emergency conditions, especially during night hours.

5.3 First aid for laboratory hazards

Knowing what to do immediately if an accident occurs can help to reduce suffering and the consequences of serious accidents. In some situations, first aid can be life saving, example, the control of bleeding. It can also prevent an injured person's condition from worsening, example, by protecting and treating wounds, placing a person in the best possible position, offering reassurance, and seeking immediate assistance.

Therefore, laboratory workers should receive a basic practical training in first aid, with particular attention being paid to the types of accidents, which may occur in the laboratory. They should also know what emergency action needs to be taken if

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

apply a pad soaked in cold water to the affected part for 10 minutes.

- Cover with a dry dressing.

Note: If the burn is severe, look for medical treatment.

A. Chemical burns of the skin

Wash immediately with large quantities of water and neutralize with a suitable chemicals as follows:

a. If an acid burns, neutralize with sodium bicarbonate if not seek medical attention.
b. If an alkaline burns, neutralize with boric acid powder.
c. Seek medical attention.

B. Chemical injury to the eye.

- Wash the affected eye as quickly as possible with large quantities of running water.
- Neutralize with a suitable chemicals as follow:
  a. If an acid injury, neutralize with 5% sodium bicarbonate solution.
  b. If an alkaline injury, neutralize with 5% acetic acid.
  c. Immediately seek medical attention.

Emergency treatment for poisoning

Swallowing of an acid or alkali:

Immediately rinse the mouth well with water and neutralize with a suitable chemicals as follows:

a. If acid has been swallowed, neutralize by drinking 8% magnesium hydroxide suspension (milk of magnesia).
b. If an alkali has been swallowed, neutralize by drinking lemon juice or 5% acetic acid.
c. Drink three or four cups of water.
d. Seek medical attention.

Note: When acid or alkali has been swallowed do not encourage vomiting.

Swallowing of other poisonous chemicals

- Rinse out the mouth well with water.
- Depending on the chemical swallowed, take a suitable chemical antidote under medical supervision.

Note: Always seek medical advice and treatment after swallowing toxic or harmful chemicals.

Swallowing of Infected materials

- Immediately seek medical treatment.

Electric shock:

- 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 alkalis such as sodium hydroxide (caustic soda) and potassium hydroxide (caustic potash).

Storage:

Corrosive chemicals should be stored at low level to avoid any serious injury, which could be caused if they are accidentally knocked off a shelf.

Safe use:

Never mouth pipette corrosive chemicals instead use

automatic pipettes. The accidental swallowing of corrosive chemicals can cause sever injury because such chemicals destroy living tissues. Always pour corrosive chemicals at below eye level, slowly and with great care to avoid splashing. When opening a container of corrosive chemicals, and when pouring it, wear protective materials.

N.B: When diluting concentrated acids in general and sulfuric acid in particular, **'ALWAYS'** add the acids to the water. This is because adding of a small amount of water to concentrated acids produces sufficient amount of heat that can break a glass container, which can cause damage and even chemical burn.

5.4.3 Toxic, harmful, and irritating chemicals

Toxic chemicals are those chemicals which can cause death or serious ill-health if swallowed or inhaled, or if the chemical is allowed to come into contact with the skin. Examples of toxic chemicals include potassium cyanide, sodium nitro-prusside, formaldehyde solution, chloroform, barium chloride and methanol. Harmful chemicals can cause ill- heath if swallowed and inhaled, or by skin contact. Example, iodine and sulphanilic acid chemicals can cause inflammation and irritation of the skin, mucous membranes, and respiratory tract.

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;
- Walls should be smooth, free from cracks, impermeable to liquids and easily washable;
- Doors of the of the lab, should be opened to the out side direction;
- Sectioning of the lab, into separate rooms or working areas with definite places (for patients, visitors, and reception of specimens);
- Bench surfaces should be without cracks, washable and resistant to the disinfectants and chemicals used in the laboratory;
- An adequate number of hand basins with running water is essential.
- Provision of protective clothing; Example, gown
- Fire extinguishers should be placed at accessible points. If extinguishers are not available several buckets of sand must be provided;
- Ensure that all work in the laboratory is done with a safety conscious attitude;
- All staff must ensure that the conditions of their work do

not create any hazard for those working near by;

- The chances of an accident occurring in the laboratory are much reduced if:
    - Every one works in a tidy fashion;
    - Every one works with out rush;
    - Benches are clean;
    - Reagents returned to the shelves after each use;
    - No eating, drinking or smoking in the laboratory.

N.B: Eat, drink and be merry but not in the laboratory!

- Laboratory coats should be fully buttoned up while working and removed before leaving the laboratory.

5.6 General precautions for avoidance of laboratory accidents

1. Handle acids and alkalis firmly up right with a dry hand. Never pour water in to strong acids because of the danger of reaction and splashing.
2. Do not keep acids and alkalis in bottles with ground glass stoppers. This is because they may get stuck.
3. Use small measuring cylinders for measuring acids and alkalis where possible.

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 should be true positive and true negative. However, false positive and false negative can be obtained. Therefore, we should minimize conditions associated with false positive and false negative results using good quality assurance program.

Quality assurance program is a coordinated effort to bring

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 practiced by every one and every time within the laboratory.

Quality assurance must be:

- Comprehensive, to cover every step in the cycle from collection of the specimen to sending of reports;
- Rational, to concentrate on the most critical steps in the cycle;
- Regular, to provide continuous monitoring of test procedures;
- Frequent, to detect and correct errors as they occur.

6.1 Types and causes of errors in medical laboratories

Any measurement can be subjected to error and the magnitude of the error will depend on the type of measurement being made, the apparatus used, and the technique of the person performing the measurement. Based on a set of control limits, the total error of any analytical procedure consists of unpredictable and predictable errors.

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

individuals.

Sensitivity can be calculated using the following formulae:

Sensitivity = Total number of true positive results X 100

Total number of infected patients.

$\Rightarrow$ True Positive X 100

True positive + False negative

The greater the sensitivity of a test, the fewer the number of false negative results

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).

Therefore, specific method will not give false positive result.

Specificity = Total number of true negative results X 100

Total number of uninfected patient

$\Rightarrow$ True negative X 100

True negative + False positive

The greater the specificity of a test, the fewer the number of false positive results. For example, Ziehl Neelson staining of

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.
17. **Titration: -** is the process of making a weaker solution from a stronger one.
18. **Sterilization: -** A process that kills and / or removes all classes of microorganisms and spores.

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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 of the scale. This is done by moving the weight that is located on the front of the balance, move the weigh on the screw left or right.
3. By moving the weights on the sliding scales find the desired weigh.
   For example, if you wish to weigh 25.0 gms, the weight on the lower sliding scale is put on the 20 gm mark and the weight on the upper sliding scale is put on the 5.0 mark.
4. Add the reagent to the paper on the left hand pan until the pointer comes to rest at the mid point of the scale.
5. Transfer the reagent to a container.
   Discard the papers and replace the weights to the zero points on their sliding scales

Preparation of chromic acid cleaning solution

Stir about 20 gm of powdered sodium dichromate with just enough water to make a thick paste. Slowly and carefully add 300 ml of concentrated sulfuric acid, stirring well.

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.

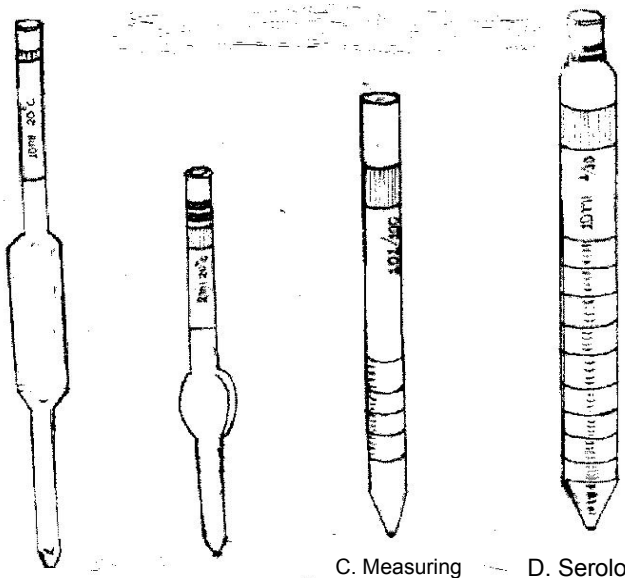
Cleaning of p^H Probe

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

1. If Inorganic substances are deposited on the glass bulb: Immerse the p^H probe tip in 0.1 M HCL for five minute.
2. In case of Organic oil/ grease films: Wash probe tip with liquid detergent and water or suitable solvent such as ethanol or acetone.
3. When the glass bulb contaminated with protein: Soak the p^H probe tip in 2% pepsin in 0.1M HCL for 24 hours or a ready made protein cleaning solution. After cleaning, rinse the probe tip with distilled or deionised water and soak in storage

solution for 24 hours.

Laboratory wares

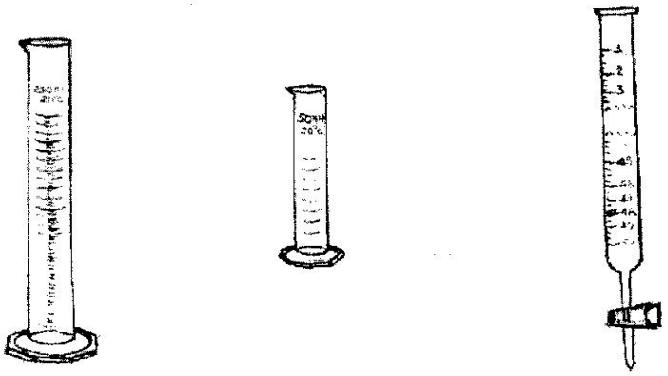


A. VOLUMETRIC (transfer)

B. Ostwald folin (transfer)

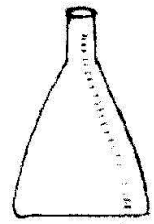
C. Measuring (Mohr)

D. Serological (Graduated to

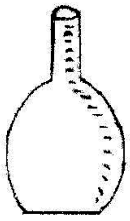


Measuring Cylinder

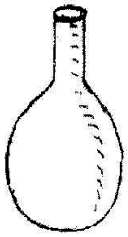
Burret with glass stop cork



Conical



Flat bottomed



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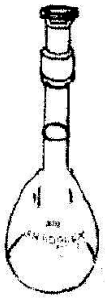
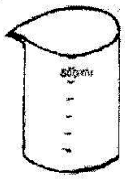
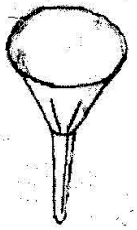


Volumetric

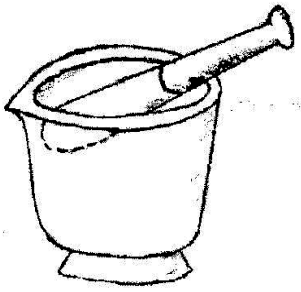


Beakers

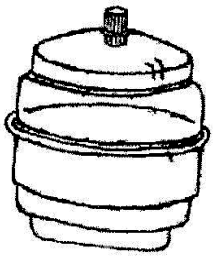


A. Filter

B. Separating

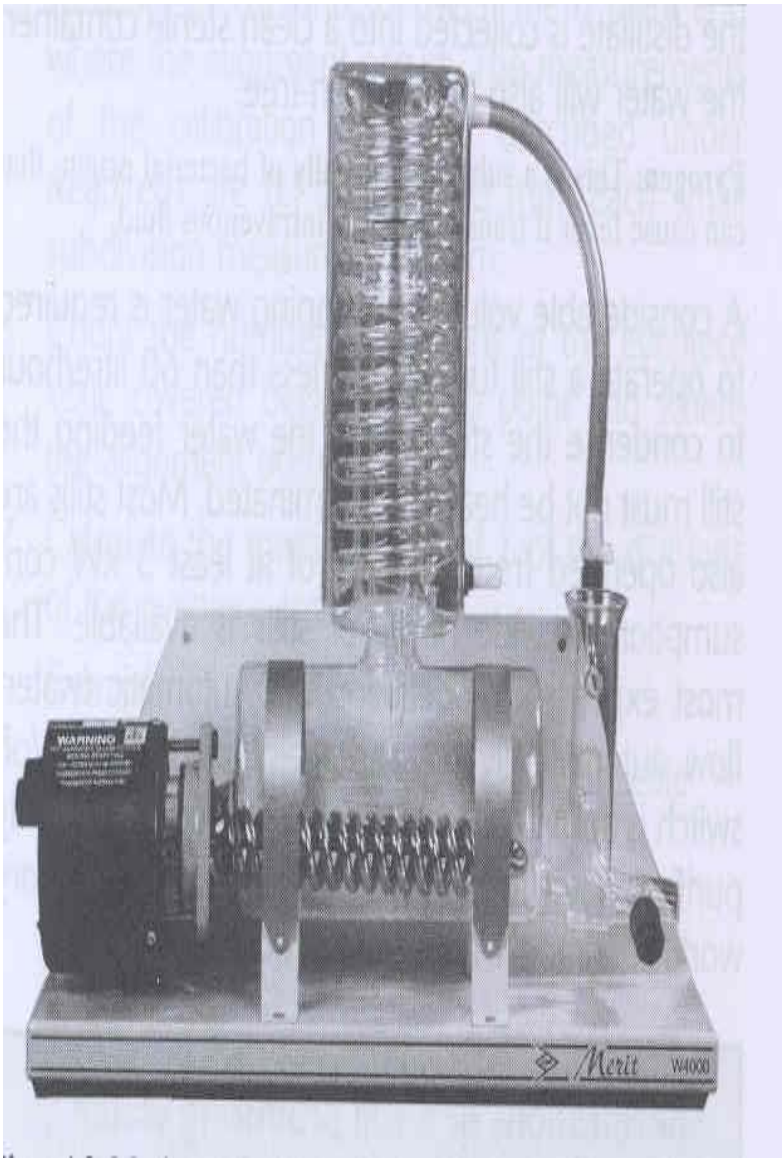


Pestle and mortar



Dessicator

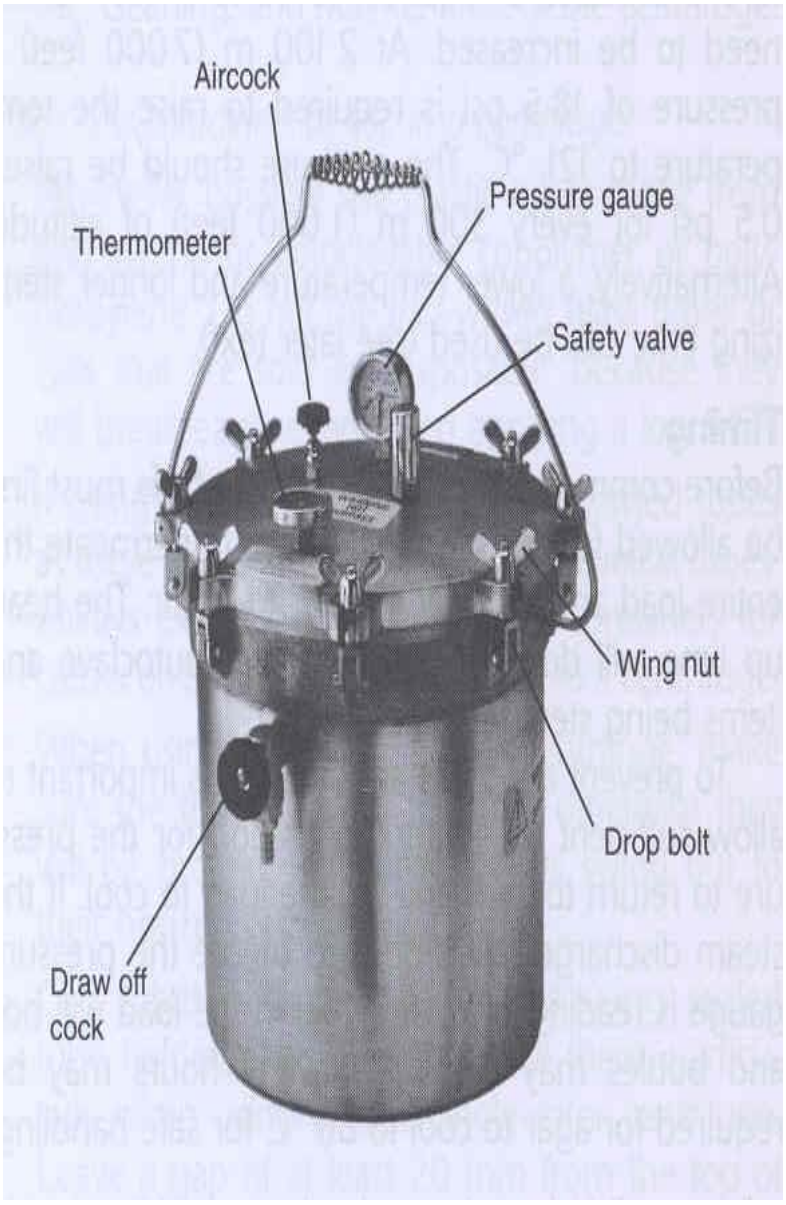
Laboratory equipment



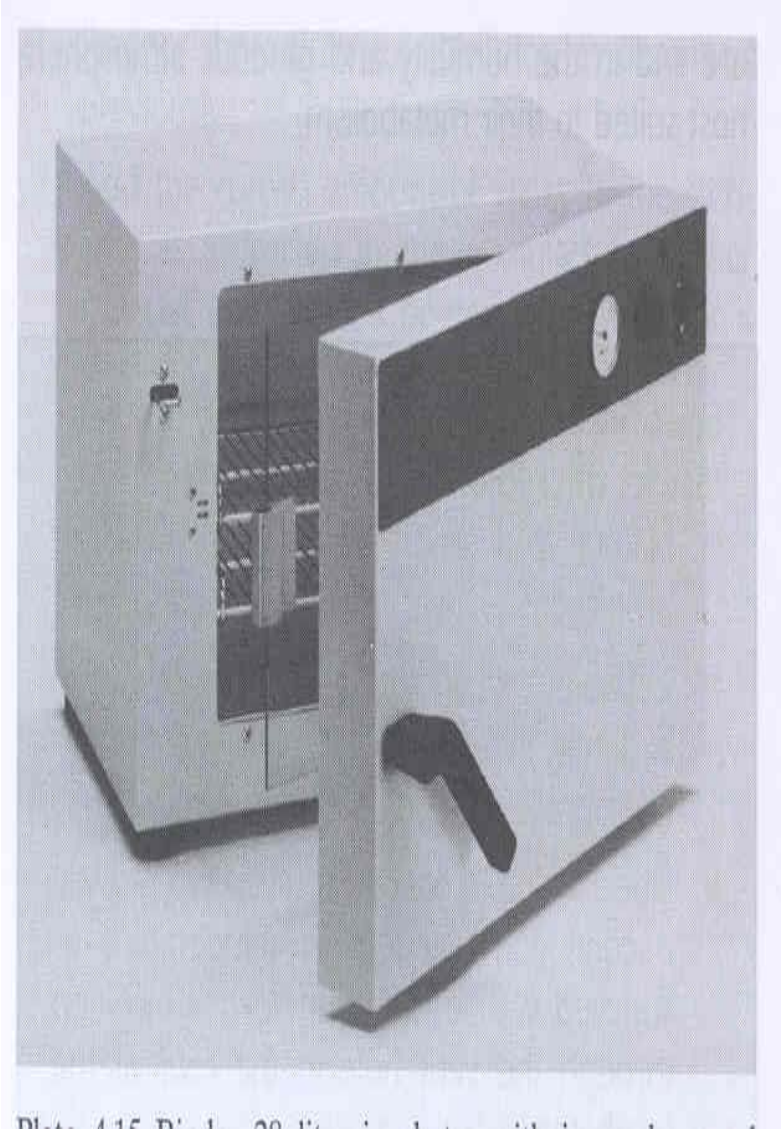
Water distiller

Plate 4.4 Ohaus 311 mechanical beam balance, readability of

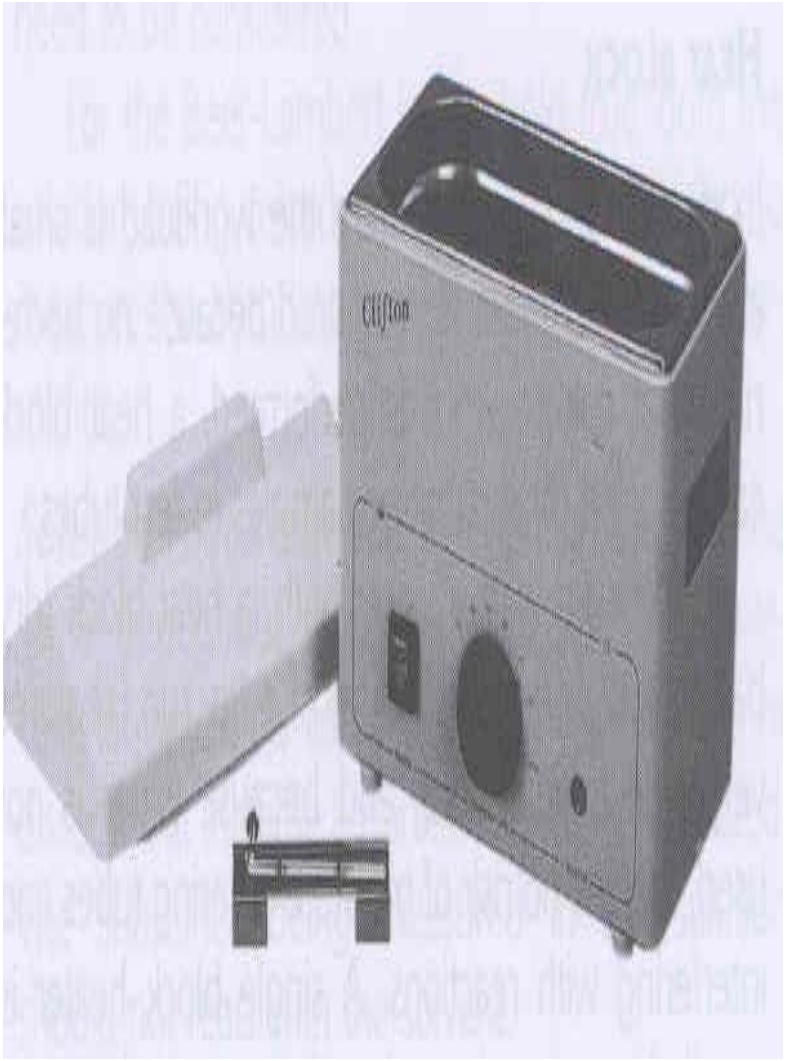
Mechanical beam balance


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

Autoclave



Incubator



Safety signs and symbols

Safety signs and symbols