COURSE GUIDE

PHS 303
INTRODUCTION TO CLINICAL LABORATORY TECHNIQUES

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INTRODUCTION

Introduction to Medical (Clinical) Laboratory Techniques (PHS 303) is a second semester two-credit unit course available to students of Bachelor of Science, (B.Sc.) Public Health Science in the National Open University of Nigeria (NOUN).

Medical (Clinical) Laboratory Techniques entail the Concepts, Principles, Procedures and Equipment used in a professional Clinical Laboratory, by extension assist in the diagnosis and treatment of diseases by performing qualitative ,quantitative or screening test procedures or examination on materials derived from the human body. This course provides the fundamental knowledge of the Clinical Laboratory practice.

WHAT YOU LEARN IN THIS COURSE

The course consists of three Modules and nine Units and a Course Guide. This Course Guide tells you briefly what the Course is all about , what Course Materials you will be using and how you can work with the materials. In addition, it advocates some general guidelines for the amount of time you are likely to spend on each unit of the Course in order to complete it successfully.

Furthermore, it gives you guidance in respect of your Tutor-Marked Assingment which will be made available in the assignment file. There will be regular tutorial classes that are related to the Course. It is advisable for you to attend these tutorial sessions. The Course will prepare you for the challenges you will meet in Medical Laboratory Practices as you go fully into your professional practice.

COURSE AIMS

The course aims at providing an understanding of the basic techniques employed in clinical laboratory practices.

COURSE OBJECTIVES

Each Module and unit have specific objectives which are stated at the beginning of the unit. It is advised that you read the objectives before going into the main content as this will enable self-monitoring of your understanding of the module and unit. Reading the objectives again at the end of each unit will give room for self-assessment of your understanding of the unit. After going through the course, you should be able to:

(a) distinguish between the functions of the different clinical laboratory divisions or Sections (departments).
(b) explain the basic approach to the laboratory in the laboratory diagnosis.
(c) collect clinical specimens of acceptable quality suitable for laboratory testing for public health intervention or fore pidemiological studies.
(d) comprehend the general principle and techniques employed in clinical laboratory practice.
(e) have the basic knowledge required to perform some common and simple laboratory procedures.
(f) highlight the essentials of managing a clinical laboratory.

WORKING THROUGH THIS COURSE

In this course, it is required of you to read each unit and any other materials that may be provided by the NOUN. The text book"Introduction to Medical Laboratory Technology" by Baker & Silverton (Seventh Edition), Butterworth: Heinemann publishing Co.. and also, the Manual of Basic Techniques for a Health Laboratory (2^{nd}Edition). World Health Organisation, Geneva could be found useful. Medical laboratory Science, J OCHEI, A KOLHATKAR., other related books and articles .

The course should take a total of 10-12 weeks, at the end of which shall be an examination. This course is divided into three modules and nine study units. There is Tutor-Marked Assignment at the end of each unit, you are advised to answer the assignments in your own words (avoid copying directly from the course material). You are also required to submit them to your facilitator for grading.4-5 visits to a clinical laboratory within the community is recommended with the view of getting acquainted with some common laboratory equipments and some laboratory tests, discussed in the modules and units respectively. The reports of the visits must be submitted to the facilitator for assessment. The report is expected to show the procedures of activities learnt during the visits.

THE COURSE MATERIALS

The components of the Course are:

1. The Course Guide
2 Modules
2. Study Units
3. References/Further Reading
4. Assignments
5. Presentation Schedule

STUDY MODULES/UNITS

This course consists of six Modules and thirteen units. Unit 1 of Module 1 is about Clinical/Medical laboratory while unit 2 discusses Safety in the Laboratories and 3 major causes of laboratory hazards. Module 2; Unit 1 discusses collection of specimens in the laboratory while unit 2 focuses on the Principles and Techniques ,as 3 emphasises on the management of Laboratories .Module 3 dealt with Microbiology department. Unit 1 focuses on bacteriology while unit 2 discusses Parasitology. Module 4; unit1 and 2 discuss haematology and Blood Group Serology while Module 5; unit 1 and 2 acquaint you with basicMajor Techniques, collection of samples, Principles and how to run Tests/Analysis in Clinical Chemistry and module 6 , unit 1 discusses Histopathology laboratory . You are expected to give a brief report/account of at least two visits to your nearest Clinical Laboratory; showing the activities learnt/participated . A diagram of equipments seen is also expected,an average of 2 ½ weeks is assigned for the study of each unit.

TEXTBOOKS AND REFERENCES

The textbook "Introduction to Medical Laboratory Technology" by Baker & Silverton (Seventh Edition), Butterworth: Heinemann publishing Co.. andalso, the Manual of Basic Techniques for a Health Laboratory (2ndEdition). World Health Organization, Geneva could be found useful.Medical laboratory Science, J OCHEI, A KOLHATKAR., other related books and articles .

ASSIGNMENT FILE

The three aspects of assessment ascribed to this course are as follows:

Report of a minimum of 4 visits to a clinical laboratory recommended in the course material. (10%)
Tutor-Marked Assignments ,(20%)
Final Examination, (70%).

The report of the visits is expected to cover the followings:

(a) Collection of blood samples by venipuncture
(b) Diagram and principles of operation of any 2 equipments seen in the laboratory visited .
(c) Report of test procedures learnt during the visits. (Stool microscopy, blood group by tile method, urinalysis, occult blood test etc) as done in the laboratory visited.

TUTOR-MARKED ASSIGNMENTS

The TMA is a continuos assignment component of your Course. It accounts for 30% of the total score. You will be given 4 TMAs to answer. Three of these must be answered before you are allowed to sit for the end of Course examination. The TMAs will be given to you by your Facilitator and returned after you have done the assignment. Assignment questions for the units in this Course are contained in the assignment file. You will be able to complete the assignments from the information and material contained in your reading, references and study units. However, it is desirable in all degree level of education to demonstrate that you have read and researched more into your references, which will give you a wide view point and provide you with a deeper understanding of the subject.

Make sure that each assignment reaches your facilitator on or before the deadline given in the presentation schedule and assignment file . If for any reason you can not complete your work on time , contact your facilitator before the assignment is due to discuss the possibilty of an extension. Extension will not be granted after the due date unless there are exceptional circumstances.

FINAL EXAMINATION AND GRADING

The end of Course of Examination for Introduction to Clinical Laboratory Techniques will be for about 2 hours and it has a value of 70 percent of the total course work. The examination will consist of questions, which will reflect the type of self-testing, practice exercise and tutor-marked assignment problem you have previously encountered. All areas of the course will be assessed.

You are advised to use the time between finishing the last unit and sitting for the examination to revise the whole course . You might find it useful to review your self-tests, TMAs and comments on them before the examination. The end of Course examination covers information from all parts of the Course .

PRESENTATION SCHEDULE

Your course materials have important dates for the early and timely completion and submission of your TMAs and attending tutorials. You should remember that you are to submit all your assignments by the stipulated time and date. You should guard against falling behind in your work.

COURSE MARKING SCHEME

Assingnment Marks
Assignment 1-920%
Report of visits to the laboratory (minimal 4 visits)10%
End of course exam70%
Total 100%

HOW TO GET THE MOST FROM THIS COURSE.

In distance learning, the study units replace the university lecturer. This is one of the huge advantages of distance learning mode; you can read and work through specially designed study materials at your own pace and at a time and place that suit you best.

Think of it as reading from the teacher, the study guide tells you what to read, when to read and the relevant texts to consult. You are provided exercises at appropriate points, just as a lecturer might give you an in-class exercise.

Each of the study units follows a common format. The first item is an introduction to the subject matter of the unit and how a particular unit is integrated with the other units and the course as a whole. Next to this is a set of learning objectives. These learning objectives are meant to guide your studies. The moment a unit is finished, you must go back and check whether you have achieved the objectives. If this is made a habit, then you will significantly improve your chances of passing the course.

The main body of the units also guides you through the required readings from other sources. This will usually be either from a set book or from other sources.

Self -assessment exercises are provided throughout the unit, to aid personal studies and answers are provided at the end of the unit. Working through these self- tests will help you to achieve the objectives of the unit and also prepare you for tutor marked assignments and examinations. You should attempt each self- test as you encounter them in the units.

The following are practical strategies for working through this course

1. Read the Course Guide thoroughly.
2. Organize a study schedule. Note the time you are expected to spend on each unit and how the assignment relates to the units. Important details, e.g. details of your tutorials and the date of the first day of the semester are available. You need to gather

together all these information in one place such as a diary, a wall chart calendar or an organizer. Whatever method you choose, you should decide on and write in your own dates for working on each unit.

3. Once you have created your own study schedule, do everything you can to stick to it. The major reason that students fail is that they get behind with their course works. If you get into difficulties with your schedule, please let your tutor know before it is too late for help.
4. Turn to Unit 1 and read the introduction and the objectives for the unit.
5. Assemble the study materials. Information about what you need for a unit is given in the table of contents at the beginning of each unit. You will almost always need both the study unit you are working on and one of the materials recommended for further readings, on your desk at the same time.
6. Work through the unit, the content of the unit itself has been arranged to provide a sequence for you to follow. As you work through the unit, you will be encouraged to read from your set books.
7. Keep in mind that you will learn a lot by doing all your assignments carefully. They have been designed to help you meet the objectives of the course and will help you pass the examination.
8. Review the objectives of each study unit to confirm that you have achieved them. If you are not certain about any of the objectives, review the study material and consult your tutor.
9. When you are confident that you have achieved a unit's objectives, you can start on the next unit. Proceed unit by unit through the course and try to pace your study so that you can keep yourself on schedule.
10. When you have submitted an assignment to your tutor for marking, do not wait for its return before starting on the next unit. Keep to your schedule. When the assignment is returned, pay particular attention to your tutor's comments, both on the tutor-marked assignment form and also that written on the assignment. Consult your tutor as soon as possible if you have any question/s or problem/s.
11. After completing the last unit, review the course and prepare yourself for the final examination. Check that you have achieved the unit objectives (listed at the beginning of each unit) and the course objectives (listed in this course guide).

FACILITATOR/TUTORS AND TUTORIALS

Information about the hours of tutorials, dates, times and location of the tutorials as well as the name and contact of your facilitator will be communicated to you as soon as you are allocated into a tutorial group.

All assignments are expected to be mailed to your facilitator. You can contact your facilitator for any assistance or clarification.

You should endeavour to read well, ruminate over what you have read, go through the Self- Assessment Exercise and TMA provided in each Study unit. You will definitely appreciate this course.

SUMMARY

Introduction to Clinical Laboratory Techniques is a Course that has been planned to provide a general view and understanding of Laboratory techniques and procedures that are most commonly seen in the Clinical Laboratory facilities in Nigeria and elsewhere that you will come across in the course of learning and practising your profession. Upon completing this Course , you will be equiped with the basic knowledge of Clinical Laboratory Techniques like urine analysis, Haemoglobin estimation, Pregnancy test, collection of blood samples and others eg Urine, Stool or Faeces, Sputum etc. In addition, you will be able to answer the following questions:

The real meaning of Clinical Laboratory Techniques and Procedures You will be able to understand and define some Principles behind some techniques and Procedures You will be able to carry out simple tests like stool or faecal analysis.

Wishing you success in the course and I hope you will find the Course very interesting.

MAIN COURSE

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MODULE 1 CLINICAL ORMEDICAL LABORATORY

UNIT 1 THE CLINICAL OR MEDICAL LABORATORY AND DIAGNOSTIC SKILLS IN HEALTH PRACTICE

CONTENTS

1.0 INTRODUCTION

Clinical or medical laboratory provides laboratory information and services needed for the diagnosis and treatment of diseases .This unit discusses the Clinical or Medical laboratory and diagnostic skills in health practice.

It will give you the overall view of what Clinical or medical laboratory is all about, as well as the role of the Medical or Clinical Laboratory in diagnosis of diseases.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- know what medical or clinical laboratory is all about,
- discuss medical or cclinical laboratory and diagnostic skills in health practice.
- eexplains the key elements of diagnostic skills
- eexplain the basic approach of the laboratory in diagnosis of diseases.

3.0 MAIN CONTENT

3.1 The Clinical Laboratory

Clinical laboratory or medical laboratory is a facility that provides controlled conditions in which tests are done on body fluids in order to acquire information about the health of an individual (or patient) for the purpose of diagnosis, treatment, and prevention of disease or medical research.

The major role of a medical laboratory is to assist in the diagnosis and treatment of diseases by performing qualitative, quantitative or screening test procedures or examinations on materials derived from the human body. The laboratory plays a vital role in the overall comprehensive health care system by:

(i) confirming provisional diagnosis of the clinician,
(ii) ruling out a diagnosis,
(iii) detecting diseases
(iv) regulating therapy .

In recent times, the increased demands on the laboratory has led to the introduction of more specialized and sophisticated procedures including automation and computerization.

3.1.2 Test Utilization

Test utilization should be defined as a strategy for performing appropriate laboratory and pathology testing with the goal of providing high-quality, cost-effective patient care. A test utilization program must be focused on patient care, ultimately leading to a more efficient and cost-effective laboratory diagnostic approach to answer the clinical questions being asked. A test utilization initiative cannot be driven as a pure cost-control process. If the primary motive is financially instead of patient care driven, then any utilization program will either be short-

lived or ineffective in its outcome. High-quality medical practice must be the driving force if a test utilization program is to be successful.

3.1.2 Laboratory informatics

Laboratory informatics is the specialized application of information technology to optimize and extend laboratory operations.

Laboratories today are held together by a system of software programs and computers that exchange data about patients, test requests, and test results known as a Laboratory information system or LIS. The LIS is often interfaced with the hospital information system, and Laboratory instruments. This system enables hospitals and labs to order the correct test requests for each patient, keep track of individual patient or specimen histories, and help guarantee a better quality of results as well as printing hard copies of the results for patient charts and doctors to check.

3.1.3 The Laboratory (Design)

The design, size and organization of a medical laboratory depends on the nature of work to be carried out, and the availability of funds and space. Basically, the laboratory, no matter the size, must have enough rooms for full movement of the people working in it . An overcrowded and cramped laboratory easily leads to accidents, damaging of instruments and endangering of life.

The floor of the laboratory must be well constructed, washable, non-slip, impermeable to liquids and resistant to the chemicals used in the laboratory. Floor drains are recommended. The walls must be smooth, washable and free from cracks. At each end of the laboratory, there must be swing doors so that the laboratory staff may not be trapped during fire or other emergencies. A modern laboratory should be effectively air-conditioned. This eliminates strong currents of wind which may cause problems of air-borne infections, aerial contaminations and menace of pests and insects, and which may also constantly deflect the flame of a Bunsen burner.

The laboratory should be sectioned into separate rooms or working areas with designated places and rooms for patients, reception of specimens, visitors and staff rest room. The working benches should be smooth-surfaced, impervious, washable and resistant to disinfectants and chemicals used in the laboratory. The working benches should be about 60-65cm, high and 50-60cm, wide. To give good working space and facilitate effective cleaning of the bench tops should not be cluttered. Cupboards and shelves can be constructed to fit in under and above the

benches respectively .A fully air-conditioned and fire proof store for reagents and chemicals must be part of the laboratory. Hand-wash Basins are to be located at strategic corners of the laboratory and paper towels and soaps should always be available near them. The laboratory should have adequate lighting arrangement. A steady supply of electric power and running tap water are essential for the laboratory to function effectively . An adequate and safe waste disposal system for the laboratory is also important. In addition fire-extinguishers must be conspicuously located in the laboratory, and fume cupboards/safety cabinets must be provided. It is now the practice to use modular designs for the laboratory layout. This is because the concept of modular designs offers much more flexibility in overall layout of a busy laboratory.

3.2 Medical Diagnosis

Diagnosis is defined as the determination of the nature of an illness or disorder in a patient through physical examination, medical tests or other procedures.

Medical diagnosis (abbreviated D_S or Dx) can also be defined as the process of determining which disease or condition explains a person's symptoms and signs. It is usually called just diagnosis when the medical context is implicit (unless its distinction from a nursing diagnosis is being clarified).

The information required for diagnosis is typically collected from a history and physical examination of the person seeking medical care. Often, one or more diagnostic procedures, such as diagnostic tests, are also done during the process. Diagnosis is often challenging, because many signs and symptoms are nonspecific. For example, redness of the skin (erythema), by itself, is a sign of many disorders and thus doesn't tell the healthcare professional what is wrong. Thus differential diagnosis, in which several possible explanations are compared and contrasted, must be performed. This involves the correlation of various pieces of information followed by the recognition and differentiation of patterns. Occasionally the process is made easy by a sign or symptom (or a group of several) that is pathognomonic (of a sign or symptom) specifically characteristic or indicative of a particular disease or condition.

There are two parts of the above definition of diagnosis,

(a) determination of an illness or disease through:
(i) Physical examination
(ii) Medical tests or other procedures.

One cannot identify a thing, without a prior information or knowledge about it. This therefore, implies that skills are needed in making medical diagnosis. These skills, call diagnostic skills, are knowledge and experience required in identifying and understanding cause-and-effect relationship between symptoms and signs of disease and the underlying sources. Signs and symptoms of disease or illness in patients are obtained by the health care provider (or health professional) through interaction with the patient in a process called physical examination.

(b) Physical examination (also called Clinical Examination);This refers to the process or a form of interaction between the health professional (or health care provider) and the patients during which the following take place:

(1) The health professional obtains medical history from the patient through which symptoms of disease complained by the patient(experienced or being experienced) are noted. Symptom is defined as subjective evidence of disease perceived by the patient.

(2) The body of the patient is checked (examined) with a view of detecting signs of disease on the patient. Sign is defined as objective evidence of disease perceptible to the examining health professional.

(3) Vital signs, which are measures of various physiological statistics that assess the most basic body functions, are taken (or measured). Vital signs include the following:

1. Body temperature
2. Pulse rate
3. Respiration rate
4. Blood pressure.

3.2.1 Medical Tests

A medical test is a form of medical procedure performed to diagnose or evaluate or monitor disease, disease processes and susceptibility and also to determine a course of treatment.

By utilization:
Medical tests can be classified by what the test result will be used for, mainly including usage for diagnosis, screening or evaluation, as separately detailed below: Diagnostic;

A diagnostic test is a procedure performed to confirm, or determine the presence of disease in an individual suspected of having the disease,

usually following the report of symptoms, or based on the results of other medical tests. Such tests include**:**
Utilizing nuclear medicine techniques to examine a patient having a lymphoma.

Measuring the blood sugar in a person suspected of having diabetes mellitus, after periods of increased urination.

Taking a complete blood count of an individual experiencing a high fever, to check for a bacterial infection. Monitoring electrocardiogram readings on a patient suffering chest pain, to diagnose or determine any heart irregularities.

Screening
Screening refers to a medical test or series of tests used to detect or predict the presence of disease in individuals at risk for disease within a defined group, such as a population, family, or workforce. Screenings may be performed to monitor disease prevalence, manage epidemiology, aid in prevention, or strictly for statistical purposes.

Examples of screenings include measuring the level of TSH in the blood of a newborn infant as part of newborn screening for congenital hypothyroidism, checking for Lung cancer in non-smoking individuals who are exposed to second-hand smoke in an unregulated working environment, and Pap smear screening for prevention or early detection of cervical cancer.

Monitoring
Some medical tests are used to monitor the progress of, or response to medical treatment.

Medical tests include:
(i) Medical Imaging
(ii) Medical Laboratory Tests
(iii) Electrocardiography

The inevitable roles of medical tests in making diagnosis is corroborated by Tony (2010) who showed that over-reliance on physical examination Alone can lead to missed diagnosis and poor diseases outcome.
This is because:

(a) many signs and symptoms may not be specific for any particular Disease.
(b) some diseases may be asymptomatic (present no symptom).
(c) patients often forget or mispresent past symptoms.
(d) ssymptoms are subjective and hence may be psychological.

Therefore, medical tests are often needed as an aid to medical diagnosis. Information or data obtained by the attending health professional during the clinical examination, and the results of the medical tests are skillfully interpreted to arrive at the diagnosis.

3.2.2 Key Elements of Diagnostic Skills

Diagnostic skills have earlier been defined as the knowledge and experiences required in identifying the cause-and-effect relationship (between symptoms and signs) and the underlying sources.

The first step in diagnostic reasoning which is based on knowledge and experience is:

Data Acquisition: The attending health care provider acquires or gathers data during clinical examinations. Such data include:

(i) History
(ii) Signs and symptoms
(iii) Results of medical tests (laboratory or imaging studies).

Creation of Mental Abstraction: This is a brief summary of the case presented by the patients, in defining the specific case in abstract terms. This is also called problem representation. Creating a concise and appropriate problem representation depends on the ability to recognize the information (or data) that points to a particular diagnosis while ruling out other possibilities and also on the ability to recall conditions, syndromes, diseases and other relevant information that are connected to problem representations.

Generation of Hypothesis: Accurate problem representation triggers generation of hypothesis. Generation of hypothesis implies forming a hypothetical diagnosis and other data to the possible disease. Gathering and Selection of Illness Script: The hypothesis generated triggers search for more information on defining features of the specific illness being diagnosed. Such information being recalled from the memory store or being drawn from the wealth of experience of the health care provider serves as hypothesis testing leading to final diagnosis.

3.2.3 Laboratory Diagnosis of Diseases

Diseases can generally be grouped into two: communicable diseases and Non-communicable diseases.

Communicable diseases

Communicable diseases are illnesses caused by specific infectious agents or their toxic products. It arises through transmission of that agent or its products from an infected person, animal, or inanimate reservoir to a susceptible host, either directly or indirectly (through an intermediate plant or animal host, vector, or the inanimate environment). Control of disease is the reduction of disease incidence, prevalence, morbidity, or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction. Control is to be contrasted with elimination (reduction to zero of the incidence of a specified disease in a defined geographic area as a result of deliberate efforts; continued intervention measures are required), eradication (permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed), and extinction (the specific infectious agent no longer exists in nature or the laboratory).Communicable diseases may be classified according to the causative agent, the clinical illness caused, or the means of transmission. Often all three characteristics are used (e.g., food-borne *Salmonella* gastroenteritis). Causative agents include bacteria, viruses, and parasites. Examples of bacterial diseases include pneumococcal pneumonia and gonorrhea.

Viral diseases include influenza, measles, and ebola. Parasitic diseases include malaria and schistosomiasis. Other communicable diseases may be caused by other types of microorganisms such as fungi (e.g., histoplasmosis). The types of illness include pneumonia, diarrhea, meningitis, or other clinical syndromes.

Various categorizations of means of transmission have been used. The American Public Health Association uses these categories: direct transmission, indirect transmission, and airborne. Direct transmission refers to direct contact such as touching, biting, kissing, or sexual intercourse, or the direct projection of droplet spray into the eye, nose, or mouth during sneezing, coughing, spitting, singing, or talking. This projection usually is limited to a distance of 1 meter or less. Examples of direct contact transmission include rabies and sexually transmitted HIV (human immunodeficiency virus). Direct projection is responsible for transmission of diseases such as measles and influenza.

Indirect transmission may occur through a vehicle or an arthropod vector. The causative agent may or may not multiply or develop in or on the vehicle. Examples of possible vehicles include water, food, biological products, or contaminated articles (such as syringe needles). Water-and food borne diseases have the potential for causing outbreaks involving thousands of persons. Before the causative agent was

identified, many cases of HIV resulted from blood transfusion. Since all donor blood in the United States is now screened for HIV, this is no longer a significant means of transmission. However, sharing of needles by injection drug users remains an important factor in the AIDS (acquired immunodeficiency syndrome) epidemic. Arthropod vectors can spread disease mechanically (as a result of contamination of their feet or passage of organisms through the gastrointestinal tract) or biologically (in which the agent must multiply or go through one or more stages of its life cycle before the arthropod becomes infective). Mechanical spread by arthropod vectors is uncommon. However, arthropod-borne diseases such as malaria (in which the parasite develops within the mosquito vector) are still responsible for millions of cases and hundreds of thousands of deaths each year in tropical countries.

Some infectious agents can be spread through the air over long distances. Airborne spread requires that infectious particles are small enough to be suspended in the air and inhaled by the recipient. Tuberculosis and histoplasmosis are bacterial and fungal diseases spread in this fashion. Airborne transmission could also be used to disseminate agents of biological warfare or bioterrorism. Anthrax and smallpox have been considered among the most likely biological weapons. Diseases of animals that can be spread to humans are called zoonoses. Some zoonotic diseases include rabies, plague, and tularemia (rabbit fever).

Methods of Control

Communicable diseases occur only when the causative agent comes into contact with a susceptible host in a suitable environment. Prevention and control efforts for communicable diseases may be directed to any of these three elements. Communicable diseases affect both individuals and communities, so control efforts may be directed at both. Treatment of persons with communicable diseases with antibiotics typically kills the agent and renders them noninfectious. Thus, treatment is also prevention. A simple way to prevent the occurrence of communicable diseases is to eliminate the infectious agent through, for example, cooking food, washing hands, and sterilizing surgical instruments between use. Assuring the safety of drinking water through filtration and chlorination and treating sewage appropriately are other important means of preventing the spread of communicable diseases. For most communicable diseases there is an interval between infection and occurrence of symptoms (the incubation period) in which the infectious agent is multiplying or developing. Some persons who are infected may never develop manifestations of the disease even though they may be capable of transmitting it (inapparent infection). Some persons may carry (and transmit) the agent over prolonged periods (carriers) whether or not they develop symptoms. Treatment during the incubation period may cure the infection, thereby preventing both disease and

transmission. This preventive treatment (chemoprophylaxis) is often used in persons who have been exposed to sexually transmitted diseases such as syphilis and gonorrhea. It also is effective in persons who have been infected with tuberculosis, although the preventive treatment must be given for several months.

The susceptibility of the host to a specific infectious agent can be altered through immunization (e.g., against measles) or through taking medications that can prevent establishment of infection following exposure (chemoprophylaxis). Since malnutrition and specific vitamin deficiencies (such as vitamin A) may increase susceptibility to infection, ensuring proper nutrition and administering vitamin A can be more general ways of increasing host resistance. If persons survive a communicable disease, he or she may develop immunity that will prevent the disease from recurring if re-exposed to the causative agent.
The environment may be rendered less suitable for the occurrence of disease in a variety of ways. For example, food can be kept hot or cold (rather than warm) to prevent multiplication of organisms that may be present. Individuals can use mosquito repellents or mosquito nets to prevent being bitten by infected mosquitoes. Breeding places can be drained or insecticides used to eliminate vectors of disease. Condoms can be used to prevent sexually transmitted diseases by providing a mechanical barrier to transmission. Reduction of crowding and appropriate ventilation can reduce the likelihood of droplet or airborne transmission. Respiratory protective devices can be used to prevent passage of microorganisms into the respiratory tract.

The socio-cultural environment is also important in affecting the occurrence of communicable diseases. For example, in the 1980s there was a change in the social norms in men who have sex with other men on the West Coast of the United States, where unprotected anal intercourse had been the norm and was responsible for considerable transmission of HIV. As a result of a variety of educational and social marketing approaches, the social norm changed to the use of condoms and the rate of new HIV infections (and of rectal gonorrhea) declined.

Similarly, aggressive social marketing of condom use in Uganda has led to a change in sexual practices and a decline in new HIV infection rates. Other societal approaches to control of communicable diseases include safe water and food laws, provision of free immunization and chemoprophylaxis through public health departments, enactment and enforcement of school immunization requirements, isolation of individuals with communicable diseases to prevent transmission, and quarantine of individuals exposed to communicable diseases to prevent disease transmission during the incubation period if they have been infected.

Preventive Measures

Vaccine-Preventable Diseases: Some communicable diseases can be prevented by the use of vaccines. The word vaccine comes from *vaccinia*, the Latin name for cowpox. The first vaccine was developed by Edward Jenner, an eighteenth-century English physician and naturalist who noticed that milkmaids who had acquired cowpox (a condition that caused lesions to appear on the udders of cows) on their hands did not seem to be affected by smallpox. He believed that infection with cowpox would protect against smallpox, a serious, often fatal epidemic disease. In 1796 he took material from a skin lesion on the hand of a milkmaid and inoculated it into the arm of a young boy. The boy was subsequently exposed to smallpox and did not become ill. Thus began the vaccine era.

It was nearly one hundred years until the next vaccine (rabies) was developed by Louis Pasteur. In the twentieth century, a number of vaccines were developed; many more are under development as a result of the biotechnology revolution. Widespread use of vaccines in children has had a dramatic impact on the occurrence of the diseases.

Because smallpox has been eradicated, smallpox vaccination is no longer carried out. The last case of naturally occurring smallpox in a human was in 1977, and in 1980 the World Health Assembly certified that smallpox had been eradicated from the face of the earth. Stocks of smallpox virus have been maintained (under security) in both the United States and Russia, though the debate continues whether they should be destroyed. Concerns have arisen about the possibility that some groups or nations have retained the smallpox virus and developed it for use in biological warfare or bioterrorism.

Chemoprophylaxis: Chemoprophylaxis refers to the practice of giving anti-infective drugs to prevent occurrence of disease in individuals who are likely to be exposed to an infectious disease or who might have already been infected but have not developed disease. For example, individuals traveling to areas where malaria is common can take anti-malarial drugs before arriving, during their stay, and for a few weeks after leaving and thus protect themselves against malaria. Similarly, persons who have been exposed to syphilis can be given penicillin to prevent the possibility of their developing syphilis, and persons who have been infected with tuberculosis can be given six months of treatment to prevent the development of tuberculosis.

Antibiotics and Resistance: Antibiotics are compounds that are produced by microorganisms that kill or inhibit the growth of other microorganisms. Those that kill bacteria are called bactericidal; those that prevent multiplication (and rely on the body's defense mechanisms

to deal with the limited number of living organisms) are called bacteriostatic. Some antibiotics are effective against a limited number of micro-organisms, others may have more widespread effect.
Because microorganisms are continually in a state of evolution, strains may evolve that are resistant to a particular antibiotic. In addition, resistance characteristics can be transferred from some microorganisms to others (this is particularly true of organisms that inhabit the gastrointestinal tract). The likelihood that resistance will develop is increased if antibiotics are used in an indiscriminate manner and in inadequate amounts (either in terms of individual dosage or in length of therapy). Antimicrobial resistance is a growing problem: organisms that once were exquisitely sensitive to a particular antibiotic may now have developed significant (or total) resistance to it. This necessitates either increasing the dose of the antibiotic administered (in the case of partial resistance) or developing totally new drugs to treat the infection (in the case of total resistance). A few microorganisms (such as *enterococcus*, an organism that lives in the intestinal tract and is particularly likely to cause infections in gravely ill patients with compromised immune systems) have developed such widespread resistance that it is a real challenge to treat them effectively, resulting in a need to develop even more antibiotics.

Emerging and Re-Emerging Infectious Diseases
New infectious diseases continue to be recognized and others, once thought under control, are reemerging as significant problems. To cite a few examples of "new" diseases, the following have been recognized for the first time since 1975: legionnaire's disease, ebola virus, HIV/AIDS (acquired immunodeficiency syndrome), toxic shock syndrome, *Escherichia coli* O157:H7 (cause of hemolytic-uremic syndrome), Lyme disease, *Helicobacter pylori* (major cause of peptic ulcer), hepatitis C, and Hantavirus. Some of these are conditions previously known but without known infectious causes (e.g., peptic ulcer) while others represent apparently new clinical syndromes that have not occurred or have not been recognized in the past.

Old diseases, such as tuberculosis and malaria, are reemerging in areas where they were once under control. This may be a result of the lack of continued application of known effective interventions but also may result from ecological changes. Some of the factors involved in the increase in infectious diseases, whether new or old, include population shifts and growth (and encroachment on previously unpopulated areas); changes in behavior (e.g., injection drug use, sexual practices); urbanization, poverty, and crowding; changes in ecology and climate; evolution of microbes; inadequacy of the public health infrastructure to deal with the problems; modern travel and trade; and the increasing numbers of persons with compromised immune systems (whether as a

result of HIV/AIDS, chemotherapy for cancer, or immune suppressive therapy for organ transplants). Many of these factors are interrelated. In addition to these new and reemerging diseases, there may be specific interactions between diseases. This is particularly true with HIV and tuberculosis (TB), in which each infection is a very potent co-factor for worsening the other: Persons with HIV infection who become infected with TB are more likely to develop TB disease that is serious and rapidly progressive than persons without HIV infection, and persons with TB who contract HIV infection are very likely to have a rapid progression to full-blown AIDS.

In the United States, the incidence of food borne disease has received increasing attention in the past several years. This relates in part to improved surveillance but also relates to changes in patterns of food production, distribution, and consumption. With modern transportation, it is possible to get fresh vegetables and fruits at all times of the year. This means that salad ingredients purchased at a modern supermarket (and eaten raw) may have been grown in a developing country, where the average American traveler would not eat raw vegetables. The consolidation of producers of prepared foods makes possible large interstate outbreaks of food-borne disease such as the 1994 outbreak of *Salmonella* infections associated with ice cream that affected an estimated 224,000 persons nationwide. It is currently estimated that food-borne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year.

Epidemic Theory and Mathematical Models of Infectious Diseases

Based on observed characteristics of infectious diseases, epidemiologists have attempted to construct mathematical models that would make it possible to predict the pattern of spread of a condition within the population. Some diseases have constant features, which make mathematical modeling particularly attractive. Measles, for example, has a predictable incubation period (ten to fourteen days) and limited duration of infectivity of a given patient (four to seven days). In addition, it is highly infectious (nearly every susceptible person who comes in contact with an infectious person will become infected), and nearly everyone who is infected develops clinical illness. Lifelong immunity follows infection. There is no nonhuman reservoir. Given these relatively constant parameters, it is possible to predict the pattern of transmission if measles is introduced into a population, using different estimates for the proportion of susceptible persons in the population, the distribution of these susceptibles (e.g., randomly dispersed, clustered together), and the likelihood of contact between the infectious patient and the susceptibles. Because of the extreme infectiousness of measles, models indicate that it is necessary to reach very high levels of immunity in a population (on the order of 95 percent

or greater) in order to prevent sustained transmission of measles. Given the fact that measle vaccine is approximately 95 percent effective, this indicates that, to eradicate measles, it will be necessary to reach 100 percent of the population with a single dose of the vaccine or to reach 90 percent of the population on each of two rounds of vaccination (assuming that the second round will reach 90 percent of those who were not reached by the first round). Since babies are being born all the time, this also must be an ongoing process.

The major reason for continuing debate over whether measles eradication is an achievable goal using current vaccines is the necessity to achieve and maintain such high levels of immunity.

Non-communicable diseases (NCDs) are medical conditions or diseases that are non-infectious or non-transmissible. NCDs can refer to chronic diseases which last for long periods of time and progress slowly. Sometimes, NCDs result in rapid deaths such as seen in certain diseases such as autoimmune diseases, heart diseases, stroke, cancers, diabetes, chronic kidney disease, osteoporosis, Alzheimer's disease, cataracts, and others. While sometimes (incorrectly)

Risk factors referred to as synonymous with "chronic diseases", NCDs are distinguished only by their non-infectious cause, not necessarily by their duration. Some chronic diseases of long duration, such as HIV/AIDS, are caused by infections. Chronic diseases require chronic care management as do all diseases that are slow to develop and of long duration.

NCDs are the leading cause of death globally. In 2012 they cause 68% of all deaths (38 million) up from 60% in 2000. About half were under age 70 and half were women. Risk factors such as a person's background, lifestyle and environment increase the likelihood of certain NCDs. Every year, at least 5 million people die because of tobacco use and about 2.8 million die from being overweight. High cholesterol accounts for roughly 2.6 million deaths and 7.5 million die because of high blood pressure.

Risk factors such as a person's background; lifestyle and environment are known to increase the likelihood of certain non-communicable diseases. They include age, gender, genetics, exposure to air pollution, and behaviors such as smoking, unhealthy diet and physical inactivity which can lead to hypertension and obesity, in turn leading to increased risk of many NCDs. Most NCDs are considered preventable because they are caused by modifiable risk factors.

The WHO's *World Health Report 2002* identified five important risk factors for non-communicable disease in the top ten leading risks to health. These are raised blood pressure, raised cholesterol, tobacco use, alcohol consumption, and overweight. The other factors associated with higher risk of NCDs include a person's economic and social conditions, also known as the "[social determinants of health]."

It has been estimated that if the primary risk factors were eliminated, 80% of the cases of heart disease, stroke and type 2 diabetes and 40% of cancers could be prevented. Interventions targeting the main risk factors could have a significant impact on reducing the burden of disease worldwide. Efforts focused on better diet and increased physical activity has been shown to control the prevalence of NCDs.

Environmental diseases

NCDs include many environmental diseases covering a broad category of avoidable and unavoidable human health conditions caused by external factors, such as sunlight, nutrition, pollution, and lifestyle choices. The diseases of affluence are non-infectious diseases with environmental causes. Examples include:

- Many types of cardiovascular disease (CVD)
- Chronic obstructive pulmonary disease (COPD) caused by smoking tobacco
- Diabetes mellitus type 2
- Lower back pain caused by too little exercise
- Malnutrition caused by too little food, or eating the wrong kinds of food (e.g. scurvy from lack of Vitamin C)
- Skin cancer caused by radiation from the sun
- Obesity

Inherited diseases

Genetic disorders are caused by errors in genetic information that produce diseases in the affected people. The origin of these genetic errors can be:

- Spontaneous errors or mutations to the genome:
- A change in chromosome numbers, such as Down syndrome.
- A defect in a gene caused by mutation, such as Cystic fibrosis.
- An increase in the amount of genetic information, such as Chimerism or Heterochromia.

Cystic fibrosis is an example of an inherited disease that is caused by a mutation on a gene. The faulty gene impairs the normal movement of sodium chloride in and out of cells, which causes the mucus-secreting organs to produce abnormally thick mucus. The gene is recessive,

meaning that a person must have two copies of the faulty gene for them to develop the disease. Cystic fibrosis affects the respiratory, digestive and reproductive systems, as well as the sweat glands. The mucus secreted is very thick and blocks passageways in the lungs and digestive tracts. This mucus causes problems with breathing and with the digestion and absorption of nutrients.

Inherited genetic errors from parents:

(i) Dominant genetic diseases, such as Huntingtons, require the inheritance of one erroneous gene to be expressed.
(ii) Recessive genetic diseases require the inheritance of erroneous genes to be expressed and this is one reason they work together.

3.2.4 Laboratory diagnosis of diseases generally entails qualitative and quantitative analyses of clinical specimens (body fluids, tissues and other solid matters from the body).

Laboratory diagnosis of communicable diseases primarily involves methods and techniques of identifying the specific causative organisms (pathogens) in the specimens. This is a major responsibility of clinical microbiology laboratory. The technique involves:

(a) microscopic visualisation of the pathogenic agents in the specimen .
(b) growing the causative microorganisms on or in a medium that support such growth (culture media).
(c) identification of organisms using their phenotypic characteristics i.e. the visible characteristics of an organism resulting from the Interaction between its genetic make-up and the environment. For example:
(1) Characteristic behaviour of bacteria or its metabolic products when exposed to chemical agents.
(2) Cytopathic effect of viral agents in tissue culture (characteristics deteriorating action of virus products on tissue upon which it is grown in the laboratory).
(3) Microscopic morphology characteristics of fungi or parasite (i.e. characteristics appearance of fungi and parasite when view edunder the microscope).

Recombinant DNA technology (DNA sequencing and DNA hybridisation): This technique depends on the basic fact that the primary structure of DNA is known to be unique for every known organism. Moreover, the gene responsible for pathogenic ability of all known pathogens have been identified, sequenced and made available in various genetic databases across the globe. One of the ways by which

this technique is applied in laboratory diagnosis of pathogens is by sequencing the DNA of pathogen in the specimen and compared (or matched) it with the selected reference sequences in order to identify the pathogen. Another technical approach to identification of pathogen using Recombinant DNA technology is called DNA hybridisation. In this approach, the DNA of the infecting organism (in the clinical specimen) is allowed to form a hybrid with a diagnostic probe, which is a labeled DNA sequence complementary to the DNA of the suspected organism. The hybrid formed, which is an indication of the presence of the suspected pathogen is identified by specific colour formation or by fluorescence depend on the type of substance used to label the diagnostic probe.

Laboratory diagnosis of non-communicable disease essentially involves detection, measurement (or both) and analysis of molecular substances(or metabolites) relevant to one or more biochemical (or metabolic)reactions in the body system. Tests that detect or measure such relevant substances are carried out on clinical specimens collected from patients.

Chemical pathology, Haematology and Histopathology are the major Laboratories where such tests are done. Methods employed by these Laboratories in the diagnosis of non-communicable diseases involve the Following:

(i) detection of metabolic products in clinical specimens (e.g. urine analysis).
(ii) measurement of relevant metabolic products in clinical specimens e.g. (measurement of glucose in the blood in the diagnosis of diabetic mellitus).
(iii) Analysis of relative composition of cellular components of blood (full blood count in the diagnosis of blood diseases).
(iv) Histopathological examination of tissues e.g. (microscopic Examination of histology sections in the diagnosis of tumour and cancers).

SELF-ASSESSMENT EXERCISE

i. Mention 5 examples of infectious diseases.
ii. List 5 examples of non- communicable diseases.
iii. 3 Define medical test and classify it based on its usage .

4.0 CONCLUSION

In this unit, you have learnt what clinical/medical laboratory is, the diagnostic skills, medical test, you can now discuss communicable and non-communicable diseases, their prevention, control and diagnosis.

5.0 SUMMARY

The clinical laboratory has been defined as the facility which provides controlled conditions in which tests are performed on clinical specimens in order to acquire information about the health of an individual (or patient) for the purpose of diagnosis, treatment, prevention of diseases or for various medical researches. Identification off the cause(s) and nature of disease in patient (diagnosis) is done by the health care professionals through clinical examination and medical tests. Knowledge and experience required in identifying and understanding the cause-and-effect relationship between symptoms and the underlying sources is called Diagnostic skills. The clinical laboratory diagnosis of communicable diseases employs techniques and methods that identify the causative pathogens, while the laboratory diagnosis of non-communicable diseases involves techniques and methods that:

(a) measure the levels of relevant macromolecules in clinical specimens.
(b) detect the presence of various metabolic products in clinical specimens.
(c) analyze the relative composition of blood cellular components.
(d) assess the effects of diseases on histological architecture of tissues or cells (histopathology or cytopathology).

6.0 TUTOR-MARKED ASSIGNMENT

1. Define clinical laboratory and briefly discuss medical diagnosis.
2a. Define Diagnostic skills
b. Mention two methods each employed by the clinical laboratory in the diagnosis of communicable diseases.
c. List the key elements of diagnostic skills

7.0 REFERENCES/FURTHER READING

Baker, F.J.; Silverton, R.E. & Pallister, C.J. (2011). “Introduction to Haematology”, *In: Introduction to Medical Laboratory Technology*. (7th ed.). Butterworth: Heinemann. International laboratory Accreditation cooperation Harvard; Guide to Diagnostic Tests from Harvard Health .

Barsky, A.J. (2014). Forgeting, Fabricating and Telescoping: *The Instability of the Medical History Arch Intern Med*. 162(a): 981-984. BusinesssDictionary.Com.

Christine, A. H.. Ellen Dyer, J.O.. Richard, K.O & Kent, R. O. (2013). Making a Diagnosis of Herbal-related Toxic Hepatitis. *West J. Med.* 176(1): 39-44.

Deborah, M. Winn. Marsha, E, R. & Elainc, G. (2014*).* Epidemiological Issues in the Design and Use of Biological Specimen Banks. *Epidemiologic Review*, 12: 56-59.

Domandis, E.P. Sequencing with Microarray Technology: A Powerful New Tool for Molecular Diagnostic. *Clinical Chemistry* 2000, 6: 1523-1525.

Farr, J. M. & Laurence, Shatki (2016). Best Jobs Lists Overall, for Different Groups of People and Various Criteria (pgs. 11-31). *In: Best Jobs for the 21st Century* (2nd ed.). JISF Works: Clinical Laboratory Technologists and Technicians, pp. 304-305. Histopathology. http://en.wikipedia.org/wiki/histopathology

John, P. Langlois (2015). 'Making a Diagnosis'. *In: Fundamentals of Clinical Practice.* (2nd ed.). Mark B. Mergel, Warve Lee Holleman, Scott A. Fields. Pg. 198.

Judith, L. Bowen (2014). Educational Strategies to Promote Clinical Diagnostic Reasoning. *N Engl. J. Med*. 355: 221 Thumbnails of Human Subjects. *Intl. Journal of Nuclear Medicine and Biology,* 4(2): 128-132.

Tony Nicholson (2015). Physical examination: The Limitation of the "Stenford 25" *British Med. Journal, pp* 340: 288.

Timothy.J.Pagana(2013).Osby's Diagnostic and Laboratory Test Reference, 11th ed.

Louise.M.Malarkey and Mary .E.McMorrow.Saunders Nursing Guide to Laboratory and Diagnostic Tests, 2nd edition.

UNIT 2 SAFETY IN MEDICAL /CLINICAL LABORATORY

CONTENTS

1.0 INTRODUCTION

Workers in medical/clinical laboratories are exposed to many dangers, not only from infected material, dangerous compounds and apparatus which they use routinely, but also from the common dangers that apply to any home, office or factory. Precautions must be observed by all members of the staff, not only for themselves as individuals but for the safety of all concerned . While the application of safety precautions is mainly a matter of common sense, it is necessary to lay down general rules for guidance which must be observed at all times .

2.0 OBJECTIVES

By the end of this unit you should be able to know what is safety in the laboratory, its purpose, simple safety precautions you can take in the event of any danger in the laboratory and why the health of staff is paramount.

3.0 MAIN CONTENT

3.1 Safety in the Medical or Clinical Laboratory

The practice of medical/clinical laboratory science is associated with hazards and accidents. The laboratory worker is very much at risk of

acquiring transmissible diseases through contact with patients or handling of clinical specimens.

Though accidents do occur in the best of laboratories, a poorly designed and overcrowded laboratory has the increased possibility of hazards and accidents. It should be noted however, that bad laboratory practices are the major causes of laboratory accidents. It follows therefore, that the laboratory should be manned by well-trained, dedicated and meticulous staff . Every laboratory must have an appropriate code of safe laboratory practice.

In some laboratories, the conditions are no more dangerous than in any other room. In many labs, though, hazards are present. Laboratory hazards are as varied as the subjects of study in laboratories, and might include poisons; infectious agents; flammable, explosive, or radioactive materials; moving machinery; extreme temperatures; lasers, strong magnetic fields or high voltage. In laboratories where dangerous conditions might exist, safety precautions are important. Rules exist to minimize the individual's risk, and safety equipment is used to protect the lab user from injury or to assist in responding to an emergency.

The Occupational Safety and Health Administration (OSHA) in the United States, recognizing the unique characteristics of the laboratory workplace, has tailored a standard for occupational exposure to hazardous chemicals in laboratories. This standard is often referred to as the "Laboratory Standard". Under this standard, a laboratory is required to produce a Chemical Hygiene Plan (CHP) which addresses the specific hazards found in its location, and its approach to them.

In determining the proper Chemical Hygiene Plan for a laboratory, it is necessary to understand the requirements of the standard, evaluation of the current safety, health and environmental practices and assessment of the hazards .The CHP must be reviewed annually. Many laboratories employ safety, health, and environmental specialists, such as a Chemical Hygiene Officer (CHO) to develop, manage, and evaluate their CHP. Additionally, third party review is also used to provide an objective "outside view" which provides a fresh look at areas and problems that may be taken for granted or overlooked due to habit.

Inspections and audits like also be conducted on a regular basis to assess hazards due to chemical handling and storage, electrical equipment, biohazards, hazardous waste management**,** chemical waste, and emergency preparedness, radiation safety, ventilation as well as respiratory testing and indoor air quality. An important element of such audits is the review of regulatory compliance and the training of individuals who have access to and/or work in the laboratory. Training

is critical to the ongoing safe operation of the laboratory facility. Educators, staff and management must be engaged in working to reduce the likelihood of accidents, injuries and potential litigation. Efforts are made to ensure laboratory safety videos are both relevant and engaging.

Standards for Personnel Safety
The safety of all laboratory staff is paramount to avoid laboratory accidents that may jeopardize acquisition of infectious agents through handling of blood, as an example. Although exposure cannot always be avoided, every precaution must be taken to provide a safe work environment.

3.1.1 Hazards in the Laboratory

The hazards and accidents in the laboratory are discussed under the following areas:

(i. Infection
(ii) Burns
(iii) Cuts and Pricks
(iv) Hazards of toxic chemicals
(v) Electric shocks

Infection/ Biological Hazards
Infection is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce.[1]Infectious disease, also known as transmissible disease or communicable disease, is illness resulting from an infection.

Infections are caused by infectious agents including viruses, viroids, prions, bacteria, nematodes such as roundworms and pinworms, arthropods such as ticks, mites, fleas, and lice, fungi such as ringworm, and other macroparasites such as tapeworms.

Infections in the laboratory can occur in different ways, but the commonest causes are;

(a) Inhaletion : of pathogens in air-borne droplets (aerosols) which are released during breakage or spilling of infectious fluids, centrifuging, dispensing or pipetting of infectious materials : and snap-opening and closing of specimen containers .
(b) Ingestion of pathogens from contaminated food or fingers .
(c) Ingestion of pathogens by mouth-pipetting .
(d) Pathogens finding their way into the body through needle pricks, cuts, scratches, insect-bites, sores or skin lesions .

Signs and Symptoms
The symptoms of an infection depend on the type of disease. Some signs of infection affect the whole body generally, such as fatigue, loss of appetite, weight loss, fevers, night sweats, chills, aches and pains. Others are specific to individual body parts, such as skin rashes, coughing, or a runny nose.

In certain cases, infectious diseases may be asymptomatic for much or even all of their course in a given host. In the latter case, the disease may only be defined as a "disease" (which by definition means an illness) in hosts who secondarily become ill after contact with an asymptomatic carrier. An infection is not synonymous with an infectious disease, as some infections do not cause illness in a host.

3.1.2 Biological Hazards

Biological hazards, also known as **biohazards**, refer to biological substances that pose a threat to the health of living organisms, primarily that of humans. This can include medical waste or samples of a microorganism, virus or toxin (from a biological source) that can affect human health. It can also include substances harmful to other animals.

The term and its associated symbol are generally used as a warning, so that those potentially exposed to the substances will know to take precautions. The biohazard symbol was developed in 1966 by Charles Baldwin, an environmental-health engineer working for the Dow Chemical Company on the containment products.

It is used in the labeling of biological materials that carry a significant health risk, including viral samples and used hypodermic needles.

Directive on the protection of workers from risk related to the exposure to biological agents at work, classifies biological agents which are known to be pathogenic to humans, but it excludes those agents only pathogenic to plants or animals. The lists of classified agents is based on the effects on health workers. The directive classifies biological agents into the following risk groups:

Group 1: Biological agents unlikely to cause human disease.

Group 2: Biological agents that can cause human disease, and might be a hazard to workers but are unlikely to spread to the community.

There is usually effective prophylaxis or treatment available. An example of a pathogen in this group is Listeria monocytogenes.

Group3: Biological agents which may cause severe human disease, presenting a severe hazard to workers and may present a risk of spreading to the community. There is usually effective prophylaxis or treatment available. An example of a pathogen in this group is Mycobacterium bovis .

Group 4: Biological agent which cause severe human disease, presenting a severe hazard to workers and are likely to spread to the community. There is usually no effective prophylaxis or treatment available. An example of a pathogen in this group is Marburg virus .

Levels of Bio-hazard

Biosafety level. The United States Centers for Disease Control and Prevention (CDC) categorizes various diseases in levels of biohazard, Level 1 being minimum risk and Level 4 being extreme risk. Laboratories and other facilities are categorized as BSL (Biosafety Level) 1-4 or as *P1* through *P4* for short (Pathogen or Protection Level).

- **Biohazard Level 1:** Bacteria and viruses including *Bacillus subtilis*, caninehepatitis, *Escherichia coli*, varicella (chicken pox), as well as some cell cultures and non-infectious bacteria. At this level precautions against the biohazardous materials in question are minimal, most likely involving gloves and some sort of facial protection.
- **Biohazard Level 2:** Bacteria and viruses that cause only mild disease to humans, or are difficult to contract via aerosol in a lab setting, such as hepatitis A, B, and C, influenza A, Lyme disease, salmonella, mumps, measles, scrapie, dengue fever, HIV. "Routine diagnostic work with clinical specimens can be done safely at Biosafety Level 2, using Biosafety Level 2 practices and procedures. Research work (including co-cultivation, virus replication studies, or manipulations involving concentrated virus) can be done in a BSL-2 (P2) facility, using BSL-3 practices and procedures.
- **Biohazard Level 3:** Bacteria and viruses that can cause severe to fatal disease in humans, but for which vaccines or other treatments exist, such as anthrax, West Nile virus, Venezuelan equine encephalitis, SARS virus, tuberculosis, typhus, Rift Valley fever, Rocky Mountain spotted fever, yellow fever, and malaria. Among parasites *Plasmodium falciparum*, which causes Malaria, and *Trypanosoma cruzi*, which causes try panosomiasis, also come under this level.
- **Biohazard Level 4:** Viruses and bacteria that cause severe to fatal disease in humans, and for which vaccines or other treatments are *not* available, such as Bolivian and Argentine

hemorrhagic fevers, Marburg virus, Ebola virus, MER Shanta viruses, Lassa fever virus, Crimean–Congo hemorrhagic fever, and other hemorrhagic diseases. Variola virus (smallpox) is an agent that is worked with at BSL-4 despite the existence of a vaccine. When dealing with biological hazards at this level the use of a positive pressure personnel suit, with a segregated air supply, is mandatory. The entrance and exit of a Level Four biolab will contain multiple showers, a vacuum room, an ultraviolet light room, autonomous detection system, and other safety precautions designed to destroy all traces of the biohazard. Multiple airlocks are employed and are electronically secured to prevent both doors opening at the same time. All air and water service going to and coming from a Bio safety Level 4 (P4) lab will undergo similar decontamination procedures to eliminate the possibility of an accidental release.

3.1.3 Burns

A burn is a type of injury to flesh or skin caused by heat, electricity, chemicals, friction, or radiation.[1] Burns that affect only the superficial skin are known as superficial or first-degree burns. When damage penetrates into some of the underlying layers, it is a partial-thickness or second-degree burn. In a full-thickness or third-degree burn, the injury extends to all layers of the skin. A fourth-degree burn additionally involves injury to deeper tissues, such as muscle or bone.

Burns sustained in the laboratory may be caused by:

(i) Inflammable substances catching fire .
(ii) Fires from burnsen burners, spirit lamps or from faulty or overloaded electric circuits.
(iii) Swallowing of corrosive substances during pipetting or spilling, such substances on the skin.

The treatment required depends on the severity of the burn. Superficial burns may be managed with little more than simple pain relievers, while major burns may require prolonged treatment in specialized burn centers. Cooling with tap water may help relieve pain and decrease damage; however, prolonged exposure may result in low body temperature. Partial-thickness burns may require cleaning with soap and water, followed by dressings. It is not clear how to manage blisters, but it is probably reasonable to leave them intact. Full-thickness burns usually require surgical treatments, such as skin grafting. Extensive burns often require large amounts of intravenous fluid, because the subsequent inflammatory response causes significant capillary fluid

leakage and edema. The most common complications of burns involve infection.

While large burns can be fatal, modern treatments developed since 1960 have significantly improved the outcomes, especially in children and young adults. Globally, about 11 million people seek medical treatment, and 300,000 die from burns each year. In the United States, approximately 4% of those admitted to a burn center die from their injuries. The long-term outcome is primarily related to the size of burn and the age of the person affected.

Signs and Symptoms

The characteristics of a burn depend upon its depth. Superficial burns cause pain lasting two or three days, followed by peeling of the skin over the next few days. Individuals suffering from more severe burns may indicate discomfort or complain of feeling pressure rather than pain. Full-thickness burns may be entirely insensitive to light touch or puncture. While superficial burns are typically red in color, severe burns may be pink, white or black. Burns around the mouth or singed hair inside the nose may indicate that burns to the airways have occurred, but these findings are not definitive. More worrisome signs include: shortness of breath, hoarseness, and stridor or wheezing. Itchiness is common during the healing process, occurring in up to 90% of adults and nearly all children. Numbness or tingling may persist for a prolonged period of time after an electrical injury. Burns may also produce emotional and psychological distress.

Management/Treatment

Burns can be very painful and a number of different options may be used for pain management. These include simple analgesics (such as ibuprofen and acetaminophen) and opioids such as morphine. Benzodiazepines may be used in addition to analgesics to help with anxiety. During the healing process, antihistamines, massage, or transcutaneous nerve stimulation may be used to aid with itching. Antihistamines, however, are only effective for this purpose in 20% of people. There is tentative evidence supporting the use of gabapentin and its use may be reasonable in those who do not improve with antihistamines. Intravenous lidocaine requires more study before it can be recommended for pain.

Intravenous antibiotics are recommended before surgery for those with extensive burns (>60% TBSA). As of 2008, guidelines do not recommend their general use due to concerns regarding antibiotic resistance and the increased risk of fungal infections. Tentative evidence, however, shows that they may improve survival rates in those with large and severe burns. Erythropoietin has not been found effective

to prevent or treat anemia in burn cases. In burns caused by hydrofluoric acid, calcium gluconate is a specific antidote and may be used intravenously and/or topically. Recombinant human growth hormone (rhGH) in those with burns that involve more than 40% of their body appears to speed healing without affecting the risk of death.

3.1.4 Cuts and Pricks

CUTS is severed skin or making an opening (incision) or break the surface of skin with a sharp tool especially knife or any other sharp tool, while pricks is an act of piercing the skin with a sharp point e.g needle .
CUTS AND PRICKS may result from :

Edges of broken glasswares.
Edges of a knife.
Accidental pricking with needle or any other sharp instrument.
Walking on glass chippings.

3.1.5 Hazard of Toxic Chemicals

A hazardous chemical is defined as any chemical that is a health hazard or a physical hazard.

Health Hazard
Health Hazard is a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. Chemicals covered by this definition include carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents that act on the hematopoietic system, and agents that damage the lungs, skin, eyes, or mucous membranes.

Physical Hazard
A physical hazard is a chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable (reactive), or water-reactive.

Chemical hazards and toxic substances pose a wide range of health hazards (such as irritation, sensitization, and carcinogenicity) and physical hazards (such as flammability, corrosion, and reactivity).

Routes of Entry
Toxic materials are substances that may cause harm to an individual if it enters the body. Toxic materials may enter the body in different ways.

These ways are called the route of exposure. The most common route of exposure is through inhalation (breathing it into the lungs). Another common route of entry is through skin contact. Some materials can easily pass through unprotected skin and enter the body. Ingestion is another, less common, route of exposure in the workplace. Ingestion often occurs accidentally through poor hygiene practices (e.g. eating food or smoking a cigarette using contaminated hands).

Health Effects of Toxic Chemicals

There are many materials used in the workplace that can be hazardous. However, in order for them to affect your health, they must contact the body or be absorbed into the body. When assessing the potential health effects from working with a particular material it is necessary to understand difference between "toxicity" and "hazard".

1. **Toxicity** is the ability of a substance to produce an unwanted effect when the chemical has reached a sufficient concentration at a certain site in the body.
   The more toxic a material is, the smaller the amount of it necessary to be absorbed before harmful effects are caused. The lower the toxicity, the greater the quantity of it necessary to be absorbed. The toxicity of a chemical is generally measured by experiments on animals (*quite often rats*). If it is measured in terms of the amounts of material necessary to cause death in 50% of the test animals. These values are called LD50 (*lethal dose*) or LC50 (*lethal concentration*), and are usually given in weight of material per kg of body weight or airborne concentration of material per set time period respectively.
2. **Hazard** is the probability that this concentration in the body will occur.

Toxicity is an inherent property of the material. A material may be very toxic, but not hazardous, if it is handled properly and is not absorbed into the body. On the other hand, a material may have a very low toxicity, but be very hazardous.

Example:

1. An open container of an acid is much more hazardous than a closed container of the same material.
2. Two liquids may possess the same degree of toxicity but present different degrees of hazard:-
   One material may be non-irritating to the eyes and nose and odourless. The other may be irritating to the eyes or respiratory system and possess a pungent odour.
   The latter material, because of its warning properties presents a lesser degree of hazard.

3.1.6 Electric Shocks

Electric shock is the physiological reaction or injury caused by electric current passing through the (human) body. Typically, the expression is used to describe an injurious exposure to electricity. It occurs upon contact of a (human) body part with any source of electricity that causes a sufficient current through the skin, muscles, or hair.

Very small currents can be imperceptible. Larger current passing through the body may make it impossible for a shock victim to let go of an energized object. Still larger currents can cause fibrillation of the heart and damage to tissues. Death caused by an electric shock is called electrocution.

Electric shocks, usually due to ignorance or carelessness can be caused by:

Faulty electrical circuits.
Incorrect installation of equipment .
Touching exposed live wires .

An electrical injury has many consequences to a body as the electrical currents can travel through the nervous system and burn out tissue in patches along the way. This can leave bizarre symptoms anywhere on the body and may lead to complex regional pain syndrome. Wiring or other metalwork which is at a hazardous voltage which can constitute a risk of electric shock is called "live", as in "live wire".

Shocks can be caused by direct or indirect contact. Contact with an exposed conductive part under fault conditions is called indirect contact. IEC requires certain degrees of ingress protection against direct contact. Indirect contact protections can be achieved by earthed equipotential bonding and automatic disconnection of supply by using fuses for example.

3.1.7 Safety Precautionary Measures in Clinical Laboratory

Garner (1997) defined Universal Basic Precaution as the prevention of Transmission of blood pathogens through strict respect of rules concerning care and nursing. Gerberding *et al.*, (1995) also defined universal precaution as the routine use of appropriate barrier and techniques to reduce the likelihood of exposure to blood, other body fluid and tissue that may contain blood borne pathogens.

All specimens arriving in the laboratory should be regarded as being potentially pathogenic. It is a very wrong notion to think that only

specimens meant for bacteriological investigation are infectious. A specimen of cerebrospinal fluid sent for glucose estimation may be a part of the same specimen sent for bacterial meningitis investigation. The same is true of a specimen of blood sent for haemoglobin or packed cell volume measurement which may contain infectious micro-organisms. The laboratory worker, must therefore, observe some dos and don'ts in order to prevent laboratory acquired infections. Some of the rules for the laboratory worker are :

(a) He/She should wear protective clothing (laboratory coats/gowns) over normal clothing: preferably, wear closed shoes; and not walk barefooted in the laboratory.
(b) All specimens and infected materials should be handled with care.
(c) He/She should avoid eating , drinking or chewing gum in the laboratory.
(d) He/She should refrain from smoking in the working zone of the laboratory and also refrain from applying cosmetics in the laboratory .
(e) Nothing should be pipette with the mouth. Gummed labels should not be licked neither, should pens or pencils be put in the mouth, or stuck in the hair .
(f) Protective gloves or plastic aprons should be worn when collecting blood sample for hepatitis, AIDS or viral haemorrhagic fever investigations .
(g) Used needles should be inserted back into its guard immediately after use . But due to the increased awareness of the risks of infection from needle pricks , it is no longer advisable to recap used needles . Instead, sharp safe, containers should be made available for immediate disposal of used needles . The containers are finally incinerated .
(h) When handling specimens or culture containing highly infectious pathogens, gloves should be worn .
(i) Process specimens or cultures containing highly infectious pathogens in the safety cabinet .
(j) Any cuts, insect bites, open sore or wounds should be covered with water-proof adhesive dressing .
(k) Finger nails should be kept short .
(l) All infected or contaminated materials should be disinfected before disposal.
(m) There should be a jar of disinfectant on each bench at the start of the day's work. The disinfectant must be changed everyday .
(n) In case of any spillage, disinfectant solution should be poured to cover the spilled material and left for 15 minutes before cleaning up .

(o) Infected glasswares should be disinfected by soaking overnight in hypochlorite solution and cleaning thoroughly under running tap water .
(p) At the end of the day, all the work benches should be cleaned with disinfectant.
(q) Before leaving the laboratory for any length of time, hands should be washed with soap and water thoroughly .

Biosafety in clinical/medical laboratory

Biosafety involves safe handling and disposal of hazardous biological materials in the laboratory. These materials consist of infectious agents themselves as well as substances actually or potentially contaminated with them. A large number of laboratory workers handle such materials as part of their daily routine work. These workers should be aware of the risks of infection while handling the specimens, and must know appropriate laboratory practices to avoid these risks.

In 1984, the Centre for Disease Control (CDC) and the National Institute of Health (NIH) issued guidelines for use in working with infectious agents (laboratory-acquired infections, including brucellosis, tuberculosis, typhoid, streptococcal infections, hepatitis and HIV .) in the laboratory . It has been recommended that all specimens from all patients should be considered potentially infectious. The approach is referred to as, UNIVERSAL PRECAUTIONS, and eliminates the need to identify the patients infected with HUMAN IMMUNODEFICIENCY VIRUS (HIV) or other blood borne pathogens. Infectious agents such as HIV have been isolated from blood, semen, saliva, urine, cerebrospinal fluid, tears, breast milk, cervical secretions and tissues of infected persons. A laboratory worker should consider skin (especially when scratches, cuts, abrasions or other lesions are present) and mucous membranes of the eye, nose , mouth and respiratory tract as potential pathways for entry of infectious agents. Needles and other sharp instruments must be carefully handled and properly discarded . Spilling and splashing of infected materials should be avoided .

Safety Equipment

Safety equipment has been developed specifically for use in the clinical laboratory. Equipment for accident prevention and control includes fire-extinguishers, fire blankets, first- Aid supplies, safety showers, eye-wash stations, spill kits, and hazard labeling systems.

For personal protection, safety glasses or protective work shields should be used to protect the eyes and face from splash and impact . Gloves and rubberized sleeves or gowns should be used to protect the hands, arms, or body. Closed footwear should be used.

4.0 CONCLUSION

This unit has discussed safety in the medical/clinical laboratory, which includes hazard in the laboratory, burns, biosafety ,toxic chemicals and others. I, now urge you to read further in order to acquire more knowledge in this topic.

5.0 SUMMARY

The practice of medical/clinical laboratory science is associated with hazards and accidents . The laboratory worker is very much at risk of acquiring transmissible diseases through contact with patients or handling of clinical specimens . This topic has dealt with the hazard in the laboratory, types of hazards, routes of entry of toxic chemicals, management of burns, safety measures in the laboratory, biosafety and safety of equipments .

6.0 TUTOR-MARKED ASSIGNMENT

(1) Discuss in detail biosafety in clinical laboratory and explain the simple meaning of Universal precaution in the laboratory .
(2) Define burns and discuss fully in your own words, how you can reduce the pain of a patient with burns .
(3) Biological hazards are also known as biohazards. Discuss the classification and levels of biohazards.

7.0 REFERENCES / FURTHER READING

Lloyd, E.C; Rodgers, B.C; Michener, M; William M.S (2014): Outpatient burns; Prevention and Care.Amer. Family Physician 85(1);25-32.

Potter, P. (2013); Summer buzz. Emerg. Infect. Dis. 19(3); 1184.

Joshua M Peace(2014) : Open-source laboratory; How to Build Your Own Hardware and Reduce Research cost. Elsevier.

J. Ochei; A Kolhatkar (2012): Medical Laboratory Science :Theory and Practice. Tata McGraw-Hill . New Delhi.

Baker.F.J; ,Silverton.R.E; Pallister.C.J.(2011): Introduction to medical laboratory Technology. 7th Edition, Bounty Press Limited .

UNIT 3 MAJOR CAUSES OF LABORATORY HAZARDS

CONTENTS

1.0 INTRODUCTION

For good management of laboratory hazards, the laboratory staff must know the sources and causes of these hazards in order to plan for effective prevention. This unit discusses the major causes of laboratory hazards, pay attention and go further than what is in the unit .

2.0 OBJECTIVES

By the end of this unit , you should be able to know the major causes of laboratory hazards, for instance the most dangerous chemical, fire, careless use of equipments etc .

3.0 MAIN CONTENT

3.1 Major causes of Laboratory Hazards

The prevention of common accidents is very well documented in many laboratory manuals .It is very necessary that all members of the laboratory staff are conversant with the nature of these hazards and their prevention.

The most common accidents even in the smallest laboratories are mainly due to the following:

(1) Dangerous chemicals
(2) Fire
(3) Careless use of laboratory equipment, dangerous chemicals and reagents.

3.1.1 Dangerous or Hazardous Chemicals

A hazardous chemical is a chemical for which there is statistically significant evidence (based on at least one study conducted according to established scientific principles) that acute or chronic health effects may occur in exposed employees, or if it meets one or more of the following: any chemical which is a physical hazard or a health hazard.

(i) any chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, organic peroxide, pyrophoric, unstable (reactive), or reacts violently with water;
(ii) any chemical, for which there is statistically evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees, is classified as a health hazard. Health hazards include chemicals that are carcinogens, toxin or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopietic systems; and agents that damage the lungs, skin, eye or mucous membranes .
(iii) any chemical, for which the evaluation performed by the chemicalmanufacturer/importer, as listed on the Material Safety Data Sheet (MSDS), indicates it is hazardous;
(iv) "Toxic and Hazardous Substances" or Environmental l Protection Agency (EPA), "Identification and Listing of Hazardous Waste.";
(v) any chemical listed in the "Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment", American Conference of Governmental Industrial Hygienist (ACGIH)"The Registry of Toxic Effects of Chemical Substances",
(vi) any chemical regulated by the U.S. Department of Transportation.
(vii) any chemical that is capable of causing environmental or health damage if disposed of improperly as specified in the U.S. Environmental Protection Agency, Title 40 Code of Federal Regulations (Resource Conservation and Recovery Act); and
(viii) any mixture untested as a whole to determine whether it is a physical or health hazard is assumed to present the same physical or health hazard as the individual components that compromise one percent or greater (by weight or volume) of the mixture. An exception is the mixture shall be assumed to present a carcinogenic hazard if it contains a component which is considered to be a carcinogen in concentration of 0.1 percent or greater. In most cases, the labels indicate the type of hazards if

the chemical is hazardous. Look for key words like “caution”, “hazardous”, “toxic”, “dangerous”, “corrosive”, “irritant”, or “carcinogen”. A label is any written, printed, or graphic material displayed on, or affixed to, containers of chemicals. Labels or other forms of hazard warnings, such as tags or placards, provide immediate warning of potential danger.

Dangerous chemicals are used in the laboratories either directly or incorporated into reagents and stains. These dangerous chemicals include the highly inflammable ones like ether or alcohols, highly corrosive ones like phenol or sulphuric acid, toxic ones like formaldehyde solution, carcinogenic ones like benzidine or explosive ones like picric acid.

To minimize accidents caused by the chemicals, it is mandatory for manufacturers to label dangerous chemicals with hazard symbols and supply simple instructions for use and storage.

It is also important to label clearly those reagents prepared from the dangerous chemicals. The labeling should include the nature and strength of the reagents or solutions, date of preparation, expiry date and any other safety warning applicable. It is advisable that only chemicals required for daily use should be kept in the main laboratory, and the others in the store room. It is also recommended to examine the containers periodically to detect in time any possible buildup of the pressure which may cause the container to burst.

Store rooms in which bulk stocks of chemicals are kept should be designed to reduce the risk of fire.

Fire extinguishers should be placed just outside the store rooms. They should also be well ventilated and adequately lit.

Radioactive substances require proper supervision of their storage as provided b for by the law.

The prevention of hazards and accidents due to dangerous chemicals and reagents lies mainly in the careful observance of safety precautions during handling and storage.

Flammable chemicals: These chemicals should be stored in the fire-proof metal boxes at ground level preferably in an outside, cool, locked store. Only small amounts of flammable chemicals should be left inside the laboratory. A container of flammable liquid should never be opened near an open flame.

A bottle of ether should be opened at least three meters away from naked flame. No smoking rule must be enforced in the laboratory at all times.

Corrosive Substances

Corrosive chemicals such as strong acid (eg, concentrated sulphuric or nitric acid) and caustic alkalis (eg, Sodium hydroxide, Potassium hydroxide) should be stored at the floor level. Never mouth pipette a corrosive liquid: always use an automatic pipette or dispenser. Eyes must be protected from the fumes of corrosive substances .When mixing; acid should always be added slowly to the water, but never the reverse. In case of spillage, wear protective footwear when clean up the area. Wash acid and alkali burns under a free stream of running cold water.

Toxic and Irritating Chemicals

Toxic chemicals are equally irritating and can cause death or serious ill-health if swallowed, inhaled or allowed to come into contact with skin. Some of this chemical cause irritation of the skin and mucous membranes. Highly toxic chemicals such as potassium cyanide should be kept in locked cupboards . Wear protective gloves when handling a toxic substance. Always wash hands immediately after using a toxic or harmful chemical. Chemicals such as formaldehyde or ammonia with an irritating or harmful vapour should be used in a fume cupboard or safety cabinet. Always use an automatic pipette or dispenser to measure a toxic chemical.

Carcinogenic Chemicals

These chemicals are capable of causing cancer when inhaled, or ingested or when they come in contact with the skin. The chance of being affected depends on the length and frequency of exposure to, and concentration of the chemical. The carcinogenic substance should be stored in a closed container. Wear protective gloves and a face mask when handling a carcinogen. Wash everything used in handling the carcinogen thoroughly in cold water.

Explosive Chemicals

An explosive chemical can explode as a result of heat, flame or friction. Chemicals such as picric acid should be stored under water. Never leave such an explosive chemical in a dry state.

Radioactive Chemicals
All areas where radioactive materials are stored or used must be posted with caution signs. Trafic in this area should be restricted to essential personnel only. Decontamination of laboratory equipment, glassware and work areas should be routinely done on a regular basis.

3.1.2 Fire

Fire hazards include all types of live flames, causes of sparks, hot objects, and chemicals that are potential for ignition, or that can aggravate a fire to become large and uncontrolled. Fire hazards also include all types of potential threats to fire prevention practices, firefighting, built-in fire safety systems and situations that restrict the escape of people from an affected building or area in the event of a fire.
Fire can break out in any laboratory if proper safety precautions are not observed. It is essential that the laboratory staff is properly educated on the causes and controls of fires. The fire can be caused by:

(a) Ordinary combustibles such as paper, wood, etc.
(b) Flammable liquids such as ether, alcohols, etc.
(c) Gases such as methane
(d) Metals such as magnesium, potassium, etc .
(e) Faulty electric circuits or appliances .

Precautionary measures against fire:
(1) The laboratory staff should have fire drills from time to time .
(2) Fire extinguishers for all types of fire must be kept handy in the laboratory. The fire extinguishers in common use are water, foam, dry powder, carbon dioxide and halogen. (The use of halogen may be discontinued due to its adverse effects on the atmospheric ozone layer.)
(3) Fire extinguishers should always be free and in good working condition .
(4) Keep flammable liquids away from naked flame .
(5) In case of a major fire , someone should be delegated to take charge of fighting the fire . The practice of regular fire drills will help minimize casualties.

Fire Emergency Measures

In the event of fire, the following steps should be taken:
(i) Sound fire alarm
(ii) evacuate from the immediate vicinity of fire.
(iii) Close all the doors and windows and turn off all gas and electrical appliances.
(iv) Attack the fire if safe to do so with the appliances available .

The following equipment for dealing with all minor fires and controlling larger ones should be available in all laboratories . All members of the staff should be familiar with the location of the fire apparatus adjacent to their own laboratory and trained in their appropriate use . Inappropriate use of fire equipment can be extremely dangerous.

Hoses
Fire hoses must be checked regularly to ensure that they are in good working order.

Water and Sand Buckets
Water and sand buckets must be kept filled and covered. They should be checked periodically.

Fire Blankets
Fire blankets must be fixed in easily accessible positions . They are effective in smothering and preventing the spreading of fires . They are particularly useful for extinguishing fires involving clothing or cotton wool.

3.1.3 Fire Extinguishers

Foam Type
Foam fire extinguishers should be used on organic solvent fires, e.g; xylene. They should not be used where live electrical circuits are exposed .

Soda-Acid Type
Soda-acid fire extinguishers may be used on fires involving solid material or water miscible solvents, but not on electrical fires .

Carbon Dioxide Type
Carbon dioxide fire extinguishers may be used on small fires and where live electrical circuits are exposed. They should not be used on fires involving flammable liquids .

3.1.4 Careless Use of Laboratory Equipment, Dangerous Chemicals and Reagents (Equipment or Instrument-Related Accidents)

Accidents occur as a result of incorrect positioning, installation and wrong use of equipment. Poor maintenance and irregular servicing of equipments also lead to accidents. It is important to position the equipment in such a way that safety and convenience are given priority over other considerations. It is therefore essential not to overcrowd the

bench with too many equipments. Equipments like refrigerators and freezers should not be placed too close to the wall.

Electrical equipment is better installed by the supplier, a biomedical technologist or a well trained laboratory instrument technician.

It should be ensured that the correct voltage is applied ; that the power required can be supplied by the power supply circuit of the laboratory ; that overloading is avoided; that the wire flex is not unnecessarily long; and that there is no leakage on the flex.

To avoid breakdowns and inefficient functioning, laboratory equipment should be properly maintained and regularly serviced, worn out components, loose connections and corroded parts are some of the causes of accidents. Relevant spare parts must be well stocked for prompt replacement of worn-out parts . Instruments such as Microtome knives require careful handling. Carelessness in handling equipment leads to damage to equipment and risk of accidents.

Safety Equipment

Safety equipment has been developed specifically for use in the Medical or Clinical Laboratory. Equipment for accident prevention and control includes fire extinguishers, fire blankets, first-aid supplies, safety showers , eye-wash stations, spill kits and hazard labeling systems .

For personal protection, safety glasses or protective work shield should be used to protect the eyes and face from splash and impact . Gloves and rubberized sleeves or gowns protect the hands, arms or body. Closed footwear should be used.

4.0 CONCLUSION

Collective protection measures must be provided, including the supply of appropriate protective clothing . The work processes and control measures must prevent or minimize the risk of the release of the biological agent into the work place . Also there must be a proper procedure for the safe collection and disposal of contaminated waste including the use of secure and identifiable containers .

5.0 SUMMARY

The major causes of Laboratory hazards have been discussed, the sources which include dangerous chemicals, fire and equipment-related ones, the type of fire extinguishers and fire emergency measures ; precautions against fire and dangerous chemicals as sources of hazards have also been mentioned. You now read further to acquire more .

6.0 TUTOR-MARKED ASSIGNMENT

1. Mention three common sources of accidents in the laboratory and discuss anyone of them in your own words .
2. Briefly describe four equipments use in dealing with minor fires and controlling larger ones .
3. Write short note on safety equipment .

7.0 REFERENCES /FURTHER READING

F. J. Baker; R.E. Silverton; C. J. Pallister (2011): Introduction To Medical Laboratory Technology.7th edition. Bounty Press Limited .

Ochei.J; Kolhatkar.A. (2014); Medical Laboratory Science; Theory and Practice. Tata McGraw-Hill Publishing CO. Limited. NEW Delhi.

Edwin. G .F. (2013) :Occupational safety and health.

MODULE 2 COLLECTION, TECHNIQUES MANAGEMENT

Unit 1 Collection of specimens in the clinical laboratory diagnosis
Unit 2 Principles and Techniques use in the clinical laboratory tests/diagnosis/analysis
Unit 3 Clinical/Medical Laboratory Management

UNIT 1 COLLECTION AND REPORTING OF SPECIMEN IN THE CLINICAL/MEDICAL LABORATORY

CONTENTS

1.0 INTRODUCTION

In all routine laboratories the quality of the specimen has an important effect on the tests that are performed and their results . For example, a sputum for bacteriological examination would be of little value, if the specimen was mainly saliva and it is necessary to observe certain details to ensure an accurate report with the minimum delay .The quality of the specimen to be tested depends so much on:

(i) The patient being in the correct state required for such tests
(ii) Use of appropriate specimen containers.
(iii) Correct handling of the sample.
(iv) Collection of the appropriate sample for the investigation needed.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the essential information required to prepare patients for clinical laboratory testing as necessary
- select appropriate specimen containers for various tests
- state ways of handling and transporting clinical specimen correctly

3.0 MAIN CONTENT

Proper specimen procurement and handling is an integral part of obtaining a valid and timely laboratory test result. Specimens must be obtained in the proper tubes or containers, correctly labeled and promptly transported to the laboratory

3.1 Clinical Specimens

The samples (or specimens) collected from patients for laboratory analysis is referred to as clinical specimens. These include:

(1) Blood
(2) Urine
(3) Faeces
(4) Body Tissues
(5) Other body fluids/secretion e.g. cerebrospinal fluids, sweat, saliva, sputum, aspirate or ascitic tap and seminal fluid
(6) Swab

One of the key factors upon which the clinical value of laboratory results depend, is the integrity of the specimen collected. A clinical specimen must be approximately collected, handled and transported such that its constituents are maintained as they are found in the patient at the time of collection.

Many different types of specimen are received daily in routine medical/clinical laboratories, and it is necessary to observe certain details to ensure an accurate report with the minimum delay:

(i) The specimen should be clearly labeled with the patient's name, hospital number, ward, date and time of collection .
(ii) A fully completed request form should accompany each specimen with the above details together with the nature and origin of the specimen, the provisional diagnosis , the

investigation required and any other relevant information which may aid the laboratory in setting up the correct test .

In all routine laboratories the quality of the specimen has an important effect on the tests that are performed and their results . For example ,a sputum for bacteriological examination would be of little value if the specimen was mainly saliva ; a urine for culture whose delivery to the laboratory had been delayed for a considerable time would again be of little value as any organism present would be multiplying within that urine, giving a false picture of possible infection , a clotted blood sample would be of little value for a white cell count etc, therefore, for a laboratory to give an adequate service, there must be two-way communication . It is essential that physicians know what specimens to send for a particular investigation, what container should be used and how quickly it should be delivered to the laboratory.

On the other hand, it is equally important that the laboratory help the physician in these matters . One way of achieving this is to issue to the medical staff list which provide the information necessary for the majority of tests commonly performed in routine laboratories.

3.1.1 Receipt of Specimens

Hospitals vary in the way in which specimens are collected from the wards, but the following requirements must be fulfilled;

(i) the specimen containers must be robust and leak-proof.
(ii) special collecting trays or boxes must be used and they be leak-proof and able to withstand repeated autoclaving or disinfection.
(iii) all specimens must be carried upright, and therefore the tray or box must have bottle or tube racks fitted .
(iv) the trays or boxes must be sterilized weekly or after any visible leak or spillage .
(v) Requisition forms should be kept separate from the specimens to prevent contamination. Plastic envelopes for specimens, with a separate sleeve for the request form, are ideal for this purpose.

Specimens that are suspected or known to contain dangerous pathogens must have a danger of infection label affixed and be inserted into a plastic envelope. The reception staff must not handle such labeled specimens. On receipt of the specimen, many hospital laboratories have a central area where the specimen is given a laboratory number, the information on the request form accompanying the specimen is checked, and the specimen and form dispatched to the appropriate laboratory.

All staff working in the reception area must wear protective clothing and must not be permitted to handle leaking or broken specimens. In most routine histopathology departments, specimens are received in a container of fixative. This should be of a suitable size to contain a volume of fixative approximately 10 times that of the specimen.

The most common incidence of unfixed specimens coming to the laboratory is when specimens for frozen section are sent; here, the theatre should make arrangements with the laboratory so that the staff and equipment are at hand to deal with the specimen . Some specimens are not placed in fixative before sending to the laboratory, such as specimens for histo-chemistry(muscle biopsies) or specimens in which cytological imprints may be made, eg , lymph nodes may be bisected, smears made and then a slice(s) of the tissue is put into fixative .

In all cases where specimens are not fixed it is essential that the laboratory staff and theatre staff have an abundantly clear understanding that these specimens must be delivered to the laboratory immediately.
Once the specimens have arrived at the laboratory, they should be correctly identified along with their request forms which have been suitably completed. In many laboratories it is usual to sign the theatre book to acknowledge receipt of correctly identified specimens and forms. Multiple specimens from a patient should be collated, in order that they will ultimately be reported together. They should then be identified self-adhesive numbered labels on the specimen container (not the lid) and request form.

In general terms, specimens received for cytology are designated gynaecological or non-gynaecological. Almost all gynaecological specimens are received in the laboratory as fixed smears labeled with a pencil. The smears should be transported to the laboratory in a suitable container together with the request form.

3.1.2 Patients Preparation Prior to Specimen Collection

Patient preparation includes all instructions, guidance or tutelage given to patients, which ensure that the right type of specimen are correctly or approximately collected, and so form part of quality assurance of the results of tests performed on such specimens. Examples of patient preparations are as follows:

Fasting
Some tests are influenced by recent food ingestion, so, samples for such tests are collected after an overnight fast. Fasting blood sugar(FBS) test, which is used in the diagnosis and management of diabetes mellitus, is an example of such tests, in which patients are instructed to have an over night fast of 10-14hrs, after which the blood sample is collected in the

morning. Measurement of blood lipids, a test used in prediction and management of heart diseases is another example of test requiring an overnight fast.

Abstinence from Sexual Intercourse
As part of preparing a male patient for seminal fluid analysis, the patient is instructed to abstain from sex for 3-4 days prior to semen sample collections.

Drugs and Dietary Restriction
Drugs known to affect some specific tests are also avoided for days prior to collection of samples for such tests. Examples of these are avoidance of meat, vegetables, vitamin C, and haematinics prior to collection of faeces for occult blood test, and avoidance of antibiotic drugs when samples for bacteriological examinations (tests) are to be collected. Female patient for occult blood test is also instructed to avoid sample contamination with menstrual bleeding.

Instructions on Various Types of Urine Specimen Collection
Patients are instructed, when necessary, on how a specific type of urine specimen should be collected. Examples are:

(a) **Mid -stream urine specimen:** This is the specimen of choice for bacteriological investigation of urinary tract infection. The first part of the urine flow is allowed to go, sample is collected mid way of the urination and the last part if the urine flow is also allowed to go. This method of collection reduces the possible cellular or microbial contamination of the urine during the sample collection.

(b) **Timed urine collection** (e.g. 24 hr urine specimen): This type of urine collection is needed when quantitative analysis of biochemical substance is to be done on urine specimen. For example, measurement of the amount of creatinine excreted by the kidney, into the urine over a specific period of time. The patient is instructed to choose a convenient time, say ***x*** a.m. of a certain day. At the chosen time, the urine present in the bladder is voided away. All other subsequent urination goes into a container, (like 5 liters keg.) until ***x*** a.m. the next day. This is called 24 hr urine specimen.

(c) **Random urine specimen:** This is a urine specimen collected without reference to any form of time, and is collected in a dry clean container It is the urine specimen most commonly sent to the laboratory for urinalysis and urine microscopy primarily because it is the easiest to obtain and is readily available. It is the

urine of choice, it is often more convenient for the patient. It is satisfactory and hence, generally acceptable and is suitable for most screening purposes. There are no specific guidelines for random urine specimen collection.

3.1.4 Specimen Containers

These are containers into which clinical samples are collected. They are also referred to as specimen bottles. Sample containers must have tight cover (or lid) and must be leak-proof. The choice of a particular type of specimen bottle is dependent on the test(s) required on the sample and the nature of the sample to be collected. Bacteriological and parasitological tests generally require sterile universal bottles. Samples for tests that require whole blood or plasma are collected into specimen bottles containing the anticoagulants. Sample for tests that requires serum is collected inside a plain bottle (i.e. without anticoagulant). Anticoagulants are chemical substances that prevent blood from clotting. Examples of common anticoagulants in clinical laboratory are Heparin, Ethylene Diamine TetraAcetic Acid(EDTA), Fluoride-oxalate mixture and citrate.

Most of the specimens for haematology tests are collected inside EDTA bottle; however, citrate anti-coagulated bottles are used in a few tests(e.g. Erythrocyte Sedimentation Rate, ESR).
The specimen bottle (container) of choice for general blood chemistry tests (chemical pathology tests) is Lithium Heparin Bottle. This specimen bottle contains lithium heparin as the anticoagulant. When centrifuged, plasma needed for general blood chemistry tests is carefully harvested (removed) with the aid of a Pasteur pipette. Blood sample for general chemistry tests can also be collected into a bottle that does not contain anticoagulant. Such bottle is called plain bottle. In this container, the blood is allowed to clot, centrifuge, and the serum is harvested. Specimen meant for blood glucose measurement is specifically collected inside a fluoride-oxalate bottle. Tissue specimen for histopathology examination is collected inside a specimen container of fixative: A fixative is a chemical substance that preserves the tissue’s shape, structure and chemical constituents in as life-like manner as possible. Example of fixative in histopathology laboratory is formal-saline.

The tissue sample is placed inside a container with sufficient fixative to submerge the tissue. Blood specimen containers can be recognised by the colour of their covers. There is an internationally agreed colour codes for blood specimen containers for laboratory tests. The colour code depicts the type of anticoagulant in the blood specimen containers.

Colour of the Cover (Type of the Bottle)	**Type of Anticoagulant**	**Tests**
1 Purple (or lavenders) colour cover	EDTA bottle	Haematology tests: Full Blood Count
2 Green cover	Lithium Heparin bottle	General bloods chemistry
3 Red cover (bottle)	Plain bottle	General blood chemistryy and Serologicaltests
4 Grey colour cover	Fluoride-oxalate bottle	Blood glucose tests
5 Blue colour cover	Sodium citrate bottle	Coagulation assays

3.1.5 Collection of Blood Samples

Blood for laboratory test may be obtained from veins, arteries or capillaries. Blood sample collected from the vein, artery and capillary are referred to as venous, arterial and capillary blood respectively. The commonly used blood specimen in clinical laboratory is the venous blood followed by the capillary blood. Arterial blood is used mainly for blood gas analyses. The step involved in obtaining an appropriate, identified blood specimen from a patient's vein is called venipuncture (arterial puncture and skin puncture for arterial and capillary blood respectively). The vein commonly used is the medium cubital vein, a superficial vein of the upper limb, located on the forearm.

Hands are washed; gloves are worn before carrying out venipuncture.

SELF ASSESSMENT EXERCISE

Visit a hospital or private clinical laboratory and observe how venipuncture and skin puncture are made. Sample collected from patient must be properly labeled and the accompany request form must also be properly filled. The following arethe basic information expected on the sample label and the laboratoryrequest form:

(i) Full name of patient
(ii) Age
(iii) Sex
(iv) Occupation (on form only)
(v) Date and time of sample collection
(vi) Clinical details (on form only)
(vii) Requested tests.

3.2 Transportation of Clinical Specimens

Specimens to be sent to the laboratory require special attention for safe packaging of the material. The packaging should be done to achieve two purposes:

i. Prevention of possible infection of the transporter, and people in the environment.
ii. Maintenance of integrity of the sample (i.e. maintaining the constituents of the sample as they were until analysis is done).

For hand-carried transportation over a short-distance, the specimen should be placed upright in appropriate racks. For long distance transportation, clinical specimens must be packaged to avoid leakage and for shock absorption during transport. In general, the basic triple packaging system should be adopted.

Triple Packaging System
The triple packaging system entails the following:

(a) A primary container which has the specimen and it must be Leak-proof and well covered. It should be kept in upright position.
(b) A secondary container which is durable and water-proof. This could be made up of metal, plastic or disposable zip lock plastic bag. A secondary container should have enough absorbent material to absorb content of the primary container in case of spoilage or leakage. On the outside of the secondary container, the details of the specimen should be posted.
(c) A tertiary container which is usually made of wood or card box. It should be capable of withstanding the shocks and trauma of transportation. Dry ice is kept in-between the secondary and primary container, along with some absorbent materials.

The dry ice keeps the environment of the sample cool for a long time. In the laboratory, samples received are registered by the reception officer(s), urine specimens are analysed within 2 hrs of reception. Plasma or serum for biochemical analyses are kept frozen, if analysis would be Delayed. Microbiological analyses expectedly are started underlay so as to prevent the organisms from dying or excessively multiplied or the cellular components disintegrated. Tissue samples collected inside an appropriate fixative can be kept for a long time.

3.3 Reporting of Clinical Laboratory Results

After the specimen has been processed in the laboratory, it is essential that the information obtained be conveyed to the physician or even the

patient. The 3Rs of reporting are reliability, rapidity and relevance. Reliability of course speaks for itself.

The results must be reliable and they must be transmitted as rapidly as possible. Relevance is of vital importance. Although many tests may be performed on a specimen, it is advisable that only those relevant to the request should be sent. For example if several antibiotic susceptibility tests are performed on an organism, it is not always advisable to give every result. Only those relevant and consistent with the antibiotic policy of the hospital should be mentioned. The need for information to be given as rapidly as possible often means telephoning result to the ward. This can be highly dangerous procedure. There are many examples of misinterpretation of results due to either the speaker not being precise, or the recipient misunderstanding. A classic example of this was when the specimen was received from the theatre for a rapid result. The result was phoned through as an adenocarcinoma which was interpreted as, had no carcinoma. If results were telephoned, it is essential for the person to read back what has been said so that no mistakes can occur. A written report should be sent as soon as possible.

Reporting systems vary from hospital to hospital and no universal reporting system would necessarily be accepted by everyone . Cumulative reporting , if carried out correctly , is probably the most helpful way both to Laboratory and Physician . This necessitates each patient having a master card for each discipline and the results are entered onto this card and photocopied, the photocopy being delivered to the ward . By this method the Physician can see at a glance any changing pattern of results and this also gives the laboratory a check on their results when compared with the previous ones . Today, more and more Laboratories are reporting by computer . This has many advantages , particularly with the storage of reports . If wards have a visual display unit (VDU) , reports can be obtained even more rapidly . Whichever system is used , an adequate check must be made that the correct details of the patient’s name, ward, hospital number etc, have been filled in, and the correct result has been entered and the report delivered to the right place .

4.0 CONCLUSION

In this unit, you have learnt what clinical specimens are, also acquainted with the importance of patient preparation before sample collection, you can now prepare patients when necessary, for clinical sample collections. Moreso, you learnt how clinical specimens are packaged for transportation. It is also essential that the information obtained be conveyed to the physician or even the patient.

Therefore, all reports of laboratory test results should be computer printed and will contain all patient identification data, patient location and other relevant information as provided in the test request. Results will be reported with reference ranges, abnormal panic flags, etc., where applicable. Emergency/stat test results, abnormal critical results and results of pathogenic organisms isolated from blood and cerebrospinal fluid or of public health importance such as *V. cholera* and *Salmonella* typhi will be reported by telephone to the staff nurse or doctor-in-charge of the patient, as indicated in the test request. Telephone reporting should be considered an interim step and requires confirmation with a printed report. The type of report e.g., preliminary, final or duplicate should be indicated.

5.0 SUMMARY

Various types of specimens from patients for clinical laboratory analysis were discussed in this unit. The quality of the clinical specimen collected is pivotal to the reliability or clinical usefulness of laboratory results. Collections of clinical specimens that will generate clinical usefulness of the results include the following:

(i) patient preparations which encompasses all instructions, guidance or tutelage given to patient to ensure that the right sample are appropriately collected .

(ii) collection of clinical specimens into correct specimen bottle i.e. identifying the appropriate specimen bottle for different laboratory testing. (e.g. EDTA bottle for general Haematological test, fluoride-oxalate for blood glucose, lithium heparin for general. Chemistry test, formal-saline fixative, sterile universal bottle for bacterial.

(iii) appropriate transportation of clinical specimen accompanied with approximately filled request form .You have also being taking through reporting system in the laboratory as well as what is known as cumulative reporting .

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe how to collect an 8 hrs urine specimen.
2. List the 3 components of triple packaging system of clinical specimens.
3. Briefly discuss how a result generated in laboratory should be conveyed to a Physician.

7.0 REFERENCES/FURTHER READING

Abayomi, Adeoye .(2013). Occult Blood in Faeces. *In: Medical Laboratory Practice*.

Donald, S. Young & Edward, W. Bermes .(2014). Specimen Collection and Processing: Sources of Biological Variation. *In: Clinical Chemistry* (eds.) Burtis & Ashword (3rd ed.). W.B Saunder Co. pp. 42-73.

Hinting, A.; Schoonjans F, & Comhaire F. (2014): Validation of a Single-step Procedure for the Objective Assessment of Sperm Motility Characteristics. *Int. J. Androl.* 11(4): 277-87.

Baker.F.J; Silverton.R.E and Pallister.C.J. (2011): Introduction to Medical Laboratory Technology.7th edition. Bounty Press Limited.

UNIT 2 PRINCIPLES AND TECHNIQUES OF CLINICAL LABORATORY TESTING

CONTENTS

1.0 INTRODUCTION

Principle is an important underlying law or assumption upon which a system is based. It is the basic science that governs any scientific system. Basic scientific law or theorems (in chemistry, physics andbiology) are employed in development of analytical methods (ortechniques). Good understanding of the scientific theorem or law upon which a particular method or equipment is based is vital to the correct use and effective troubleshooting of the method/equipment. This unit focuses on some basic principles and techniques commonly used in clinical laboratory.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- state the relationship between principles and techniques of analysis
- highlight the applications of basic sciences in clinical laboratory testing.

3.0 MAIN CONTENT

3.1 Principles of Tests

Tests performed on clinical specimens in the laboratory can be broadly grouped into two: Qualitative analysis and Quantitative analysis. A qualitative analysis is a test that detects the presence of a specific substance/matter, or reveal the various constituents matter of Importance, in a given clinical specimen e.g.

(i) Occult blood test detects the presence of blood or blood product that cannot be seen physically in faeces or urine.
(ii) Stool microscopy detects or reveals the presence of parasite in faeces.
(iii) Histological staining reveals the effect(s) of disease on histological or cytological Architecture. A quantitative analysis is any test that measures the level of a specific Substance, or shows the relative composition of the constituent matter in a clinical specimen e.g.
(1) Full blood count: A test which among others reveals the relative blood cellular compositions.
(2) Measurement of various biochemical substances in body fluid e.g. glucose, creatinine, lipids, enzymes etc. Analytical methods (qualitative and quantitative) are based or built on known scientific facts or theorem. The basic scientific fact(s) or theorem upon through which a test method is based is referred to as the principle of that method (or test). It is the chemical events, physics, or biological phenomenon taking place in there acting vessels (e.g. test tubes or slides) when the test is being carried out. The technique on the other hand referred to the skill or manipulations involved (manual or automation) in the applications of the scientific facts (or theorem) to test procedures in the laboratory. For example, a commonly used method for estimating blood glucose is glucose oxidase method. One of the scientific facts upon which this method is based is that an enzyme is specific in its action. So, glucose oxidase enzyme will act on glucose only and not on any other related monosaccharide (aldose monosaccharide). When glucose oxidase acts on glucose, gluconic acid and hydrogen peroxide (H_2O_2) are produced. Another specific enzyme, hydrogen peroxidase breaks down the hydrogen peroxide to water and oxygen. The oxygen given off is allowed to react with a colour forming compound a **chromogen**, the intensity of the colour formed, which is directly proportional to the amount of glucose present in the specimen is measured

The intensity of colour formed is therefore extrapolated with that formed from a glucose solution of known concentration in order to know the concentration of the glucose in the specimen. From the foregoing, two basic facts upon which this method is based are the following:

(i) Glucose oxidase (an enzyme) specifically oxidizes glucose, to give a product from which oxygen evolved.
(ii) The oxygen evolved oxidizes a chromogen to give a coloured compound, the intensity of which is directly proportional to the amount of glucose present is the specimen.

The above statements, which are the principle of glucose oxidase method, are also the chemical events happening in the reacting vessels when the test is being performed.

3.2 Laboratory Techniques

Laboratory Techniques refer to all skills and manipulations involved in the applications of scientific facts (from the natural sciences) to test procedures in the laboratory. While medical/clinical techniques are the ones use to carry out clinical/medical investigative procedures in the diagnosis and therapy of disease. Some important techniques employed in the clinical laboratory analysis are as follows:

(a) Optical Techniques
(b) Electrochemical Techniques
(c) Chromatography
(d) Electrophoresis

Optical Techniques
Analytical techniques that make use of light spectrum either of a specific Wavelength or as visible light spectrum can be collectively referred to as

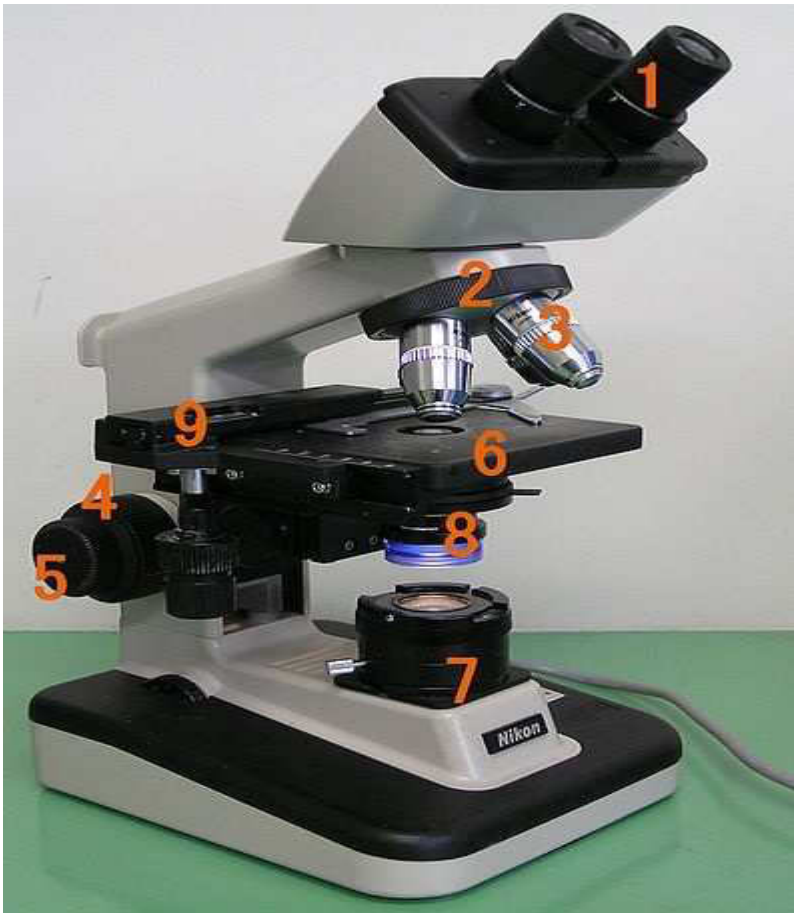
Optical Techniques
The major optical techniques used in clinical laboratory are microscopy and spectrophotometry.

Microscopy
The use of microscope to view objects that are not visible to the naked eye is referred to as microscopy. A microscope is a magnifying instrument that magnifies the image of the objects. Optical microscope, often referred to as the light microscope is a type of magnifying instrument which uses visible light and a system of lenses to magnify images of objects that cannot be physically seen in the specimen (e.g. parasite, bacteria, fungi).

Working Principle of a Microscope

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

The specimen to be viewed with the light microscope is made sufficiently thin so that light can pass through it. In many cases, specimens are stained using specific dyes and appropriate staining technique in order to enhance contrast during microscopy hence, more details of the object (specimen) is revealed.



Picture of a Microscope

Spectrophotometry

Many biochemical quantitative analyses done in clinical laboratory are based on measurements of radiant energy (light) emitted, transmitted, absorbed, scattered or Reflected when the substances being measured interact with incident light, under controlled conditions. Techniques of measuring such radiant energy (light) are termed spectrophotometric Techniques. Specific spectrophotometric techniques (or instrumentation design) depend on whether the interaction between the incident wavelength of light and the substance being measured results into light absorption, (or transmission) reflection or scattering. In colorimetric method, (an example of spectrophotometric), light of a specific wavelength is made to pass through a solution of which concentration is to be determined. The amount of light absorbed by the solution is measured (absorbance). A known standard solution of the substance being measured is treated same way and its absorbance is measured, the concentration of the test solution is derived by simple extrapolation. The specific wavelength of light made to pass through the solution is dependent on the colour of the test solution. Complementary colour of that of the solution is made use of: e.g. Colour of Solution Complementary Colours of Light

1. Blue Yellow (e.g. 450 nm)
2. Bluish-green Red (630 nm)
3. Purple (or pinkish) Green (e.g. 520 nm)

In colorimetric technique used in clinical laboratory, the wavelengths of light commonly employed are the visible spectrum (380-750 nm),ultraviolet (<380 nm) and infrared (800-2500 nm) are sometimes used. The basic principle of colorimetric technique is Beer-Lambert law. Beer-Lambert law states that ‘when a specific wavelength of light(monochromatic light) passes through a coloured solution, the amount of light absorbed is directly proportional to the concentration of the solution (intensity of the colour) and the length path through the solution’. The biochemical substance or analyte (e.g. glucose, cholesterol) to be measured in a clinical specimen (body fluids) is allowed to specifically react with chemical agent(s) to form a coloured product (in solution).The absorbance of the coloured solution formed is measured using spectrophotometer (an instrument used to measure the amount of light absorbed or transmitted by substances in solutions). Since absorbance of a substance in solution is directly proportional to its concentration. The concentration of the substance of interest is calculated from its absorbance and the absorbance of a standard solution can be treated the same way.

3.3 Atomic Emission Spectrophotometric Techniques

This technique is used in the quantitative measurement of sodium and potassium in body fluids. Calcium can also be measured by this technique. In this technique, an atom of an element (sodium or potassium) in the sample is heated in a hot flame. The atom absorbs energy, therefore becomes unstable, and emits the absorbed energy in form of a wave length that is characteristic of the element (e.g. sodium emit primarily a wavelength of 589 nm with a yellow colour while potassiumemits primarily, a wavelength of 400 nm and 767 nm with a violet orlilac colour light. The intensity of light emitted (emission) is proportional to the concentration of the element in the measured sample. Concentration of the element in the sample is calculated from the emission and displayed by the instrument. The instrument used in this technique is called Flame Photometer. The principle employed by this technique is based on the following chemistry: Atoms of many metallic elements, when given sufficient energy such as that supplied by a hot flame, emit energy at wavelengths that are characteristic of the element. A specific amount or quantum of thermal energy is absorbed by an orbital electron. The electrons, being unstable in this high energy (excited) state, release their excess energy as photons of a particular wavelength as they change from the excited state to their previous (ground) state. The concentration of the element is directly proportional to the quantity of the energy emitted. In other word, concentration of the element is measured by the equipment as a function of the amount of energy emitted.

3.4 Atomic Absorption Spectrophotometric Technique

Atomic absorption spectrophotometric technique is employed in the measurement of trace elements in body fluids. The principle by which this quantitative technique is based states that anatom in the ground state will absorb an amount of energy that is equal to the energy difference between the energy level of the electron in the excited state and the energy level that the electron occupies in the ground state. In this technique, the sample solution is first vapourised and atomized in a flame thereby transform it to unexcited ground state where it absorb slight at specific wavelength. A light beam from a lamp whose cathode is made of the element being measured is passed through the flame. Radiation is thus absorbed. The amount of radiation absorbed depends on the concentration of element in the sample. The concentration of the element is measured as a function of the amount of radiation absorbed.

3.5 Electrophoretic Techniques

Electrophoresis is a method of separation of mixtures based on differential rate of movement of charged particles when subjected to an electric field at a specific pH. Electrophoretic technique is typically used in the clinical laboratory for the separation of proteins. It is primarily a qualitative method of analysis, but it can be adopted for quantitative analysis.

Principle of Electrophoretic Separation of Proteins
Proteins in serum vary in there is o-electric points. I so-electric point of a protein is the pH at which there is no net charge (zero charge) on protein particles. At a pH alkaline to its is o-electric point, a protein will carry a net negative charge and therefore migrates to the anode when a current is passed, whereas at a pH acidic to its is o-electric point, it will carry a net positive charge and migrate to the cathode when a current is applied. Iso-electric point of serum proteins varies from 4.7(albumen) to 7.3(gamma globulin).Hence, at a buffered pH of say 8.6, each protein fraction will migrate at different rates when subjected to an electric field.

Electrophoretic technique can be used to detect or identify an abnormal protein present in plasma as a result of disease conditions. Example is in a disease called multiple myeloma, an abnormal protein called Bence - Jones protein can be detected by electrophoretic method. Determination of Haemoglobin genotype of an individual is also done using this technique. Sample to be tested is applied onto a solid support medium,(e.g. cellulose paper, Agarose gel). The medium carrying the sample is placed across negative and positive electrodes.

3.6 Chromatographic Techniques

Chromatography is a method of separation of mixtures which utilizes differential affinity of the separating molecules substance in the mixture, for mobile and stationary phases, over which the substances to be separated are distributed. A mobile phase may be a gas or a liquid(solvent) in which the substance (mixture) is solubilised, while a stationary phase is either a solid or a liquid supported (stationed) on a solid matter, over which the mobile phase carries the mixture. Substances (in the mixture) that have greater affinity for the mobile phase are separated first, after the order of their affinities for the mobile phase constituents of the mixture that have greater affinities for the stationary phase are separated much latter during the process. As the mobile phase carries the mixture over stationary phase (like an effluent),the separated constituent are collected as different fractions. The different fractions can be identified and also quantified.

Chromatographic technique is typically named after the mobile stationary phase e.g. Gas-liquid chromatography, or after the working principle e.g. ion-exchange chromatography. Others include:

(a) Thin layer chromatography
(b) Molecular sieve chromatography
(c) High performance liquid chromatography

3.7 Centrifugation

Centrifugation is a process that involves the use of centrifugal force for the separation of mixtures. In the clinical laboratory setting, the majoruse of centrifugation is as follows:

(1) Separation of plasma, serum and red cells from whole blood, when a particular fraction of the blood is needed for tests.
(2) Acquisition of urine sediment for microbiological examination
(3) Any laboratory procedure (test) that require separation of a particular fraction of a suspension. Many particles or cells in a liquid medium (suspension) at a given time, will eventually settle at the bottom of a container due to gravity.

However, the length of time required for such separation may be long. When a suspension is rotated at a certain speed or revolution per minute, centrifugal force causes the particulates to move away from the axis of rotation and therefore settles at the bottom of the container as a precipitate. The remaining solution or liquid is called the supernate or supernatant. The equipment used for the process is called centrifuge. There are basically two types of centrifuge used in clinical laboratory:

1. Bench centrifuge
2. Microhaematocrit centrifuge

The bench centrifuge is a general purpose centrifuge while the microhaematocrit centrifuge is designed specifically for the determination of packed cell volume (PCV) on a sample of blood collected inside a capillary tube.

4.0 CONCLUSION

The principle of clinical laboratory test methods is generally the basic science upon which the method is based, while the techniques are the skills, manipulations or instrumentation involved in the application of the principle to test procedure. Some major techniques exploited in the clinical laboratory, discussed in this unit are optical techniques (e.g. microscopy) chromatography, electrophoresis and centrifugation.

5.0 SUMMARY

Laboratory analysis can be broadly grouped into two:

(i) quantitative analysis which is any test that measures the level of a specific substance in a sample (e.g. blood glucose measurement).
(ii) qualitative analysis which is any test that detects the presence of specific substance in a given clinical specimen .The principle of a test is the basic science upon which the test method is based. Laboratory techniques are the skills, manipulations (or instrumentation) involved in the application of the basic science to test procedures.
(iii) Optical techniques make use of light either of a specificwave length or generally as visible light. These include the optical microscope and spectrophotometry whereby substances to be measured in a given clinical specimen is allowed to interact with light under controlled conditions and the light absorbed, scattered, emitted or reflected as a result is measured so as to quantified the substance of interest in the sample.
(iv) Chromatographic techniques are based on differential affinity of various constituents of a mixture for mobile and stationary phases in order to separate them. It is both a quantitative and qualitative analytical tools
(v) Electrophoretic technique is basically a qualitative analytical technique. Constituents of a sample (mixture) are separated based on differential mobility of charged particles at a particular pH, when exposed to an electric field.
(vi) Centrifugation is a technique of separation of suspension whereby with the use of an instrument called centrifuge, centrifugal force causes the particles in the suspension to settle rapidly at the bottom of the test tube or container.

6.0 TUTOR-MARKED ASSIGNMENT

1. In your own words, differentiate between principle of a test and Laboratory techniques.
2. Define qualitative and quantitative analysis with examples.
3. List 4 laboratory techniques (clinical laboratory).
4. Write the working principle of two of the techniques listed above.

7.0 REFERENCES/FURTHER READING

David, U.; Larry D. Bowers & Carl, A. Burtis 2015).Chromatography/Mass spectrophotometry *In:Clinical Chemistry* (eds.) Burtis & Aswort (3rd ed.). W.B. Saunders Co, pp. 164-203.

Green, J.M. (2014). A Practical Guide to Analytical Method Validation. *Anal. Chem. News & Features*, pp. 305A-309A.

Hargis, L.G.; Howell, J.A. & Sutton R.E. (2013). Ultraviolet and Light Absorption Spectrophotometry. *Anal. Chem.*, 68: 169R – 183R

Merle, A. Evenson (2015). "Spectrophotometric Techniques". *In*: *Clinical Chemistry*. (eds). Burtis & Aswort (3rd ed.). W.B Saunders Co, pp. 75-93.

Monica, Cheesebrough (2012). "Measurement of Blood or Plasma Glucose". *In*: *District Laboratory Practice in Tropical Countries*. Part 1. Cambridge: Cambridge University Press, pp. 340-348.

Murray, Royce W. (2014). *Chemical Analysis* Microsoft ® Encarta 2009(DVD).

Ochei, J. & Kolhartkar, A, (2007). "General Laboratory Instruments and Equipment". *In: Medical Laboratory Science*: *Theory and Practice*. (6th ed.). Tata: Mc-Graw Hill, pp 16-49.

Raymond, E. Karcher & Kern, L. Nuttall (2014). "Electrophoresis". *In*: *Clinical Chemistry* (eds.). Burtis & Aswort (3rd ed.). W.B. Saunders Co, pp 150-163.

Trinder. P. (2014). Determination of Glucose in Blood using Glucose Oxidase with an Alternative Oxygen Accepting. *Anal. Chem. Biochem*. (6th ed.). pp.24-26

UNIT 3 LABORATORY MANAGEMENT

CONTENTS

1.0 INTRODUCTION

The role of the clinical laboratory as an important component of the health care delivery system cannot be overemphasized. Constant political, social, economic and technological changes are impacting on health care delivery. It is therefore paramount that the laboratory managers obtain the fundamental management principles so that the clinical laboratories will be able to deliver in the constant changing environment. This unit will focus on the fundamental or basic elements of management as it applies to the clinical laboratory.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- highlight the basic element of management and relate them to the laboratory
- explain what planning and implementation entails in the clinical laboratory settings.

3.0 MAIN CONTENT

3.1 Definition and Principles of Clinical/Medical Laboratory Management

The first step in a systematic approach to the management and organization of a health laboratory begins with the establishment of general goals and specific objectives by the Laboratory staff. The use of such objectives for purposes of management is known as Management By Objectives (MBO). In order to achieve these objectives, the laboratory must have adequate facilities, equipment & supplies, and an adequate number of qualified personnel. As used here, goals are those general and qualitative statements of overall philosophy of the organization. An example of a goal is " a commitment by the hospital laboratories to be a vital component of a hospital whose goal is to provide a patient care environment of excellence to serve the community, and to serve as a setting for clinical teaching.

The goal should be consistent with the organizational structure, the management style of the laboratory director, and the available resources. In turn, such goals should influence the future programs of the laboratory and the activities of the director and lab staff. The types of goals set for a laboratory will vary greatly. For instance, the goals for operation of an office laboratory with two physicians are different from those of a reference laboratory serving thousands of patients over a large geographic area. A useful exercise for a new laboratory is to write the overall goals of the laboratory after discussions with appropriate persons in the organization. As part of this process, laboratory directors should encourage written input from each organization level toward the development of the goals and objectives. Such written goals may be organized as follows ;

(1). A statement of the primary external goals of the laboratory
(2). A statement of the secondary and tertiary goals of the laboratory in reference to service, research, or education.
(3). A statement in reference to the management philosophy of and need for cost effectiveness.
(4). A statement as to what kind of environment is desired in the laboratory with respect to interpersonal relationships, working conditions, and attitudes toward teaching and scholarly activities . Such overall goals, once established, should be reviewed every year and appropriate modifications should be made. In contrast to the general goals mentioned above, objectives should be in quantifiable statements which are achievable over a designated period of time. An example of an objective might be " to evaluate available approaches to automation of antibiotic susceptibility

testing and also to implement the optimal approach by the end of the fiscal year". Allowing concerned personnel to have input into formulating such objectives generally enhances the success of this approach of management by objectives.

Management by objectives is a process of formulation, performance and assessment, and as such it provides means of focus on pertinent factors and issues that affect the practice of laboratory medicine. As a tool of management, MBO encourages discussion, interaction, and consensus decision making among all organizational levels of the laboratory. Good management means getting work done. A well-organized laboratory service is efficient, and produces work of high standard in a safe and pleasant working environment. The main steps to good management are:

- Setting up the main working room
- Arranging stocks of laboratory items
- Establishing routine procedures for disinfection and disposal.
- Establishing good communication with clinicians
- Organizing patient flow
- Keeping laboratory records
- Ordering laboratory supplies
- Organizing staff activities
- Establishing a reliable quality control system
- Setting planned programme for laboratory personnel trainings to the highest possible qualification .
- Setting plans for enhancing the laboratory activities to the highest possible technical level.

3.1.1 Fundamentals of Management

The basic elements of management of any business or organization applicable to laboratory management are listed below:

(1) Management functions
(2) Human resources management
(3) Planning and execution of goals
(4) The control and improvement of the laboratory processes
(5) Management of finances and supplies
(6) Management of equipment

3.1.2 Management Functions

In the management of clinical laboratory, the basics functions are as follows:

(a) **Planning:** This is the ongoing process of developing the laboratory's mission and objectives and determining of how these mission and objectives will be accomplished.
(b) **Organising:** This involves making optimum use of the resources required to enable the successful carrying out of plans. The focus is on division, coordination and control of tasks and the flow of information within the laboratory. It is in this function that the manager distributes authority to jobholders.
(c) **Staffing:** This function of the laboratory manager involves employment of qualified personnel in all positions in the laboratory. Staffing also includes training, hiring, evaluating and compensating.
(d) **Directing:** This function has to do with determining what needs to be done in a situation and getting people to do it. This is achieved by influencing people's (workers) behaviour through motivation, communication, group dynamic, leadership and discipline.
(e) **Controlling:** Controlling as one of the basic functions of management deals with checking progress against plans, modifying when necessary, based on feedback. It is the process of establishing performance standards based on the laboratory objectives, measuring and reporting actual performance, comparing the two and taking corrective or preventive action as needed.

3.1.3 Human Resources Management

Generally in management, people are regarded as the most valuable assets of any organisation. So, goal fulfilling oriented organization provides an environment that challenges employee to assume increasing responsibilities consistent with their training, experience and personal aspiration.

In practice, this includes:
(a) Building a position culture
(b) Providing employees with essential management tools.

A. Building Positive Culture

A positive culture is an environment that nurtures capable, dedicated an informed employees. Essentials of building a positive culture include:

(i) **Developing a clear mission and vision statements:** The worker must be carried along with the mission and vision (or goals) of the organisation (laboratory).This will enable them

to effectively contribute purposefully. It also unifies the leaders and directs their daily decisions.

(ii) **Providing leadership by example and attitude:** This includes punctuality, decorum, work habits, ethics on the part of the leader or manager. Leadership by example has a great influence on the employees.

(iii) **Recognizing the contributions of employees:** Positive recognition of the employees accomplishment, contributes to a work culture in which there is mutual respect irrespective of individual job assignments, titles or credentials. Techniques of recognition in human resource management includes:
- Personally greeting an employee especially in the presence of peers
- Sending personal notes
- Telling peers of an individual's accomplishments
- Starting an employee of the month award
- Having an employee recognized at an appropriate meeting.

(iv) **Implementing a team approach to problem solving:** Problem must never be ignored, but must also not be too overemphasized, as this will create a negative nonproductive. Environment. Problem resolution should be handled in a manner that preserves personal dignity for all concerned.

(v) **Provision an environment of open communication:** This can be affected by holding monthly operational meetings

B. Management Tools

The common management tools are:

(i) Meeting
(ii) Memoranda
(iii) Letters
(iv) Reports

Meetings are invaluable management tools for the following:
- Decision making for the laboratory
- In-house training of personnel
- Communication of vital information
- Clarification of issues
- Motivation of the staff

Letters, memoranda and reports are important management tools for information dissemination and also for follow-up when needed.

3.1.4 Planning and Execution of Goal

A plan is any procedure used to achieve an objective, or a set of intended action through which a goal is expected to be achieved.

Planning is both the organisational process of creating and maintaining intended action through which a goal would be achieved.

Planning in the clinical laboratory is anchored on the laboratory's mission, vision and objectives. That is, the mission and objective of the laboratory must first be clearly stated in writing, planning can should also be made based on them. The mission defines the fundamental purpose of an organization (laboratory). It can be a short or long term mission. Vision defines the desire or intended future state of the organisation (laboratory). It could answer the question: "Where do we want to go"? It concentrates on the future and provides clear decision making criteria. Planning process involve some key member of staff. In the laboratory setting, planning and implementation entail the followings:

(1) **The business plan:** This deals with the laboratory's mission, vision, objectives and their goals.
(2) **The marketing plan:** This describes the customers, type or nature of tests and services to be rendered.
(3) **The operation plan:** It describes the facilities and equipment, and the staffing of the laboratory.
(4) **The financial plan:** It describes needs for capital, financial resources and budget.
(5) Strategies for implementation.

3.1.5 Control and Improvement of the Laboratory Process

This is monitoring and evaluation of the entire laboratory process. This is achieved through Quality Assurance programme and Quality Control program. Quality Assurance refers to a broad spectrum of spelt out plan, policies, procedures or activities within and outside the laboratory that together ensured a quality service delivery from the laboratory, while Quality Control is the quantitative technique aspect of quality assurance that is Primarily concerns with the control of errors in the performance of tests and verification of test results. Quality assurance programme put in place by the laboratory include guidelines or procedure that would prevent introduction of errors at thethree phases of laboratory testing. These phases are:

(a) **Pre-Analytical Phase:** This includes guidelines and information about specimen collection and transportation.
(b) **Analytical Phase:** The laboratory must spell out the standard procedure for carrying out all testing (following standard operating procedure). Performance of tests must be assigned to qualified personnel.

(c) **Post-Analytical Phase:** The laboratory must put in place guidelines or procedure that will prevent transcriptional error in reporting of results, ensure timely dispatch of results to the patient's record, and appropriate interpretation of the results.

3.1.6 Financial Management

Financial management of clinical laboratory requires a detailed accounting system that collects, identifies and codify financial data. Such accounting system provides three keys information:

i. Information necessary for ongoing control of operational expenses.
ii. Information needed in making strategic decisions
iii. Summary information.

Areas of financial management that must be considered in the financial management of clinical laboratory are:

(a) Budgeting
(b) Test Cost Accounting
(c) Capital Expenditure
(d) Expense Report
(e) Make-versus-Buy Decision

(**Budgeting:** A laboratory budget is a financial plan that predicts laboratory expenditures by section and category for the upcoming fiscal year. The laboratory manager is expected to develop a realistic and manageable budget which contributes to the financial goals of the laboratory (independent laboratory) or financial goals of the entire organisation, (if the laboratory is under a larger organisation) and then, present it to the management.

Test Cost Accounting: It is imperative for the laboratories to know their tests cost because of market competitive and growing intolerance for excessive health care costs. Such data of cost per test allows managers to make decisions of profitable pricing or decision on introduction of new tests or instruments.

Capital Expenditures: This involves making profitable investment decision for capital expenditures. Such decision usually includes consideration for procuring new analytical system. In practice, a laboratory will choose either to procure a batch or random access system. Labour and supply costs often differ widely between these systems.

Expense Report: For effective management of laboratory finance, expenses must be closely tracked so that expense report would be

generated and compared with budgeted expenses. Significant variances can then be regularly addressed and communicated to the management.

Make-versus-Buy Decisions: This means determination of whether it is advantageous to make a particular item in-house, or to buy it from a supplier. Examples of make-versus-buy decisions that a laboratory might face include:

- Should a specific test currently being sent to a reference laboratory be developed in-house?
- Should low volume tests performed in-house be sent to another laboratory?
- Should cleaning services be developed in-house or contracted out?
- Should test reagents be prepared in-house or purchased from a vendor? Etc.

The manager is responsible for evaluating alternatives and making decisions that bring the greatest benefit to the laboratory.

3.1.7 Management of Equipment and Supplies

To effectively manage laboratory equipment, equipment management policy is mandatory. Such policy includes the following:

- Specifications and standard of equipment required must be stated
- Preferred place of purchase must be identified.
- Maintenance schedule must be put in place and record of Maintenance must be kept.
- Procedure for reporting faults and getting the repairs quickly must be put in place

4.0 CONCLUSION

Management of the clinical laboratory is a relatively new field of medicine. It is a rapidly evolving and developing field in keeping with social, political and technological challenges. The laboratory manager must acquire fundamental understanding which must be appropriately applied in the management of the laboratory.

5.0 SUMMARY

The focus of this unit is the basic or fundamental elements of Management. These are:

(1) management functions (planning, organizing, staffing, directing and controlling).

(2) human Resources Management, which entails providing an environment which challenges employees to assume responsibilities consistent with their training, experience and personal aspiration. Such an environment can be attained through building positive culture and by providing employee with essential management tools

(3) planning and Implementation that will achieve the desired goals must be based on the laboratory's mission, vision and objectives. Such planning includes business plans, marketing plans, operational plans, financial plans and strategies for implementation

(4) control and improvement of the laboratory process. This monitoring and evaluation is achieved by putting in place quality assurance programme and quality control in the laboratory.

(5) financial management which requires the laboratory to have an accounting system and to include in considerations other areas of financial management: budget, test cost accounting, capital expenditure, expense report and make-versus-buy decision

(6) management of equipment and supplies requires the laboratory to have a management policy for their equipment and supplies.

6.0 TUTOR-MARKED ASSIGNMENT

1. State the role of laboratories in Public health activities
2. What are the main steps to be followed in the management of health laboratories.
3. List the fundamental elements of management applicable to laboratory management.
4. Briefly discuss the management function.
5. Planning is the road map to achieving a desired goal; briefly Discuss this in relation to clinical laboratory management.

7.0 REFERENCES/FURTHER READING

James A.F.Stoner (2012) : Management .6th ed. Prentice Hall of India.
Cheesbrough M.(2012) : Medical Lab. Manual for tropical countries. Vol.1

Cheesbrough. M (2013) : District Lab. Practice in tropical countries. Part 1

Ash, K.O. (2014). Impact of Cost-cutting on Laboratories and its Role in Healthcare. *Clin. Chem*. 42: 822-826.

Farewell, D.C. (2014). Hospital Laboratory Consolidation. *Clin. Lab. Manag. Rev*. 9:411-420.

Patterson, P.P. (2013). Cost Accounting in Hospital and Clinical Laboratories. Part 1. *Clin. Lab. Manag. Rev.* 2:343-346.

Ronald, L., Weiss & Owen, K, Ash (2014). 'Laboratory Management'. *In: Tietz Textbook of Clinical Chemistry*. Burtis & Ashwood (3rd ed.). W.B Saunder Co, pp. 369-383.

MODULE 3 MICROBIOLOGY AND HISTOPATHOLOGY LABORATORIES

UNIT 1 BACTERIOLOGY

CONTENTS

1.0 INTRODUCTION

The Microbiology Laboratory is one of many laboratories in the Department of Laboratory Medicine. The main purpose of this laboratory is to assist in the diagnosis of infectious disease eg : bacteria, parasites, fungi , viruses and the latest type of infectious disease called prion . The microbiology laboratory also helps in empirical studies of micro-organisms.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- know what microbiology laboratory is,
- define microbiology laboratory,
- know the type of diagnostic tests obtainable in microbiology laboratory and

- the types of infectious micro-organisms expected to be seen in medical microbiology laboratory .
- also the types of specimens associated with different types of microorganisms.

3.0 MAIN CONTENT

3.1 Microbiology Laboratory

Medical microbiology is a branch of medicine concerned with the prevention, diagnosis and treatment of infectious diseases , this field of science, study various clinical applications of microbes for the improvement of health, there are four kinds of microorganisms that cause infectious diseases : Bacteria (bacteriology), Parasites (parasitology), Fungi (mycology), Virus (virology), one type of infectious proteins called Prion .

3.2 Bacteriology/Fungal/Viral Laboratory

Bacteriology laboratory is where diagnosis of specimen for bacterial organisms are examined and cultured to make accurate species identification of important isolates, to perform antibiotic susceptibility tests when indicated, and to evaluate serological samples for infectious diseases .

This starts with the patient entering the healthcare system and presenting with certain signs and symptoms consistent with an infectious disease. The patient's physician then orders specific tests to clarify the presence of an infectious disease. These diseases can include those caused by bacteria (e.g., Group A streptococcus that may cause strep throat); those caused by viruses (e.g., influenza A that cause the flu); those caused by fungi (e.g., Candida, which may cause vaginal yeast infections).

In order for bacteriology to aid in diagnosing the correct infectious disease, a number of steps occur. The first step is the collection of the appropriate specimens to diagnose the patient's disease. For instance, if an infection of the bladder is a possibility, the physician may order a urinalysis that looks at the cell types present in the urine, such as red and white blood cells, as well as a urine specimen for a culture to grow the organism that is causing the infection.

Specimens may be collected from a number of different sources, including blood, urine, and swab specimens of the throat. Sometimes a more invasive technique using a needle or other special instrument is required to collect the specimen. This first step in microbiology diagnosis of infectious disease is very important! A good quality

specimen means the best chance of diagnosing the infectious disease. After the collection of the specimen and input from the physician about what the possible infectious disease may be, the laboratory decides what technique is best to identify the infectious disease and the organism likely causing the problem. All the techniques look for some product of an organism. The time it takes to identify the organism is dependent on the technique used, as well as how complicated the testing process may be. For the patient, this means the laboratory may be able to provide an answer in anywhere from 15 minutes to eight weeks!

3.3 Microscopic Examination (of bacteria)

Microscopic examination of stained or unstained preparation of bacteria is usually one of the essential steps in the long process of isolating and identifying bacteria. Microscopy of stained smears helps to differentiate cellular constituents of and render them more visible. Staining also helps to classify organisms by placing them in their separate groups, based on the staining reaction of the organism.

Making a Wet Preparation
Microscopic examination of urine is performed on urine sediment . That is, urine that has been centrifuged to concentrate the substances in it at the bottom of the tube. In practice, urine microscopy is not usually performed as part of routine urinalysis, but usually requested separately. When urine is microscopically examined, the following substances are Usually sought for: white blood cells, red blood cells, epithelia cells, Bacteria, yeast, egg of schistosoma haematobium, trichomonas, casts and crystals

Procedure for Urine Microscopy
(1) Mix the urine (in the specimen container) gently
(2) Pour about 2-4mls into a centrifuge tube.
(3) Centrifuge the sample at low speed (2500g) for 5 minutes
(4) Decant the supernatant and mix the sediment at the bottom
(5) Using a Pasteur pipette, add a drop of the sediment on a slide
(6) Apply a cover slip
(7) Observe first under 10X objective, then
(8) Examine the observed objects using 40X objective
(9) Report (record) your findings.

Making a Wet Preparation for Other Materials
(i) On a clean microscope slide, place a drop of the material to be examined. Mix a small portion of the specimen such as vaginal secretion/smears in a drop of saline.
(ii) Carefully place a cover-slip over the preparation, making sure that there are no air bubbles trapped under the cover-slip.

(iii) Examine immediately. If there is going to be a delay in examining the preparation. It is recommended to seal the edges of the cover-slip with nail varnish or petroleum jelly.

Preparation of smears (stained)

Stained preparations are needed to examine microorganisms microscopically in order to study their morphology and observe their cellular constituents. Smears (or tissue sections) are made and stained by any one of the recorgnised staining methods e.g gram, zeelnelsen. Smears can be made from solid or liquid cultures or from clinical specimens.

Smears from Solid Media

(i) sterilise the wire loop in Bunsen flame.
(ii) Place one drop of sterile saline on a clean slide with the sterilized loop.
(iii) Re-sterilize the loop
(iv) With the wire pick a small portion of bacterial growth and emulsify it in the drop of saline and spread to give a thin homogeneous film or smear on the slide, then sterilize the loop.
(v) Allow the smear to dry in air, fix and stain.

Smears from Liquid Media

(i) Sterilize wire loop in the Bunsen flame.
(ii) Using aseptic technique, remove a loopful of the culture
(iii) Place the culture on a clean slide and spread it with the loop to give a fairly thick film of culture. Sterilize the loop.
(iv) Allow the film to dry, fix and stain.

Smears from Clinical Specimens

Smears are made from original clinical specimens directly on the slide if it is a swab or with a loop if it is fluid.

3.4 Differential Staining Methods

(1) Gram's Stain

In 1884, Christian Gram, a Danish bacteriologist, described this staining method which is the most important stain in routine bacteriology. It divides bacteria into two groups: the Gram positive and the Gram negative.

The Gram positive organisms must have an intact cell wall. A damaged cell wall Gram stains reaction. The Gram's stain reaction is based on the ability of the organism to resist decolourisation with acetone, alcohol or aniline oil after the initial staining with one of the rosaniline basic dyes and then

treating with a mordant (a mordant such as iodine results in the formation of a complex with the primary stain) . The rosaniline dyes commonly used as crystal violet, methyl violet and gential violet. Iodine is the mordant used.

Gram positive bacteria: In a Gram stained smear, Gram positive bacteria which retain the primary stain, appear violet or deep purple in colour. The Gram positive reaction is due to the presence of technoic acid in the cell wall. The technoic acid forms a mesh which prevents the primary stain-mordant complex from being washed away by the decolourising agent.

Gram negative bacteria: These appear red. Gram negative bacteria have a high lipid content which dissolves in the decolourising agent. This allows the primary stain-mordant complex to be washed off . The red counterstain makes the decolourised Gram negative bacteria visible in a contrast colour.

Gram Stain Procedure

(i) Make a smear, allow drying and then fixing with gentle heat by passing the slide two or three times over a Bunsen flame or placing the slide on a heater at 60C.
(ii) Stain with crystal violet for 30 seconds to one minute
(iii) Replace solution 1with Lugol's iodine and allow to act for 30-60 seconds.
(iv) Rinse with ethyl alcohol or acetone and continue application until no more colour appears to flow from the preparation.
(v) Wash with water
(vi) Apply dilute carbol fuschin for 30seconds.
(vii) Rinse with water, blot carefully and dry with gentle heat .
(viii) Examine microscopically.

Acid-Fast Stain

Acid-fast staining is another example of a differential stain used in Bacteriology. It divides bacteria into two groups : acid-fast and non-acid-fast. Members of the genus Mycobacterium are acid-fast in nature. Like the Gram reaction, the acid fastness is also cell wall dependent. Mycobacteria have high lipid content, especially, mycolic acid, in their cell wall. The ordinary aniline dye solutions cannot penetrate the mycobacterial cell wall. Strong staining solutions containing phenol preferably with application of heat, are used for staining them . Once stained, they resist decolourisation with mineral acid . Therefore, they are called acid-fast bacilli (AFB). The degrees of acid-fastness vary with species. *Mycobacterium tuberculosis* can resist decolourisation with 20% sulphuric acid, or 3% hydrochloric acid in 95% ethanol. Hence, they are also called acid-alcohol-fast (AAFB), *Mycobacterium leprae*

resist 5% sulphuric acid while Norcadia species are acid-fast only with 1% sulphuric acid.

Procedure for AFB (Ziel-Neelsen (ZN) Method

(i) Make and fix smear by heat
(ii) Flood the slide with the strong carbol-fuschin solution and gently until heat rises. Do not allow the stain to boil, stain for 5 to 10 minutes.
(iii) Wash with water.
(iv) Decolourise with 3% acid alcohol, continue to apply until the preparation is colourless or a faint pink colour.
(v) Wash with water.
(vi) Counterstain with o.5% methylene blue or malachite green for 20-30seconds.
(vii) Wash with water, blot carefully and dry with gentle heat.
(viii) Examine under oil immersion lens.

3.5 Microbial Culture

Microbiological culture is the primary method used for isolating infectious disease for study in the laboratory. Tissue or fluid samples are tested for the presence of a specific pathogen, which is determined by growth in a selective or differential medium.

The 3 main types of media used for testing are:

- **Solid culture**: A solid surface is created using a mixture of nutrients, salts and agar. A single microbe on an agar plate can then grow into colonies (clones where cells are identical to each other) containing thousands of cells. These are primarily used to culture bacteria and fungi.

- **Liquid culture**: Cells are grown inside a liquid media. Microbial growth is determined by the time taken for the liquid to form a colloidal suspension. This technique is used for diagnosing parasites and detecting mycobacteria.

- **Cell Culture**: Human or animal cell cultures are infected with the microbe of interest. These cultures are then observed to determine the effect this new microbe has on the cell. This technique is used for identifying viruses.

- **Specimens for Cultures:** The ability of the laboratory to correctly diagnose an infection very much depends on the quality of the clinical specimen. This is because the results of many laboratory tests are closely related to the selection, timing and

method of collection of specimens. The following basic concepts should be taken into consideration when collecting specimens:

(i) The clinical specimen should be taken from the actual infection site. Here are a few guidelines:

(a) Sputum specimen should be from the lower respiratory tract and not salivary

(b) Mid-stream urine sample from a female patient should follow adequate cleaning of the perineum and other surrounding tissues.

(c) Depths of wounds and draining sinuses should be sampled without touching the adjacent skin .

(d) Deep abscesses should be aspirated with needles or cannulas.

(ii) Optimal times for specimen collection should be established, the recovery of certain bacteria is enhanced if optimal time of their release is known .

(iii) The quantity of specimen must be adequate . A guideline as to what is sufficient amount of material should be established.

(iv) Proper and sterile collection devices and specimen containers must be used . This is to avoid contamination. Containers should be made for ease of collection, eg; narrow mouthed containers are not suitable for urine, sputum or stool collection.

Tightly fitted caps or lids must be provided to prevent contamination and leakage during transportation Swabs are commonly used for collection of materials for culture. Swabs may be transported in transport medium to the laboratory; or they may be coated with charcoal or albumen to neutralize the toxic effect of long chain fatty acids in the cotton wool.

(v) Prompt delivery of specimens to the Laboratory is important .

(vi) Obtain all the necessary specimens prior to the administration of antimicrobial drugs . This is particularly true if organisms such as Neisseria gonorrhoeae, Beta-haemolytic streptococci and Haemophilus influenza are suspected .

(vii) The clinical specimen container must be properly labeled with;

- Patient’s name
- Type of specimen
- Hospital/Laboratory number
- Ward/Clinic
- Time and date of collection.

A request form containing the above information and the type of tests required must accompany each specimen.

Macroscopic Examination

Few clinical specimens are subjected to macroscopic examination in bacteriological investigations. These may include:

(i) CSF and other body fluids – appearance and presence of clot
(ii) Sputum – appearance and presence of blood
(iii) Stool – Consistency, colour and presence of blood and mucus .

Microscopic examination is done on stained and unstained preparations of the specimen. The unstained preparation may be in the form of a wet mount preparation, e.g; when looking for cells and casts in urine. Gram staining is the single most useful stain in diagnostic bacteriology. Other special stains such as Ziehl-Neelsen stain for Acid-fast bacilli and Albert's stain for metachromatic granules in Corynebacteria, may be useful and even diagnostic in some cases. Microscopic examination of stained materials is not definitive but only suggestive.

4.0 CONCLUSION

Bacterial organisms are found almost everywhere in nature and are in constant interaction in bacteriologylaboratory; medical bacteriology laboratory, hence is an important arm of healthcare helping to provide a conducive environment where these microbes be it bacteria, virus, fungi,, whichever one is/ are identified for medical treatment, evaluation and control .

5.0 SUMMARY

Bacteriology laboratory is where all the tests for the identification of all the bacterial oorganism eg; bacteria, , virus, fungi etc are carried out.

The important techniques and methods have been discussed, also various samples associated with the organisms have been mentioned.

6.0 TUTOR-MARKED ASSIGNMENT

(i) Define microbiology laboratory.
(ii) What are the steps involved in the collection of appropriate specimen for the purpose of diagnosis in microbiology laboratory
(iii) Describe the procedure for Ziel-Neelsen staining

7.0 REFERENCES/FURTHER READING

Ochei .J & Kolhartkar. A. (2008) : Medical Laboratory Science Theory and Practice. Tata McGraw- Hill .

F.J. Baker; R. E. Silverton and C. J. Pallister (2011) : Introduction to Medical Laboratory Technology. 7th edition Bounty Press Limited.

Daniel. S.S (2015): Microbiology Laboratory. What is the microbiology laboratory? ; Methods in Microbiology laboratory. J.Infect. Dis. Prev.

UNIT 2 PARASITOLOGY LABORATORY

CONTENTS

1.0 INTRODUCTION

Parasitology laboratory is a section or part of microbiology laboratory in Laboratory Medicine. The main purpose of this section is to assist in the diagnosis of parasites and parasitic diseases, for example; Hookworm , Ascriasis; Teaniasis; Filariasis;Amoebiasis; Giardiasis ; Malaria etc. It also helps in empirical studies of parasitic diseases and parasites.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- know what parasitology laboratory is,
- define parasitology laboratory;
- know the type of diagnostic tests obtainable in parasitology;
- the type of parasitic organisms expected to see in medical parasitology laboratory;
- also different types of specimens associated with different types of parasitic organisms.

3.0 MAIN CONTENT

3.1 Parasitology Laboratory

This is a laboratory where parasites like Hookworm, Plasmodium species, Schistosoma species and others are detected using specimens like faeces, blood, urine and other body fluids.

Faeces/Stool
The most commonly used specimen for the detection of intestinal parasites is faeces. Feacal specimens are examined for the presence of trophozoites and cysts of protozoa, eggs and larva of helminthes, whole adult worms or segments of some worms may also be seen. Trophozoites, cysts, eggs and larva can be seen only with the microscope, but the adult worms or segments of Tapeworms can be seen with the naked eye. Samples of faeces must be properly collected, processed and examined for the detection of parasites.

Collection of Faeces
A faecal specimen should be collected in a clean, wide-mouthed container with a tightly fitted lid. Jars may also be used, but containers such as match boxes are not suitable.

Water that may contain free-living organisms, or with urine which can cause trophozoites to lose their motility or to undergo lysis. Sufficient quantity of the specimen should be obtained for satisfactory examination. The container should be labeled with patient's name or number, date and time of collection.

While handling the faecal specimen, it must be remembered that it can contain potentially infectious micro-organisms. Loose or semi-formed specimens should be examined within 30 minutes to 1 hour of collection. Trophozoites of protozoa cannot survive for longer periods outside the body. The formed specimen must be examined on the same day of collection. If these time limits cannot be met, a portion of the stool should be preserved by one of the methods of preservation. The rest of the specimen should be refrigerated at 3-5C and stored in closed containers to prevent dessication. At this temperature protozoan cyst, helminthes eggs and larva can survive for several days. Specimen of faeces should never be incubated or frozen before examination.

Preservation of Faecal Specimens
Sometimes it may be impossible to examine a faecal specimen within specified limits of time due to constraints such as unavoidable delay in processing the sample. The faecal specimen, in such cases, should be placed in an appropriate preservative so that the morphology of parasites

if present in the specimen, is maintained. Such a preservative is also useful if the specimen is required for teaching purposes. The preservatives which are used most frequently are:

(i) Polyvinyl alcohol (PVA) fixative ; This fixative is widely used because it allows concentration procedures and preparation of permanent stained smears . It is particularly useful for protozoan cysts.

It should be used in the ratio of three parts of fixative to one part of faecal material. The fixative remains stable for long periods at room temperature if kept in sealed containers.

(ii) 10% Formal Saline: It is a time-honoured fixative and preserves protozoan cysts, helminth eggs, and larva for long periods. Hot formal saline at 60C can prevent further development of eggs to infective stage.

It should be used in the same proportion as the PVA fixative with faecal material. The disadvantage of formal-saline is that the faeces cannot be used for permanent stained preparations.

(iii) Sodium acetate formalin (SAF) fixative. This also is very useful preservative, both for concentration methods and for permanent stained smears. The only disadvantage is that it is difficult to adhere the material to the slide after it is mixed with, SAF, Mayer's albumin, has been recommended as an adhesive for this purpose. With this fixative, staining the smears with haematoxylin gives better results than with trichrome stain. It is used in the same proportion with faecal material as PVA.

(iv) Merthiolate-Iodine formalin (MIF) Solution: MIF solution is useful especially in field surveys as it preserves the morphology of the parasites very well. However, permanent stained smears cannot be prepared from MIF fixed specimens. Another disadvantage is that the iodine component makes the reagent unstable. Solution 1 and II must be prepared and stored separately. Just before use, add 1ml of solution 11 to 13 mls of solution 1and mix with 1-2g of fresh ffaeces.

3.2 Visual or Macroscopic Examination of Faeces

Before processing the specimen of faeces, it should be visually examined. Its colour, consistency, and presence of blood, pus, mucus or parasites should be reported.

Colour
Normally, stool is brown in colour, variation from this colour may occur under certain conditions. Reddish colour may be due to bleeding from the lower gastro-intestinal (GI) tract . Consumption of beet-root may also give a red colour to the stool. Black-tarry colour may be due to bleeding from the upper gastro-intestinal tract or due to consumption of iron.

Clay-coloured stool is seen in obstructive jaundice or after barium sulphate meal . Green stool may result from the consumption of leafy vegetables such as spinach or sometimes due to oral antibiotic therapy.

Consistency
A normal stool specimen is formed or semi formed in consistency. Loose or watery stool may be seen in diarrhea. Trophozoites of intestinal protozoa are usually seen in loose or watery specimens while cysts are found in formed and semi formed ones . Eggs of helminthes may be found in any consistency. Large amounts of mushy, foul smelling, frothy specimens are seen giardiasis and other conditions associated with malabsorption.

Blood
The presence of blood in or on the specimen must always be reported. Fresh, bright red blood is often from the lower gastro-intestinal tract whereas dark red colour may indicate bleeding from the upper gastro-intestinal tract. When very small amounts of blood are being passed in faeces, it may not be visible macroscopically. Such specimens are said to contain occult (hidden) blood . Some of the causes for the presence of occult blood include iron deficiency anaemia, peptic ulcer or cancer of the gastro-intestinal tract . In suspected cases, a special request is sent for occult blood test.

Tests for Occult Blood
Chemical tests for occult blood involve oxidation of chromogenic compounds such as benzidine, O-toluidine or 4-aminoantipyrine catalyzed by haemoglobin or its derivatives. This oxidation is indicated by a coloured reaction to produce blue or blue-green colour.

Reagents

(i) (A) Reagent using 4-aminoantipyrine

4-aminoantipyrine	0,4g
Alcohol, 95 %(v/v)	15ml
Acetic acid, 10 %(v/)	1ml

Prepare the reagent just before use

(B) Reagent using benzidine or O-toluidine

Benzidine or O-toluidine 0.4g
Glacial acetic acid 10ml

Caution Both benzidines O-toluidine are **Carcinogenic**.

(ii) Hydrogen peroxide, 10 volumes solution
(ie 1 volume of H_2O_2 will give 10 volumes of oxygen on complete degradation) .

Technique:
Emulsify a pea-size (10-15 mm in diameter) specimen of faeces in 7ml of distilled water. Allow the faecal particles to settle and transfer 5 ml of the supernatant to another test tube. Layer it carefully with 5 ml of reagent (i) .Do not mix.

Add 10 drops of 10 vol. Hydrogen peroxide solution.
Allow it to stand for 1 minute Appearance of blue colour at the junction of the specimen and reagent (i) indicates a positive reaction.
Run pure distilled water, and distilled water mixed with a trace (0,05 ml) of blood as negative and positive controls simultaneously.

Interpretation;

Negative	No colour change
+	Pale blue
++	Dark blue
+++	Blue-black

The negative control shows no colour change while the positive control shows an appropriate colour change depending on the amount of blood used.

Occult Blood using Okokit Method
Based on the same principles (Peroxidase-like activity of haemoglobin or its products). Reagents for these chemical methods are commercially available. However, Okokit method is a very simple one.
Patient preparation is important before sample collection.

Materials Required
Okokit Kit: (commercially available) It consist of the followings:
a. Okokit table
b. Diluents
c. Test papers

Procedure
(1) Make a 1:40,000 dilution of whole blood in distill water
(2) Prepare a thin smear of the faeces at the center of the test paper
(3) Prepare a similar smear of the diluted blood
(4) Place one Okokit tablet on each of the smears
(5) Add two to three drops of diluent on to the tablets
(6) Add two more drops after 2 minutes
(7) Read the colour change after five minutes as follows:

- Dark blue colour around the tablet: Positive (++)
- Pale blue colour around the tablet: Positive (+)

Note: The positive control carried along is to rule out false negative result. A negative test is repeated at another two more consecutive Occasions before reporting.

Note:

PUS : Fairly large quantities of pus may be passed out in stools of patients with inflammation of the GI tract as in bacillary dysentery or chronic ulcerative colitis

Amoebic colitis is generally not associated with large amounts of pus . Whenever present, a portion of the pus must always be included in the microscopic examination.

MUCUS: A normal stool does not contain mucus. Mucus may be associated with blood and pus in many inflammatory conditions of the GI tract, and other conditions such as neoplasm.

PARASITE: Adult worms such as Enterobius vermicularis or Ascaris lumbricoides, and Tapeworm segments may be found on the surface or in the stool. They should be identified and reported.

3.3 Microscopic Examination (Wet Mounts)

The easiest and the simplest technique for the direct microscopic examination of faeces is wet mount. Wet mounts can be prepared directly from the faecal material in saline *and*iodine. Other useful wet mounts are buffered methylene blue (BMB) and eosin. The saline wet mount is used for the preliminary microscopic examination of faeces to detect protozoan trophozoites and cysts ; and helminth larvae and eggs. A charactistics motility of a parasite can be used for its identification. Excessive celluler exudates in the faeces in the form of pus (white blood cells) or blood (red blood cells), macrophages or any other significant material such as charcot-leyden crystals can also be detected in wet mounts.

In the iodine wet mounts, most cysts can usually be specifically identified because the iodine stains their nuclei and glycogen, present. However, the parasites are not motile in iodine. BMB wet mount is useful only when amoebic trophozoites are seen in a saline wet mount of a fresh, unpreserved faecal specimen. It stains amoebic trophozoites, but not the cysts, and also does not the trophozoites and the cysts of flagellates. Eosin wet mount is very good for the initial detection of cysts and trophozoites. Eosin does not stain them, but they can be much more easily seen against a pink-red background of eosin.

Technique;

(i) Place a drop of saline in the center of the left half of the slide and place a drop of iodine solution in the center of the right half of the slide.

(ii) With applicator stick, pick up a small portion (2 mg) of faeces from an appropriate site, and mix it with saline on the slide to form a uniform suspension. In the same way, prepare a suspension of faeces in the iodine on the slide.

(iii) Cover the drop of saline suspension with a coverslip by holding it at an angle and lowering it gently on to the slide to reduce the formation of air bubbles. Cover the iodine suspension with another coverslip, taking care not to mix it with the saline suspension.

Examination

(i) Focus on the wet mount using a low power (x10) objective.

(ii) Regulate the light with the substage condenser, the diaphragm and the light source . Since most of the parasites are pale or colourless, too much or too little light may not be useful. Lowering the condenser and closing the diaphragm partially can give adequate light .

(iii) Examine the entire coverslip in a systematic order. Focus the objective on the top left hand corner and move slide slowly up and down or backwards and forwards. When any parasite or suspicious material is observed, change to the high-dry objective (40X). Increase the light by opening the substage diaphragm to observe the detailed morphology.

Examine each microscopic field carefully, focusing up and down, before moving to the next field. When a slowly motile trophozoite is suspected, observe for at least 15 seconds to confirm the motility.

3.4 Blood Samples for Parasites

The parasites which can be detected in the blood are:

Plasmodia, Trypanosomes, Leishmania, Filarial worms. Some parasites such as microfilariae and trypanosomes can be detected in the direct wet mount of fresh blood by their characteristic shape and motility. However, specific identification of the parasites requires a permanent stain. For permanent staining, two types of blood films can be prepared. Thick film allows a larger volume of blood to be examined, thus making it easier to detect light infections with few parasites, while species identification is difficult. Thin films are necessary to see the morphological characteristics of the parasites and to identify them.

Collection of Specimens for Blood Films

Caution: Careful attention to safety technique is necessary at the time of collection of blood samples and preparation of blood films. A number of parasitological, bacterial,and viral and viral diseases can be transmitted through blood . Blood films should be prepared before the commencement of any treatment. It is preferable to prepare blood films with fresh blood without anticoagulant. If it is not possible, blood anticoagulated with EDTA (10mg/5ml) should be used; and blood films should be prepared as soon as possible, preferably within 1 hour of collection. The time of collection should be mentioned on the specimen as well as on the result sheet so that the results may be correlated with the fever pattern by the Physician.

Preparation of Blood Films

For accurate examination of blood films, it is necessary to use absolutely clean, grease-free slides. Well washed slides cleaned with 70% ethanol are recommended.

Thick Blood Films

To make a thick film, place two or three small drops of fresh blood without anticoagulant on a clean slide . With a corner of another slide, mix the drops in a circular motion over in an area about 2cm in diameter, continue mixing for about 30 seconds to prevent formation of fibrin strands that may obscure the parasites after staining . (if anticoagulant blood is used it is not necessary to continue mixing for 30seconds). Allow the film to dry in air at room temperature. Before staining, the thick films are laked to lyse the red blood cells and to remove haemoglobin so that the parasites can be easily detected.

To lake the films, they are either placed in buffer solution before staining or placed directly into an aqueous stain like Giemsastain. If thick blood films are to be stored, they should be laked before storage. Once fixed, it is difficult to lyse red cells and release haemoglobin in thick blood films.

Thin Blood Films

In thin films, the number of parasites is much less than in the thick films, but it permits specific identification of parasites. The thin film is prepared in exactly the same way as the one used for peripheral blood smear examination. Allow the thin blood film to air-dry. The need for fixation before staining depends on the type of stain used. Aqueuos stains, such as Giemsa, need pre-fixation of thin films with methanol; while a methanol based stain, such as Leishman stain, does not require fixation

3.5 Parasites in Urine

Parasites which can be detected in urine are:

(i) Eggs of Schistosoma haematobium
(ii) Microfileriae of Wuchereria bancrofti
(iii) Microfileriae of Onchocerca volvulus
(iv) Trophozoites of Trichomonas vaginalis.

If present in sufficient numbers, these parasites can be detected while examining centrifuged deposits of urine. In areas where Schistosomiasis is endemic, the first indication of infection is haematuria which can be detected either by chemical test or microscopically. A heavy infection with schistosoma may lead to gross haematuria which is seen visually. Milky urine may show microfileriae of Wuchereria bancrofti or Onchocerca volvulus in a centrifuged deposit of urine. Trichomonas vaginalis is a parasite of the genital system but may appear in the urinary deposit as a contaminant from genital organs.

Diagnosis of Schistosoma
Collection of urine. A special care is needed for the collection of urine for suspected schistosomiasis because the number of ova excreted in the urine varies throughout the day . It is highest in the terminal portion of the urine between 10.00AM to 2.oo PM. The last few drops of urine contain the maximum number of the eggs. Therefore the specimen should be collected between these times and should be terminal urine at least10 ml in volume. Alternatively, a 24-hour specimen may be collected with formalin (1 ml per 100 ml of urine) as a preservative for the eggs. If it is not possible to examine the fresh specimen within 1 hour of collection, this too, should be preserved with formalin using the same proportion. It is advisable to examine large volume of urine because the ova are very scanty and can easily be missed. If not preserved with formalin, the eggs may hatch to release miracidia.

The Sedimentation Technique
This technique is cheaper and simpler to perform.
(i) Collect a 24-hour or terminal urine sample and check for haematuria.
(ii) Shake the urine well and allow it to sediment for I hour.
(iii) At the end of 1 hour, carefully withdraw the supernatant without disturbing the sediment.
(iv) Transfer the sediment to a centrifuge tube and centrifuge at500rpm for 5 minutes
(v) Discard the supernatant and examine the sediment as a wet preparation, using the 10x objective to search for the ova of Schistosoma haematobium.

Special Technique for Detecting Microfileriae in Urine

Microfileriae mostly commonly found in urine are those of Wuchereria bancrofti. A chronic infection with Wuchereria bancrofti may lead to chyluria. Urine containing chyle appears creamy white or milky. The chyle consists of lymph and fat particles which gives the urine its Characteristics appearance. The fat is soluble in ether, therefore urine becomes clear when mixed with a small of ether. Other microfileriae, such as those of Onchocerca volvulus and Brugia malayi, may also be found in urine.

Sedimentation Technique

(i) collect the first early morning urine sample after waking.
(ii) If the urine is milky in appearance, add about 2 ml of ether and shake till it becomes clear .
(iii) Centrifuge 10 ml of the specimen at 500g (rpm) for 5 minutes. A very high speed is not advisable as the microfileriae may lose their sheath.
(iv) Discard the supernatant and examine the sediment.
(v) If the sediment shows the presence of blood, add one or two drops of water to lyse the red cells.
(vi) Centrifuge again, discard the supernatant, and examine the sediment after covering it with a coverslip.
(vii) Motile microfileriae can be observed under 10x objective in reduced light .

3.6 Parasites in Aspirates

Examination of aspirated material or tissue biopsies may help in the diagnosis of parasitic infection.

Aspirate from Lung or Liver Abscess

Examination of aspirates from lung or liver abscess may reveal trophozoites of Entamoeba histolytica. The parasites are trapped in the viscous pus and tissue debris at the periphery of the abscess. To see the typical motility, the aspirate should be treated with proteolytic enzyme which will which will allow the parasite to move.

Technique

(i) Add 1 ml of sputasol or 3% KOH to about 1 ml of pus , and incubate at 37C for 30 minutes , shaking in between.
(ii) Centrifuge at 500xg (rpm) for 5 minutes .
(iii) Examine the sediment microscopically as a wet mount and a permanent stained smear.

3.7 Skin Snips for Microfileriae

(i) A bloodless skin snip should be collected by using a sterile needle and a razor blade .
- clean skin with an alcohol swab and dry.
- insert the needle almost horizontally into the skin, and lift it so that a small cone about 2mm in size is formed.
- cut off the piece with a sterile razor blade.

(ii) Immediately place the skin snip in a centrifuge tube containing about 2 ml of saline .

(iii) Incubate at room temperature overnight.

(iv) Remove the skin snip with forceps, place on a slide and cover with coverslip.

(v) Centrifuge the at 500g (rpm) for 5 minutes

(vi) Examine the sediment microscopically less than 10x objective and identify the microfilaria

3.8 Examination of sputum for eggs of Paragonimus westermani

Collect a deep-sputum specimen from the lower respiratory passages, and not the saliva. In paragonimus infection, the sputum may be tinged with brown flecks or iron filings, which are clusters of eggs, and may be streaked with blood. Examine the specimen directly as a wet mount. If the sputum is too thick, digest it with an equal volume of 3%NAOH (sodium hydroxide)(w/v) solution and leave for 10 minutes to dissolve the mucus . Mix and centrifuge at 500g (rpm) for 5 minutes. Examine the sediment as a wet mount. The eggs of Paragonimus westermani are yellow-brown to brown in colour, measuring 70-100 x 50-65um, slightly flattened on one side. The operculum is flat and has shoulders.

4.0 CONCLUSION

Parasites are found almost everywhere in nature and are in constant interaction in Parasitology laboratory; medical parasitology laboratory, hence is an important arm of healthcare helping to provide a conducive environment where the parasiteswhichever one is/ are identified for medical treatment , evaluation and control .

5.0 SUMMARY

Parasitology laboratory is where all the tests for the identification of the entireparasites e.g; Hookworm, Ascaris, malaria, E.histolytica etc are carried out.

The important techniques and methods have been discussed; also various samples associated with the organisms have been mentioned.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the factors involved in the macroscopic examination of faeces .
2. Describe the technique in microscopic examination of stool sample.
3. What is the medical importance of thick and thin films in examining blood parasites.

7.0 REFERENCES/FURTHER READING

Ochei.J & Kolhartkar. A.(2008) : Medical Laboratory Science Theory and Practice. Tata McGraw- Hill.

F.J. Baker; R.E. Silverton and C. J. Pallister (2011) : Introduction to Medical Laboratory Technology. 7th edition Bounty Press Limited.

Daniel. S. S (2015): Microbiology Laboratory. What is the microbiology laboratory? ; Methods in Microbiology Laboratory. J. Infect. Dis. Prev.

UNIT 3 HISTOPATHOLOGY LABORATORY

CONTENTS

1.0 INTRODUCTION

Histopathology Laboratory refers to where the tissues to be examined are processed and examined. It can as well be where examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides take place.

Histopathology is the study of diseased tissue, for example, breast lumps or specimens of bowel removed because of suspected cancer, including examination under the microscope. Is also the study of tissues (histology) and cells (cytology) and usually includes morbid anatomy (autopsies).

Biopsies are usually obtained either during an investigation or as an arranged procedure. Examples of investigations when biopsies may be taken include an endoscopy (for example looking at the oesophagus (gullet) and stomach) or colposcopy (examination of the cervix with a magnifying instrument). An example of an arranged procedure is of a kidney biopsy when a core of tissue is obtained by passing a needle through the skin and into the kidney. Usually little information can be obtained looking at these biopsies by naked eye and the key information is only obtained under a microscope. Many biopsies are reassuring as they have been taken, by a doctor, to rule out cancer and the biopsy does not show cancer but may, for instance, show inflammation which explains the symptoms and signs. Other biopsies may show cancer and

the pathologist is able to look for features that can tell what sort of cancer is present, how aggressive it is and whether it may respond to certain types of treatment.

2.0 OBJECTIVES

At the end of this unit you will be able to:

- histopathology laboratory
- define histopathology
- know some principles and techniques involves in the procesing and examination of tissues in the l aboratory.

3.0 MAIN CONTENT

3.1 Histopathology Laboratory

Histology is the microscopic study of the normal tissues of the body, whereas histopathology is the microscopic study of tissues affected by disease.

Histopathology Laboratory is where the processing and microscopic examination of tissues, in order to study the manifestations of disease takes place. The procedures adopted for the preparation of material for such studies are known as histological or histopathological techniques
.
As a field of general inquiry and research, pathology addresses four components of disease: cause/etiology, mechanisms of development (pathogenesis), structural alterations of cells (morphologic changes), and the consequences of changes (clinical manifestations). In common medical practice, general pathology is mostly concerned with analyzing known clinical abnormalities that are markers or precursors for both infectious and non-infectious disease.

3.2 General Outline of Procedures in the Examination of Tissues

There are several ways and techniques used to process tissues for laboratory examination with the intent of making accurate and proper diagnosis. Whichever technique is employed, there are factors which govern the whole procedure: the structure to be studied, the size and nature of the tissue, whether the specimen is fresh or preserved, and the urgency of the examination. Details of each stage of the investigation may differ from laboratory to laboratory but the basic principle remains the same.

Reception: The tissue on arrival in the laboratory reception, the specimen is checked at the earliest opportunity for the following:

(i) That the specimen is for histological examination
(ii) That the container is clearly labeled and accompanied by a completed request form.
(iii) That sufficient fixative is in the container, or if the specimen is not in fixative or is in a wrong fluid.

The request form is dated and stamped; the specimen is given an identification serial number which remains with the specimen until all the investigations have been carried out. The Pathologist, usually at a set time, will examine the specimen; where necessary describe the macroscopic appearance and select the pieces from which he wants the section to be prepared. A sink and running water are essential in this area usually referred to as the , cut-up-room. The Pathologist requires rubber gloves, sponge, scalpel, and scalpel blades, large ham knife, plain and rat-tooth forceps, probes, scissors, small bone saw, steel rule and some means of weighing the specimen.

Fresh Specimen
Fresh specimens sent to the laboratory for histological examination are prepared as either squash or teased preparation, touch or frozen sections.

Squash Preparation: Small pieces of tissues not more than 1mm in diameter are placed in the center of a microscope slide; a coverslip is forcibly pressed down on them. Vital staining can be down by placing a drop of the stain at the junction of the slide and coverslip. The stain is drawn in by a capillary action and absorbed by the tissues. The purpose of squash preparation is to study the cellular contents of the tissue.

Teased Preparation: The fresh specimen of tissue immersed in saline or Ringer's solution, is dissected with mounted needles. Pieces of the tissue are picked onto a microscope slide and mounted as a wet preparation under a coverslip. The slide is then examined by the ordinary light microscope or better still by phase contrast microscope. Stains like methylene blue can be applied to enhance the recognition of cellular structures. This method allows cells to be examined in living state. A big drawback is that it is not a permanent preparation.

Smears: Smearing a piece of fresh specimen of tissue evenly on the surface of microscope slide is an acceptable practice in histopathology. The making of such smears however, depends on the type of tissue to be examined. The smear can be examined fresh in which case it is stained as for teased preparation or by using a supravital stain in conjunction with a warm stage. The preparation is never permanent.

Impression Smears: These are prepared by touching a freshly cut piece of tissue with the surface of clean microscopic slide. This way, cells are transferred and adhered to the slide. The smear can be examined with the phase contrast microscope or by using vital stain. It is also possible to fix the smears and stain accordingly.

Frozen Sections: Fresh tissue frozen on a microtome with Co2 can be cut into sections of about 10-15um in thickness. The sections are transferred to a dish and attached onto the slide before staining or from the dish carried on a glass rod through staining solutions.

3.3 Fixed Tissues

To study normal or diseased tissues microscopically, thin sections of the tissue have to be cut. Sections are normally prepared from fixed tissues. Tissues are fixed to prevent putrefaction and other post mortem changes which occur soon after removal from the body. The choice of fixative depends to a large extent on the type of investigation, nature of the specimen, urgency of the examination and whether or not serial sections are required.

3.4 Paraffin Sections

The most popular embedding medium for histological preparations is the paraffin wax . The embedding medium confers rigidity to the piece of tissue so that sections can be cut . The selected fixed pieces of tissue are first passed through a series of baths of alcohol to remove water from the tissues (dehydration). This is done because the fixative is not miscible with paraffin wax. The tissues are passed further to a bath or two of a medium that is miscible with alcohol and paraffin. This medium is the clearing agent. The clearing medium also raises the refractive index of the tissue, making it look transparent. It is this clearing medium that is eventually replaced by the molten paraffin wax. When the tissues have been sufficiently impregnated, they are imbedded in fresh wax which solidifies on cooling. It should be noted that workers refer to clearing agents as ante-media because some of them don't actually clear the tissue . Using paraffin wax allows thin and serial sections to be cut easily, allows many staining techniques and facilitates handling and storage.

3.5 Celloidin

Next to paraffin wax, Celloidin is a commonly used embedding medium. Celloidin is a purified form of nitro-cellulose which is soluble in many solvents. Celloidin allows thicker sections to be cut and that makes it very useful in the study of central nervous system. It is a better

medium for very hard tissues or tissues with varying consistency. It causes little or no shrinkage. However, it is very slow method and serial sections are very difficult to produce.

3.6 Resin Embedding

This is a new method adopted for light microscopy from electron microscopy techniques. The major advantage of using resins is that much thinner sections can be cut though; specially adapted knives and microtomes are required. The various stages of dehydration, clearing and embedding are referred to as processing and are usually done manually or by automatic processing machine..

3.7 Cutting and Staining

Sections are cut with the microtome from the paraffin wax blocks. The microtome is a most useful piece of equipment in the histopathology laboratory and microtome is as good as its knife. The cut sections are cut floated out in warm water, mounted on a clean microscope slides, dried and then stained. The stained sections are mounted in a good mountant under a cover glass, then labeled and cross-checked against the information on the request form.

4.0 CONCLUSION

Histopathology has various techniques and methods designed and employed in histopathological investigation as it is impossible to study all normal and abnormal tissue components in a single preparation, for this reason I, urge you to put more effort and get the knowledge.

5.0 SUMMARY

In this unit, Histopathology laboratory has been briefly discussed including some techniques and general outline of procedures in the examination of tissues. Try as much as possible to go through them for your benefit.

6.0 TUTOR-MARKED ASSIGNMENT

Write short notes on:

(i) Impression smears
(ii) Frozen sections
(iii) Paraffin sections

7.0 REFERENCES /FURTHER READING

Burtis, Cart A; Ashwood, Edward R ; Bruns, David E(2006): Tietz textbook of Clinical Chemistry.4th edition; Saunders .

Carson, Freida L; Christa Hladik(2009): Histotechnology. A self-instructional Text. 3rd edition. Hong; Am. Soc. For Clinical Pathology.

Robbins, Stanley (2010): Robbins and Cotran Pathology basis of disease. 8th edition, Philedelphia. Saunders /Elsevier

Cai, H; Casewell JL; Prescott JF(2014): Non culture molecular Techniques; A Diagnostic Laboratory Perspective .

Ochei.J; Kolhatkar.A. (2008): Medical Laboratory Science; Theory and Practice.

Baker.F.J.; Silver.R.E; Palister.C.J.(2011) : Introduction to medical Laboratory Technology. 7th edition; Bounty Press Limited

UNIT 4 DECALCIFICATION

CONTENTS

1.0 INTRODUCTION

Decalcification describes the technique for removing mineral from bone or other calcified tissue so that good-quality paraffin sections can be prepared that will preserve all the essential microscopic elements. Decalcification is carried out after the specimen has been thoroughly fixed and prior to routine processing to paraffin. Here are a number of options available when the histologist is required to produce sections from bone or other calcified specimens. In choosing a technique and processing method consideration must be given to the type of investigation being carried out. For example if a metabolic bone disease is being investigated and it is necessary to differentiate mineralized bone from osteoid, or if morphometric measurements are required, it may be necessary to retain and demonstrate.

The mineral content by producing sections of “un-decalcified” bone. As mineralized bone is such a hard material there are a limited range of techniques available to produce sections from it. After fixation it can be directly sawn into thin wafers and then ground using abrasive surfaces to produce thin “ground” sections. It is also possible to prepare bone specimens by infiltrating them with acrylic or epoxy resins which, when polymerized, have a hardness equivalent to that of mineralized bone. You can then produce ground sections from the infiltrated specimens or section them directly using a heavy-duty microtome and a tungsten-carbide or diamond knife. Frozen sections of mineralized cancellous bone are another possibility.

2.0 OBJECTIVE

At the end of this unit you will be able to:

- define decalcification .
- know some techniquess involved in decalcification
- know the decalcifying solutions
- know the advantages and disadvantages of the solutions.

3.0 MAIN CONTENT

3.1 Decalcification

When heavy deposits of calcium salts are present in tissue, the cutting of the sections is facilitated by decalcification. Inadequate decalcification results in poor section cutting and severe damage to the knife-edge. Calcium is normally present in large amounts in bone and teeth, but pathologically deposits may be found in varying amounts in other tissues, notably those involved in tuberculous or cancerous changes. Calcified deposits are often present also in the heart valves and walls of large blood vessels, particularly the aorta, of elderly people.

An acid is the essential constituent in most decalcifying solutions, and a second substance is often incorporated to prevent distortion of the tissue, although, this should be minimal if adequate fixation has been given. Buffer solutions of pH 4.4 to 4.5 and organic chelating agents, for example ethylenediamine tetra-acetic acid(EDTA), can also be used.

A good decalcifying agent should remove all calcium without damage to cells or tissue fibres and with no impairment of subsequent staining or impregnation. The four acids most commonly used for removing calcium salts from tissues are formic, nitric, hydrochloric and trichloracetic acids. The speeds at which calcium salts are dissolved out of the tissue is dependent upon the strength, temperature and volume of the decalcifying solution in relation to the size and consistency of the tissue undergoing decalcification. An increase in either the concentration of the acid acting as the decalcifying agent or the temperature at which decalcification takes place, can markedly decrease the time required, but this is usually attended by partial digestion of the tissue and inferior staining results. These adverse effects , produced by a higher temperature, do not apply to EDTA which may be used successfully at 40^{0C}-60^{0C}.

3.2 Selection of Tissue

Bone
Blocks of tissue suitable for sectioning are selected from the gross specimen by means of a sharp , fine-toothed hacksaw after preliminary fixation in neutral 10% formalin. To facilitate fixation and decalcification, the selected block of tissue should not exceed 5mm in thickness. Damage to the surface of the tissue and impacted bone- dust produced by sawing can be removed by trimming the decalcified tissue with a sharp knife. It is always advisable , however, to discard the first sections cut in order to avoid possible artefacts in the final preparation.

Teeth
Blocks of teeth for sectioning are usually best taking when the specimen is either completely or partially decalcified. They may then be selected with a sharp knife, thereby causing the minimum of damage and distortion to the tissue.

Calcified Tissue
Blocks of tissue suitable for processing and sectioning can usually be selected from fixed soft tissues containing calcified areas using a sharp knife. If large calcified areas are encountered a hacksaw is used to cut through the deposits and the surrounding soft tissues are cut with a knife. Damage to knife-edge and tissues will occur if the cutting of such areas is attempted by knife alone. The selected tissue block should not exceed 5mm in thickness as immersion in the decalcifying solution for too long should be avoided. Tissues should be completely fixed before commencing decalcification and neutral 10% formalin is the recommended fixative for this purpose . At least 48 hours fixation is required for tissue blocks of 5mm thickness.

3.3 Technique of Decalcification

(a) The selected tissue slice is suspended in the decalcifying solution by means of a waxed thread. This allows the solution free access to all surfaces of the tissue, while the wax protects the thread from the action of the acid. With few exceptions , the volume of decalcifying fluid should be approximately 50-100 times the volume of the tissue.
(b) The progress of decalcification should be tested at regular intervals , usually daily , but in the final stages and with some decalcifying solutions more frequent tests are made . The fluid is renewed following each positive test.
(c) When decalcification is complete the tissue is transfered directly to 70% alcohol and given several changes over 8-12 hours. This not only effectively washes out the acid , but also establishes the

first stage of dehydration for either the paraffin wax, celloidin or Low Viscosity Nitrocellulose(LVN) infiltration techniques.

(d) The tissue is the completely dehydrated and processed according to the required embedding technique. If the paraffin wax method is used, it is recommended that at least part of the wax impregnation be carried out in the vaccum oven.

3.4 Assessment of Decalcification

Tissues should be immersed in the acid decalcifying solutions only for as long as is necessary for complete calcium removal. Prolonged immersion beyond this stage will result in deterioration of cell and tissue morphology and qualityof subsequent staining reactions. The stage to which decalcification has progressed and its eventual end-point can be assessed by X-ray examination or by a chemical test. The simplicity of the chemical test has fortunately led to the abandoment of several crude methods for decalcification assessment. These included probing of tissue block by needle, knife or finger nail in an effort to detect residual gritty fragments of calcium. Such malpractices were the direct cause of tissue damage, and small spicules of often remained undetected.

(i) X-ray examination is the most satisfactory method, depending on the availability of facilities and a good relationship between laboratory and radiography department. X-ray is the only means by which tissues treated with EDTA can be adequately controlled, but it can not be used on material fixed in mercuric chloride because this fixative renders such material radio-opaque. It can also be inconvenient during the final stages of decalcification when frequent examination may be necssary.

(ii) A chemical test is a simple reliable expedient when radiography is unavailable. It is a two-stage test which depends on detection of dissolved calcium in the decacifying fluid . A positive result at either stage indicates that further decalcification of tissue in fresh fluid is required and the test should be repeated after a suitable interval.

Method

(a) Decant 5 ml of the used decalcifying fluid into a clean test-tube and add a small piece of litmus paper.

(b) Add strong ammonia (sp.gr.0.88) drop by drop while agitating the tube until the litmus paper just turns blue, indicating alkalnity.

(c) If the solution becomes turbid at this stage calcium is present in considerable amounts and the tissue should be transferred to fresh decalcifying fluid.

(d) If the solution remains clear, proceed with the second stage of the test. Add 0.5ml saturated aqueous ammonium oxalate, mix and

allow to stand for 30 minutes. Any turbidity developing during this period indicates the presence of calcium and re-immersion of the tissue in fresh decalcifying fluid is necessary. If the solution remains clear, decalcification is complete. It is important that sufficient time is allowed between tests to ensure dissolution of calcium by the fresh decacifying fluid. Intervals of 3-4 hours are adequate for most decalcifying solutions. When using the chemical test to control the degree of decalcification , it is essential that the decalcifying fluid is prepared with distilled water. Failure to observe this precaution may result in false positive readings being produced by the presence of calcium ions in tap water.

3.5 Decalcifying Solutions

(i) Formic Acidb (HCOOH)

This is recommended for post-mortem and research tissue. The time necessary for decalcification is 2-7 days.

Formula

Formic acid(sp.gr.1.20)	5 ml
Distilled water	90 ml
Formaldehyde(40%)	5 ml

Advantages

This solution permits excellent staining results and it is regarded by many workers as being the best decalcifying solution for routine purposes.

Disadvantages

At the above strength decalcification is low, and the solution is therefore unsuitable for urgent work. Decalcification may be fastened up by increasing the formic acid content up tp 25 ml. A disadvantage of using concentrations of formic acid in excess of 8%, however, is that the opacity of the solution interferes with the chemical test used in controlling the degree of decalcification. The used fluid can be diluted in order to apply this test but the final result is not as accurate as when used with a sample of the undiluted decalcifying solution.

(ii) Nitric Acid-Formaldehyde

This is recommended for urgent biopsies. The time required for decalcification is 1-3 days.

Formula

Nitric acid(sp.gr.1.41)	10 ml
Formaldehyde(40%)	5-10 ml
Distilled water	100 ml

Advantages
This is a rapidly acting decalcifying solution which permits good nuclear staining.

Disadvantages
Nuclear staining is not as good as that obtained after more slow-acting solutions. Nitric acid frequently turns yellow when used as a decalcifying agent due to nitrous acid formation. This increases the speed of decalcification but also impairs the subsequent staining reactions. The addition of 0.1% urea to the pure concentrated nitric acid temporarily arrests the discoloration without affecting the efficiency of the acid.

Aqueous Nitric acid
A rapidly acting decalcifying solution which is recommended for routine use.

Formula

Nitric acid (sp.gr.1.41)	5-10 ml
Distilled water	100 ml

Advantages
This is a rapid decalcifying solution which causes very little hydrolysis, provided that the tissue is not allowed to remain immersed beyond the stage when decalcification is completed. The subsequent staining results are good.

Disadvantages
The disadvantages of the solution are similar to those given above under nitric acid-formaldhyde. The remarks relating to the use of urea to stabilize the nitric acid also apply with this solution.

Perenyi's Fluid
This solution was introduced as a fixative for ova, but is also good routine decalcifying fluid. The time required for decacification is 2-10 days.

Formula

Nitric acid, 10% aqueous solution	40 ml
Absolute ethyl alcohol	30 ml
Chromic acid, 0.5% aqueous	30 ml

When freshly mixed the solution is yellow, but it rapidly assumes a clear violet colour.

Advantages
No hardening occurs in tissues treated with Pereny's fluid; it can be used as a softening agent , prior to dehydration, for dense fibrous tissues. Cellular detail is well preserved and subsequent staining is good. When decalcification is complete , tissues do not require washing in water and may be transferred directly to several changes of 70% alcohol.

Disadvantages
It is rather slow for decalcifying dense bone. The chemical test described earlier can not be used to determine decalcification because a precipitate is formed when ammonia is added even in the absence of calcium ions. This difficulty may be overcome, however, by a simple modification:

(i) Transfer 5 ml of used decalcifying fluid to a chemically clean test-tube and add a small square of litmus paper.
(ii) Alkalinise by dropwise addition ofammonium hydroxide solution(sp.gr.0.88).
(iii) Add glacial acetic acid drop by drop until the precipitate is dissolved.
(iv) Add 0.5 ml saturated aqueous solution of ammonium oxalate . The appearance of a white precipitate within 30 minutes indicates the presence of calcium, and that the tissue requires further treatment with fresh fluid.

Ebner's Fluid
Advantages
The use of this fluid is recommende for teeth and the time for decalcification is 3-5 days. Various formulae have been given for this method, but the following gives good results;

Formula

Sat. Aqueous sodium chloride	50 ml
Distilled water	50 ml
Hydrochloric acid	8 ml

Advantages
This is a fairly rapid decalcifying solution and subsequent staining results are usually good . It is particularly useful for decalcifying teeth. The excess acid is removed by several changes of 90% alcohol for 24 hours. Dehydration is thereby hastened.

Disadvantages
Nuclear staining is not as good as that obtained after formic acid.

Trichloracetic acid
This is recommended for small pieces of delicate tissue which require decalcification. The time necessary for decalcification is 4-5 days.

Formula

Trichloracetic acid	5 g
10% formol-saline	95 ml

Advantages
It permits good nuclear staining. The excess acid is removed by washing in several changes of 90% alcohol.

Disadvantages
It is slow decalcifying solution, and is not recommended for use with dense bone.

Citrate-Citric Acid Buffer (pH 4.5)
This is recommended when speed is not an important factor. The period required for decalcification is approximately 6 days, during which time the solution should be changed daily.

Formula

Citric acid, 7%	5.0 ml
Ammonium citrate, 7.4%	95.0 ml
Zinc sulphate,1%	0.2 ml

Add a few drops of chloroform as preservative.

Advantages
This solution produces no damage to the cells or tissue constituents and permits excellent staining results.

Disadvantages
This method is too slow for routine work.

3.6 Ion Exchange Resins

The incorporation of an Ion exchange resin(an ammonium form of polystrene resin) into the decalcifying solution has been claimed to speed up the process of decalcification and to improve staining. The principle of the method is that the calcium ions are removed from the solution by the resin , thereby increasing the rate of solubility of the calcium from the tissue. However, subsequent workers have shown that no obvious improvement in decalcification speed, preservation or staining is achieved by the use of this resins. A layer of the resin , approximately 13 mm thick , is spread over the bottom of the vessel being used and the specimen is allowed to rest on it. The decalcifying

solution is added, the volume of the solution being approximately 20-30 times that of the tissue . The end- point is determined by radiological examination , the chemical test not being applicable. The use of ion exchange resins is limited to decalcifying solutions which have a non-mineral acid as their active constituent, formic acid being the usual choice. Two baths of 0.1M hydrochloric acid followed by three washes of distilled water will regenerate the used resin for further use.

Chelating Agent
This is a very slow decalcifying solution recommended only for detailed microscopical studies where time is not an important factor. It is not suitable for use with urgent surgical specimens. The time required for decalcification is approximately 3 weeks, during which time the solution must be changed at intervals of 3 days, reducing to one day in the final stages.

Formula

Na_2EDTA	5.5g
10% neutral formalin	100ml

Advantages
Histological artifacts are minimised by the use of this solution, as no carbon dioxide bubbles are produced to destroy the partern of the remaining organic material. The subsequent staining results are also excellent.

Disadvantages
It is slow and unsuitable for urgent work. The chelating agent also tends to harden the tissue slightly.

3.7 Proprietory Decalcifying Fluids And Softening Of Dense Fibrous Tissue

Proprietory decalcifying fluids are available commercially which are more rapid than conventional fluids. The manufacturer’s instruction must be followed.

Softening of Dense Fibrous Tissue
Some specimens are composed of dense fibrous tissue which, while not containing calcium salts, is nevertheless too tough for sectioning. Blocks of tissue taken from such specimens may be softened by addition of 4-6% phenol to the dehydrating alcohols. Commercially produced reagents such as Mollifex are available for softening tissue after embedding in paraffin wax.

4.0 CONCLUSION

Decalcification has various techniques and methods designed for a particular tissue, many agents are involved. I urge you to go through all and get the knowledge.

5.0 SUMMARY

Decalcification has been described, including techniques, fluids and other agents involved. Try as much as possible to go through them for your benefit.

6.0 TUTOR –MARKED ASSINGMENT

Write short notes on:

(i) Calcified tissue
(ii) Technique of decalcification
(iii) Assessment of decalcification
(iv) Formic acid as a decalcifying agent.

7.0 REFERENCES /FURTHER READING

Burtis, Cart A; Ashwood, Edward R; Bruns, David E(2006): Tietz textbook of Clinical Chemistry. 4th edition; Saunders .

Carson, Freida L; Christa Hladik (2009): Histotechnology. A self-instructional Text . 3rd edition. Hong; Am. Soc. For Clinical Pathology.

Robbins, Stanley (2010): Robbins and Cotran Pathology basis of disease. 8th edition, Philedelphia. Saunders /Elsevier

Cai, H; Casewell JL; Prescott JF(2014): Non culture molecular Techniques; A Diagnostic Laboratory Perspective .

Ochei. J; Kolhatkar.A. (2008): Medical Laboratory Science; Theory and Practice .

Baker.F.J.; Silver.R.E; Palister.C.J.(2011) : Introduction to medical Laboratory Technology.7th edition; Bounty Press Limited

UNIT 5 DEHYDRATION, IMPREGNATION AND EMBEDDING TECHNIQUES

CONTENTS

1.0 INTRODUCTION

Once the tissue has been fixed, it must be processed into a form in which it can be made into thin microscopic sections. The usual way this is done is with paraffin. Tissues embedded in paraffin, which is similar in density to tissue, can be sectioned at anywhere from 3 to 10 microns, usually 6-8 routinely. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing.

Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water from the tissues must be removed by dehydration. This is usually done with a series of alcohols, say 70% to 95% to 100%. Sometimes the first step is a mixture of formalin and alcohol. Other dehydrants can be used, but have major disadvantages. Acetone is very fast, but a fire hazard, so is safe only for small, hand-processed sets of tissues. Dioxane can be used without clearing, but has toxic fumes.

The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium (paraffin). The commonest clearing agent is xylene. Toluene works well, and is more tolerant of small amounts of water left in the tissues, but is 3 times more expensive than xylene. Chloroform used to be used, but is a health hazard, and is slow. Methyl salicylate is rarely used because it is expensive, but it smells nice (it is oil of

wintergreen).Impregnation process involves placement of tissues with medium that will fill all natural cavities, spaces, & interstices of tissues, even inter constituent space of cell, replaces clearing agent & terminates with making of block. - Impregnation (paraffin) supports tissue from all sides with firmness without producing any injurious effects on tissues. - It allows cutting of tissues suitably thin sections without undue distortion & without alteration of spatial relationships of tissue & cellular elements.

Finally, the tissue is infiltrated with the embedding agent, almost always paraffin. Paraffins can be purchased that differ in melting point, for various hardnesses, depending upon the way the histotechnologist likes them and upon the climate. A product called paraplast contains added plasticizers that make the paraffin blocks easier for some technicians to cut. A vacuum can be applied inside the tissue processor to assist penetration of the embedding agent. A product called paraplast contains added plasticizers that make the paraffin blocks easier for some technologists to cut. A vacuum can be applied inside the tissue processor to assist penetration of the embedding agent.

2.0 OBJECTIVE

At the end of this unit you will be able to:

- define dehydration, impregnation and embedding techniques .
- know some techniquess involved in the three techniques
- know the dehydrating, impregnating and embedding solutins and other agents in the processes.
- know how to prepare some solutions for embedding.

3.0 MAIN CONTENT

3.1 Dehydration, Impregnation And Imbedding Techniques

Many fixatives, including formaldehyde, can produce harmful effects when inhaled or when in contact with the skin. A special area should therefore be set aside for the examination of all specimens. This should take the form of a stainless steel bench provided with running water and a drainage point. Extraction facilities must also be provided to remove harmful vapours. Disinfectant should always be used to wash down surfaces on which specimens have been examined. Disposable gloves should always be worn and discarded after use.

Suitable instruments must be available and should include a large slicing knife, probes, scapels, plain and toothed forceps of varying sizes, several pairs of scissors, including fine dissecting, blunt-nosed and bowel types.

A plastic rule is necessary to measure tumours and cavities. Scales are required to measure the tissue weight. Finally, bone forceps and a bone saw or fine hacksaw should be provided to take blocks of tissue from calcified specimens.

3.2 Selection of Tissue

Following fixation, pieces of tissue for histological examination are selected from the gross specimen. A brief description of the nature of the tissue and site of the origin should be recorded. The introduction of plastic embedding cassettes has greatly facilitated the processing of tissue and reduced the risk of possible error. These systems consist of small plastic cassettes, available in various sizes and colours, with an integral lid and roughened sides which permit the necessary information to be recorded in pencil. Cassette numbering are also available which engrave the specimen number onto the cassette for greater security. The cassette eventually form part of the final paraffin wax block, which means that the tissue is always identified. For special purposes where cassettes are not being used, a small cardboard ticket complete with the number identifying the block is written in waterproof ink or pencil and accompanies the specimen throughout its processing schedule.

Tissue requiring special attention should be noted and the details recorded on the working card. Frequently, blocks are to be sectioned from a particular surface. This may be identified by passing a thread through one corner of the opposite surface of the tissue to that which is to be sectioned or, the opposite surface can be identified with waterproof drawing ink. At no time after the pieces of tissue have been selected should they be separated from their identifying number. Failure to observe this rule could lead to a wrong report being issued for the wrong patient. The use of cassettes aids the elimination of this risk.

3.3 Paraffin Wax Technique

Dehydration
The original fixative solutions which were used are not miscible with paraffin wax; therefore preliminary dehydration is necessary. The solutions commonly used for this are ethyl alcohol, methyl alcohol, iso-propyl alcohol and 74^{o} OP methylated spirit. Acetone dioxane have also been used.

The Alcohol Method
This consists in passing the tissue through a series of progressively more concentrated alcohol baths. Tissues together with their identifying label, or in a cassette are transferred from one container to another at the appropriate times, allowing them to drain for a few seconds between

each change. The containers should be fitted with lids. The more delicate the tissue the lower is the grade of alcohol suitable for commencing dehydration, and the smaller the intervals there should be between the strengths of the ascending alcohols.

The strength of the initial alcohol and the time required in each grade depend on the size and type of tissue and on the fixative which was used. To ensure that the final bath of alcohol is pure , and free from water, it is advisable to keep a layer of anhydrous copper sulphate 6 mm in depth and covered with filter paper, on the bottom of the vessel used. This salt also acts as an indicator, turning blue when water is present. The alcohol should be discarded if a blue tinge becomes apparent. Isopropyl alcohol may be used for dehydration purposes.

The period necessary for dehydration may be reduced by processing at 37^{o}C instead of room temperature. This procedure is sometimes of value when sections are required urgently from small fragmentary biopsies which should be wrapped carefully in filter paper prior to processing.

Acetone Method
This is used for the most urgent biopsies. Only small pieces of tissue should be treated, and dehydration takes from 30 minutes to 2 hours. Considerable shrinkage is produced during the process, rendering it unsuitable for routine work.

Dioxane Method
Dioxane(diethylene dioxide) is a unique reagent which has the unusual property of being miscible with both water and molten paraffin wax. It produces very little shrinkage and is simple to use. It is toxic and its use is only recommended in carefully controlled conditions.

3.4 Clearing

Clearing or de-alcoholisation is the term applied to the removal of alcohl from blocks or sections of tissue by immersing them in an ante-medium. Most of the original ante-media raised the refractive index of de-alcoholised tissues, thereby imparting to them a degree of transparency which resulted in this stage of processing being designated the clearing stage and the media used as the clearing agents. Not all of the present day ante-media (eg chloroform) cause this transparent effect and the term clearing is therefore strictly incorrect.

Clearing agents must be miscible with both alchohol and paraffin wax. Common clearing agents are xylene, toluene, chloroform, 1,1,1-trichloroethane, Histo-clear and Cedar wood oil.

Xylene
A rapid clearing agent suitable for urgent biopsies. It is highly flammable. Tissues are rendered transparent by xylene and it volatilises readily in paraffin oven. Biopsies and tissue blocks not exceeding 3mm in thickness are cleared in 15-30 minutes but some materials, notably brain and blood containing tissues, tends to become brittle if immersion is prolonged. Xylene fumes must not be inhaled. The solvent should only be used with adequate protection from fumes.

Toluene
Like xylene, it is highly flammable, has similar clearing properties, but without the same brittle effect on tissues. It is somewhat more expensive than xylene. Clearing time is from 15-180 minutes, depending on the tissue type and thickness. The solvent should only be used with adequate protection from fumes.

Chloroform
An expensive , nonflammable clearing agent which causes minimal shrinkage or hardening of tissues even when the optimum clearing time is exceeded. It is relatively slow in its displacement of alcohol and tissue blocks are not rendered transparent so that the end point is difficult to assess. Most tissues of 3-5 mm thickness are de-alcoholised in 6-24 hours. It should be pointed out that chloroform vapour is both anaesthetic and toxic and in addition, it may have a deleterious effect on the rubber and synthetic sealing rings of the vaccum impregnating bath.

Cedar Wood Oil
Rarely used for routine clearing purposes because of its cost and slow action. This reagent causes little or no damage to even the most delicate tissues. It is of particular value in research laboratories and in embryological procedures. Certain tissues, notably skin and dense fibrous material, benefit from treatment with cedar wood oil in that it imparts to such tissues consistency which facilitates subsequent section cutting. Tissue-blocks become transparent after alcohol displacement, but the is difficult to eliminate in the wax bath, several changes of wax being necessary. Alternativel, the cleared tissues may be treated with toluene for 30 minutes before beinf transferred to molten paraffin wax . Cedar wood oil for histological purposes is a thin, colourless, slightly yellow fliud distinct from the more viscous type which is unsuitable for de-alcoholisation.

1,1,1-Trichloroethane
Non-flammable and of lower toxicity than most traditional solvents.

Histo-clear (National diagnostic USA)
A safe clearing agent with a strong smell of oranges produced from purified oils. Clearing in Histo-clear takes longer than most traditional solvents.

Other Agents
Carbon disulphide, carbon tetrachloride, paraffin oil, cellosolve(2-ethoxyethanol) and methyl benzoate are less commonly used as ante-media. Methyl benzoate dissolves celloidin, and is used in conjunction with it for the double impregnation of tough or fragile objects.

3.5 Impregnation with Paraffin Wax

Tissues are transferred from the clearing agent to a bath of molten paraffin wax, either in an embedding oven, or in a chamber of an automatic processing machine. During this stage, the clearing agent is eliminated from the tissues by diffusion into the surrounding melted wax and the wax in turn diffuse into the tissues to replace it. At least one change of wax should be given in order to remove the clearing agent that has been displaced from the tissue and to ensure its replacement with pur wax. The exact number of changes of wax and the time which the tissue requires in each is dependent upon the density and size of the block of tissue and clearing agent used. The wax used should be of a suitable melting point. This varies with the nature of the tissue; hard tissue requires a higher melting point wax than soft tissue to give the necessary consistency and support as sections are cut. The waxes commonly used have melting points in the range between 50^0C and 60^oC, the most popular , suitable for both the English climate and most surgical and autopsy material, having a melting point of 58^oC.

Complete wax impregnation is necessary for the production of good sections, but if tissues are subjected to high temperatures beyond this point, over-hardening may result, which is thought by some to be detrimental to sectioning. On the other hand, inadequate impregnation leads to ultimate drying and shrinking of the embedded tissue block which, being inadequately supported by wax, cracks or crumbles when section-cutting is attempted.

The interior of the wax infiltration oven should be large enough to accommodate an enamel jug and funnel, fitted with Whatman No.1 filter paper for the filtration of new or reclaimed wax, and a number of glass containers of suitable size for the wax infiltration of tissues.

The storage and dispensing of molten paraffin wax is facilitated by the use of a wax dispenser . This is an electrically heated, temperature controlled, insulated tank with an integral outlet filter, heated tap, and

loose-fitting lid. Temperature is adjustable up to 70^{o}C and safety cut-out device operating at 90^{o}C prevents accidental over-heating of the wax with its attendant fire risk. Only new wax should be stored in the dispenser unless an additional filter, suitable for the reclamation of used wax, has been installed.

Tissu Density

Dense tissue require longer immersion in molten paraffin wax to ensure complete impregnation, and therefore structures , such as bone, fibromas and brain require approximately twice as long as soft tissue such as kidney or liver. The excessive hardness of dense tissues caused by this increased exposure to hot wax is (with the exception of brain and other CNS material) undersirable because of possible difficulties during section cutting. Complete wax infiltration of such tissues can be obtained without undue hardening by the use of the vaccum impregnation techniques.

Size of the Block Tissue

The amount of clearing agent carried over into the wax depends on the surface area of the tissue-block. When treating large pieces , the effects of this contamination may be minimised by frequent changing of wax. The time required for thorough impregnation depends on the thickness of the tissue; a piece of 5mm thick, for example, takes about 3 hours, whereas a piece of 10mm thick may take up to 10 hours.

Automatic Tissue Processors

These machines decrease both the time and labour necessary for processing tissue, and produce reproducible results. The decrease in the processing time is due to the constant agitation, the application of a vaccum and the use of raised temperatures which improves penetration and produces more consistent results. A variety of these machines is manufactured. Some act on the carousel principle, with tissue blocks in baskets being transferred from one container to another. Other designs have a single central chamber into which processing fluids are transferred. The machines are equipped with electronic timers and processors which allow flexibility of programming. These machines are usually equipped with a number of safety devices which warn for example of overheating or underheating.

Tissue Containers

Special containers made of either stainless steel or plastiic are provided. Some containers are designed with, one, two, four or six divisions and are supplied with close-fitting lids and with a choice of mesh sizes. Special baskets for curettings and fragmentary tissue are available. Plastic containers are also available. These are of value when processing tissues fixed in solutions containing mercuric chloride.

Processing Schedule for Automatic Tissue Processor
The processing schedule used with automatic tissue processors varies according to the type of tissue , the nature of the work, the clearing reagent used and personal preference.

3.6 Vaccum-Impregnation Technique

The vaccum-impregnation technique depends on the production of negative pressure above the specimens in the impregnating wax. This pressure reduction hastens the extrusion of air-bubbles and of the clearing agent from the tissue block, facilitating rapid penetration by the wax. Tissue processors normally have the facility to perform vaccum impregnation.

It is useful for the following tissues:

(i) Urgent biopsies
(ii) Dense tissue
(iii) Lung tissue
(iv) Tissue which contains a large amount of lipid.

Separate vaccum-impregnation baths(or vaccum-impregnation ovens) were extensively used in the past, and may still be used for special reasons such as the impregnation of large specimens with paraffin wax. A type in common use has a vaccum compartment which was a flat-bottomed brass chamber, with a heavy glass lid resting on a thick rubber washer, to create an air-tight junction. The vaccum chamber was immersed in a thermostatically controlled water -jacket.
A valve was fitted on one side of the chamber by means of which air could be admitted when the bath was under negative pressure. On the opposite side of the chamber was a small tube by which the interior was connected to the vaccum source which was either a venturi water pump or an electrically driven vaccum pump which produced a negative pressure of 400-500 mm of mercury.

3.7 Embedding

Moulds for Embedding
A variety of moulds are available for blocking out or embedding the tissue in paraffin wax.

Plastic Embedding Cassetes
Plastic embedding cassettes are disposable products which are available in a variety of sizes and colours. A flat portion of the cassette has a matt surface which can be used to label the block either with a graphite pencil or automated block-labelling machine.

The plastic cassettes are used in conjunction with stainless steel base moulds; normally as part of an embedding centre or workstation which includes a molten wax dispenser, together with hot and cold plates. A base mould of suitable size for the specimen is placed on the hot plate, and the tissue to be embedded is positioned carefully. The plastic cassette is placed in position and the paraffin wax poured in untill it reaches the top.

After cooling on the cold plate , the base mould is easily detached, leaving the embedded tissue ready for cutting. No trimming is necessary and the wax-filled plastic cassette serves as a block holder. Following sectioning, the blocks are stored in the plastic cassettes.

Plastic Ice-Trays

These have been used, with one block being embedded in each compartment. When set , the wax blocks are easily removed by flexing the plastic tray. This may be facilitated by smearing the inside of the mould with a little glycerol or liquid paraffin. Aerosol sprays of release agent are also available.

Watch Glasses

These are ideal for embedding fragmentary biopsies. While it is not essential to smear them with glycerol before use, it is a sensible precaution as the blocks are sometimes difficult to remove.

Technique for Embedding without an Embedding Center

(i) Fill a mould with molten paraffin wax
(ii) Warm a pair of blunt-nosed forceps(electrically heated forceps are normally used), and use them to transfer the tissue from the paraffin wax bath to the mould.
(iii) Re-warm the forceps and orientate the tissue untill it is lying in the desired plane. Run the warm forceps round the tissue to ensure that any wax which may have solidified during the transfer from the paraffin bath to the mould is melted.
(iv) Remove the corresponding label from the paraffin bath, and place it against the side of the mould adjacent to the tissue.
(v) Blow on the surface untill a thin film of wax has solidified.
(vi) Transfer the mould to a container of cold water, and immerse it gently. The mould should remain submerged untill the wax hardens. This may take 10-30 minutes, but solidification may be hastened by transferring the mould to running water.

Gelatin Embedding

As a general rule, tissue from which frozen sections are to be prepared is not embedded. The freezing of the tissue provides sufficient support for

sectioning. When frozen sections are required from tough or friable tissue, it can be embedded in a supporting medium to prevent fragmentation. The usual embedding medium for this purpose is gelatin, and when embedded the blocks of tissue are transferred to formalin in order to harden them. The formalin changes the structure of the gelatin from hydrosol to the hydrogel state.

Celloidin

Celloidin is the name given to a purified form of Nitrocellulose. It is of particular value as a histological embedding medium for sectioning hard tissues of a mixed consistency, for cutting very thick sections or when the minimum of shrinkage is required and the frozen section technique is not practicable.

Celloidin is usually supplied in the form of wool dampened with alcohol. The working strengths are 2.4 and 8 percent, the solvent being in equal parts of ether and alcohol.

Necoloidine, a similar compound , may be used in place of celloidin. It is supplied as a solution of about 8% of pyroxylin in ether-alcohol, but for use should be thickened to a 16% solution. Thickening is a simple matter, the solvent being allowed to evaporate in a fume-cupboard untill the volume has become reduced by approximately half. Evaporation is a constant problem when using celloidin and the working solutions should always be stored in bottles fitted with ground-glass stoppers. An ideal bottle for this purpose is a wide mouthed bottle, fitted with a ground-glass stopper and a ground-glass covering cap. It must be remembered that ether vapour is highly dangerous and celloidin should never be used in the vicinity of an open flame.

Celloidin Impregnation and Embedding Technique

Impregnation

(i) Dehydrate tissue through ascending grades of alcohol, ending with a bath of absolute alcohol containing copper sulphate.

(ii)

(iii) Transfer the tissue to a mixture of equal parts of alcohol and ether for 24 hours to speed up subsequent impregnation.

(iv)

(v) Transfer the tissue to a thin (2%) solution of celloidin for 5-7 days.

(vi) Transfer to a medium (4%) solution of celloidin for 5-7 days and then to a thick (8%) solution of celloidin for 2-3 days.

Embedding

(i) Half-fill a suitable embedding mould with thick(8%) celloidin and place the tissue in position, with the surface to be cut uppermost. Top up the mould with more of the embedding solution. The mould should be considerably deeper than the thickness of the tissue, in order to prevent the tissue from becoming exposed, as the celloidin shrinks on hardening.

(ii) Place the mould in a desicator containing ether vapour, in order to remove all air-bubbles. Immediately all air-bubbles are removed from the embedding medium , invert the tissue so that the surface to be cut is face downwards in the mould. This prevents any air-bubble from being trapped beneath the tissue.

(iii) Transfer the mould to a second desicator containing chloroform vapour, untill the celloidin is hardened to the required consistency. This can be tested by pressing the ball of the thumb(not nail) against the surface of the block, the celloidin being hard enough when no impression is left on the surface.

(iv) Remove the block from the mould and place it in pure chloroform. The block floats at first but eventually sinks to the bottom of the solution. When the block has sunk , transfer it to a solution of 70% alcohol untill required for cutting. The block may now be trimmed with the exception of the cutting surface.

Attaching the Block to the Holder

Celloidin blocks are attached to wooden or vulcanite holders which have deep serrations cut into them. The block holder is coated with medium(4%) celloidin and the trimmed block pressed firmly into position. Pressure is maintaned by means of a lead weight or by winding a piece of thread around the holder and the block.

After about 1 hour, during which time the block and holder can be returned to the chloroform desicator , the celloidin is set firm and the block and holder should be re-immeresed in 70% alcohol for 30 minutes. The cutting surface of the block may now be trimmed with a sharp knife.

It is a common practice to store both the blocks and holders in 70% alcohol untill all work on the sections is finished. Wooden blocks should therefore be made from a hard wood and should be soaked before use in order to ensure that discoloration of the alcohol and block does not occur.

Chloroform is not always used to harden the block. Hardening is then done very slowly by placing the mould beneath a bell jar and raising one side slightly, allowing the vapour to escape and the solution to thicken. When using this method, the edge of the bell jar that is raised must be

changed periodically to ensure that even evaporation takes place and should be lowered overnight and at weekends.

Necoloidine is used in a similar manner to celloidin, but the impregnating solutions are twice as thick , 4%, 8% and 16%. The tissue is embedded in the stock solution, thickened as described in the celloidin.

4.0 CONCLUSION

Dehydration, Impregnation Embedding techniques have fully been discussed, the selection of tissue, paraffin wax technique, alcohol method and other fixative fluids, clearing and the reagents involved, impregnation and embedding and the reagents involved. I therefore , urge you to take your time and go through all to get the knowledge.

5.0 SUMMARY

This unit involves tissue processing, mentioning all the techniques, reagents and chemicals that are used in tissue processing. The dehydrating , clearing , fixing , impregnating and embedding agents and solutions mentioned. Try as much as possible to go through them for your benefit.

6.0 TUTOR-MARKED QUESTIONS

Write short note on the following:

1. Selection of tissue for processing
2. Alcohol method of dehydration
3. Xylene as a clearing agent
4. Automatic tissue processors
5. Plastic embedding cassettes
6. Plastic Ice-trays
7. Watch glasses

7.0 FURTHER READINGS/REFFERENCE

Burtis, Cart A; Ashwood, Edward R ; Bruns, David E(2006): Tietz textbook of Clinical Chemistry. 4th edition; Saunders .

Carson, Freida L; Christa Hladik(2009): Histotechnology. A self-instructional Text . 3rd edition. Hong; Am. Soc. For Clinical Pathology.

Robbins, Stanley (2010): Robbins and Cotran Pathology basis of disease 8th edition, Philedelphia. Saunders /Elsevier

Cai, H; Casewell JL; Prescott JF(2014): Non culture molecular Techniques; A Diagnostic Laboratory Perspective .

Ochei.J; Kolhatkar.A. (2008): Medical Laboratory Science; Theory and Practice.

Baker.F.J.; Silver.R.E; Palister.C.J.(2011) : Introduction to medical Laboratory Technology. 7th edition; Bounty Press Limited

MODULE 4 HAEMATOLOGY AND BLOOD GROUP SEROLOGY LABORATORY

UNIT 1 HAEMATOLOGY LABORATORY

CONTENTS

1.0 INTRODUCTION

Haematology is the scientific study of the blood. The main functions of the haematology laboratory are to detect blood disorders such as anaemia, leukaemia; to assist in the accurate characterization and diagnosis of these disorders so that appropriate treatment can be given and to monitor the progress of patient treatment.

2.0 OBJECTIVES

At the end of this study, you will be able to :

- define haematology laboratory,
- know how to collect blood samples
- types of stains in use in haematology laboratory
- know various techniques /methods involved in haematology laboratory

3.0 MAIN CONTENT

3.1 Haematology Laboratory

Haematology is the study of the morphology and physiology of blood. The haematology laboratory set-up in a healthcare setting is concerned with the diagnosis and monitoring of diseases of the blood and blood-forming organs. Biomedical scientists working in a haematology laboratory perform an array of blood tests that investigate the cellular elements of blood and a number of proteins including haemoglobin and clotting factors. Haematological tests are performed on blood samples to diagnose diseases such as leukaemia, anaemia . On any given day, working as a member of a team, a haematologist will be required to use their training to work in a variety of healthcare environments. These may include haematology, homeostasis.

3.1.1 Collection of Blood Samples in Haematology Laboratory

Inside the body (in vivo), blood is in the liquid form. But outside the body (in vitro), it clots within a few minutes. If left undisturbed in a tube, this clot begins to retract or shrink, and a During this process of coagulation or clotting, certain factors or constituents in the blood are used up and most of the cells (white, red, and platelets) are trapped in the clot . Such a blood sample is totally unsuitable for haematological investigations. Therefore, it is necessary to prevent coagulation of blood by using anticoagulants. If the anticoagulated, well mixed blood is centrifuged, it separates into three (3) main layers. The bottom layer consists of packed red cells which normally makes up for about 40-47% of the total blood volume. A thin whitish layer appears on top of this layer and normally makes up about 1% of the volume. This layer is called, buffy coat, and contains leucocytes and platelets. The uppermost liquid layer makes up about 52-57% of the total volume. This pale yellow fluid is the plasma. In a normal, healthy individual, these three constituents are in a state of equilibrium in relation to the demand and supply of the body functions. This state of equilibrium is known as homeostasis. Blood samples for haematological study are usually obtained either by finger puncture (capillary blood) or venipuncture (venous blood).

3.1.1.1 Collection of Capillary Blood

Capillary blood can be used with satisfactory results for differential blood counts, for from the tip of the finger from adults, and from the heel or the large toe from the infants. However, the use of capillary blood should be avoided as far as possible because of the high risk of sampling error and of infection. Repeat testing is usually restricted

because of the smallness of the quantity of blood collected. Capillary blood should be used only when the venous blood is not advisable, for example, in new-born infants, burn cases, amputees or in patients whose veins prove to be difficult to locate.

Technique: Select an appropriate site for puncture. The ball of the middle finger is usually satisfactory. Clean the area vigorously with 70% alcohol and allow it to dry.

This disinfects the skin and promotes circulation. For skin puncture, various types of disposable lancets are available. Use of non-disposable lancets is not recommended because of the risk of cross-infections. Make a firm, quick stab with the lancet simultaneously applying a little pressure, this ensures a free flow of blood. Wipe away the first one or two drops of blood using a dry cotton swab. Carefully draw blood into an appropriate pipette (eg; haemoglobin, RBC or WBC pipette) by applying gentle suction through the mouth-end of the rubber tubing attached to the pipette. Draw blood exactly up to the mark, avoid air bubbles. Wipe the outer surface of the pipette and deliver the blood into an appropriate diluents by blowing slowly. Mix to prevent coagulation.

3.1.1.2 Collection of Venous Blood

The most commonly used sites for venipuncture are the veins inside the bend of the elbow (the antecubical fossa) . The three main veins in this area are the cephalic, median cubital and median basilica veins. Other sites such as the veins in the wrist or ankle may be used if necessary.

Technique
Apply a tourniquet to the upper arm sufficiently tightly to restrict the venous flow and make the veins stand out. The patient should be asked to keep the arm straight and clench the fist. Usually, the veins are obvious by this time. It is advisable to feel the veins so that the most suitable one can be selected. A little tapping or gentle messaging the arm from the wrist to the elbow help in dilation of the veins. Swab the selected vein and site with 70% alcohol and allow it to dry.

Prepare the syringe and appropriate containers. Usually a 21 gauge needle is appropriate for very fine veins, 22 or 23 gauge needles may be used only if necessary. Using the left thumb, press just below the puncture site to anchor the vein. Insert the needle smoothly with the bevel facing upwards, at an angle of 20degrees to 30degrees to surface of the arm, and in a direct line with the vein. When the needle has entered the vein, blood is withdrawn into the syringe and tourniqued released. When a sufficient quantity of blood is collected, loosen the tourniqued, place a wad of cotton wool at the puncture site and withdraw

the needle gently. The puncture site should be kept pressed to stop the flow of blood. Ask the patient to release the clenched fist. Detach the needle and discard in an appropriate disposal container. Dispense the blood in the sample tubes as required. Mix the blood to be anticoagulated. Apply a strip dressing at the puncture site.

The investigations in haematolgy laboratory include Complete Blood Count (CBC), under the heading; complete blood count may vary in different laboratory set-ups, but generally include the following:

(a) Estimation of Haemoglobin (Hb) .
(b) Total red cell count
(c) Packed cell volume (PCV)
(d) Red cell indices
(e) Total white cell count
(f) Peripheral blood smear examination including the Differential Leucocyte Count (DLC) In some cases, other tests may also be included:
(g) Total eosinophil count
(h) Platelet count
(i) Reticulocyte count

3.1.2 Estimation of Haemoglobin

The purpose of estimating haemoglobin is to determine the oxygen carrying capacity of blood. Haemoglobin estimation also helps in citing changes in the haemoglobin concentration before and after operation and blood transfusion. Several techniques have been used to determine haemoglobin in the form of oxyhaemoglobin, carboxyhaemoglobin, cyanmethaemoglobin, acid and alkaline haematin, based on their oxygen carrying capacity, iron content or colour.

Haemoglobin is expressed as grams per litre, grams per 100 ml or grams per deciliter (dl) of blood. A major problem in accurate measurement of haemoglobin content is the non -availability of a suitable standard. Some visual methods, such as Sahli’s, use artificial standard. Recommended Cyanmethaemoglobin, prepared according to strict specification, as a suitable standard. Cyanmethaemoglobin is very stable and the standard is available commercially.

3.1.2.1 Cyanmethaemoglobin Method

This is a colorimetric method, and therefore, more accurate than Sahli’s. A commercial cyanmethaemoglobin standard is used for comparison.

Principle
The haemoglobin is treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium hydrogenphosphate. The ferricyanide forms methaemoglobin which is converted to cyanmethaemoglobin by the cyanide . All forms of haemoglobin except sulfhaemoglobin are converted to cyanmethaemoglobin .

ReagentsPotassium cyanide----------500mg
Potassium ferricyanide----------200mg
Potassium dihydrogen phosphate----------140mg
Distilled water----------1 litre
pH----------7.0-7.4
Store in a dark bottle. The solution keeps for several months. (2) Cyanmethaemoglobin Standard: It is available commercially in sealed ampoules. The concentration is indicated on the label and is usually about 60mg per 100 ml.

Technique: Add 0.02 ml (20ul) of blood to 5 ml of Drabkin's solution in a test tube (1:250 dilution) . Mix well and allow standing for 10 minutes. Read the absorbance colorimetrically at 540 nanometers (green filter) with Drabkin's solution as blank. Read the absorbance of the standard in the same way.

3.1.3 Haemocytometry (Counting of Cells in Blood)

Counting of blood cells, such as red blood cells (RBCs), White blood cells (WBCs) and platelets is a fundamental measurement in a haematology laboratory. These cells can be counted either manually by microscopy or with the help of automated electronic devices.

Manual Cell Counts
The manual method of counting involves :

(i) dilution of blood in an appropriate diluting fluid
(ii) use of haemocytometer to count the number of cells per unit volume of blood ie one cubic millimeter(mm3, ul) or 1 litre(L).

Use of Counting Chamber (Haemocytometer) for Cell Counting
A haemocytometer consists of a counting chamber , a coverglass for the counting chamber and the diluting pipettes . Many types of counting chambers are available. Improved Neubauer and Fuchs Rosenthal are the two most commonly used chambers in Laboratories.

Charging the Counting Chamber for Cell Counts
The counting chamber should be set up correctly so that depth of the counting chamber is uniform. To facilitate this , moisten the raised

shoulders of the chamber and slide the coverslip onto the shoulders with both thumbs . If correctly placed, the coverslip should not off even after inverting the counting chamber and should show rainbow colours (Newton'srings) .

To fill up the chamber, care must be taken that the diluted blood does not overflow into the H, shaped trough around the counting surface.
Fill the counting area by touching a drop of well mixed diluted blood to the edge of the coverglass and the fluid is drawn in by capillary action. It isnecessary to charge both the counting areas. Before counting, allow the cells to settle in a moist chamber. A simple moist chamber can be prepared by placing a wet cotton pad or filter paper in a petri dish.

Counting of Cells
While counting the cells in the squares include those that touch the lines on the left side or on top of the squares, and exclude those that touch the lines on the right side and at the bottom of the square . For squares outlined with a triple set of lines, the central line denotes the boundary of the square . This system of counting avoid the chances of a cell being counted twice or of being omitted.

3.1.4 Haematocrit or Packed Cell Volume (PCV)

The PCV or Haematocrit is a percentage of the total volume of whole blood occupied by packed red blood cells, when a known volume of whole blood is centrifuged at a constant speed for a constant period of time. Value thus obtained is used in the determination of the red cell indices; mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC); and Mean corpuscular volume (MCV).

Microhaematocrit Method
For this method, special non-graduated glass capillary tubes are used . These tubes are 7cm in length and 1mm in internal diameter. They can be purchased pre-coated internally with dried heparin for capillary blood. Plain capillaries (without heparin) can be used for anticoagulated blood specimens. (Plain capillary tubes may be coated with heparin by filling them with 1: 1000 dilution of heparin and drying at 56C).

Fill the capillary tube two-thirds full of either with well mixed venous blood or directly from a capillary puncture. Seal one end of the capillary tube with modeling clay (plasticine) . The filled tubes are then placed in the microhaematocrit centrifuge and spun at 12,000g(rpm) for 5 minutes .Place the spun tube into a specially designed scale , and read the PCV as a percentage .

3.1.5 Erythrocyte Sedimentation Rate (ESR)

ESR, though not part of complete blood count, is usually requested with it. The basic principle of the ESR is based on the fact that blood is essentially a suspension of formed elements such as red and white blood corpuscles in plasma. Therefore, when whole blood is mixed with an anticoagulant and placed in a perpendicular tube, the red blood cells sink to the bottom because they are heavier than the plasma in which they are suspended. The speed at which the red blood cells in normal blood settle is relatively slow.

However, in many diseases, eg; inflammatory or degenerative cell diseases, changes occur in physicochemical properties of the plasma. These changes include alterations in the positive charges of the plasma colloids, increase in plasma fibrinogen,and variation in concentration of plasma protein fractions and in the ratio of the various plasma protein fractions to one another. Changes may also occur in the erythrocyte surface. Change in the surface electric charge of the red blood cells causes erythrocytes to aggregate, clump, or to form rouleaux. The large clumps of cells thus formed fall at a faster rate . The changes in the proportion of the soluble constituents of plasma such as increased fibrinogen or globulin also results in increased rate of erythrocyte fall
.
Sequentially, there are three stages in erythrocyte sedimentation:

(i) The initial period of a few minutes, during which rouleaux formation occurs
(ii) A period of approximately 30 to 120 minutes, depending on the tube length, during which sedimentation occurs at a fairly constant rate.
(iii) A period of slower rate of fall during which packing of the sedimented red cell column occurs. For these reasons, the sedimentation rate test must be set up within 2 to 6 hours of blood collection.

Westergren Method
Requirements

(i) Westergren ESR tube: This tube looks similar to a one ml pipette. It is 300mm long with an internal diameter of 2.5 mm. It is graduated from the base over a 200mm scale in mm divisions.

Technique: Aspirate the blood into the westergren tube to the top mark (0). The tube is then stood vertically in a stop rack with spring clips that hold the lower end of the pipette tightly against a rubber mat in a vertical position. Leave for 1 hour, then note the readings, noting the level of red cells from 0 at the top.

4.0 CONCLUSION

The word haematology literally means study of blood and in a clinical set-up, a haematology laboratory is concerned with the abnormalities of the constituents of blood namely the plasma and the blood cells . For this reason haematology is an essential and integral part of a full health set-up that will always contribute much to the diagnosis of diseases especially those related to blood.

5.0 SUMMARY

This unit has looked briefly into haematology; haematology . A number of things have been considered including the meaning of haematology, collection of blood samples from the veins, capillaries; estimation of haemoglobin using cyanmethaemoglobin method; counting of cells in blood; Packed cell volume (PVC); Erythrocyte sedimentation rate(ESR) using westergren method; . Please sit down and study them for your own good.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define Haematology laboratory.
2. Discuss the technique in use in the collection of blood from blood capillary.
3. The investigations in haematology laboratory include Complete blood count.
4. Discuss the estimation of Haemoglobin using cyanmethaemoglobin method

7.0 REFERENCES/FURTHER READING

Ochei.J.O.; Kolhatkar.A.A. (2008): Medical Laboratory Science; Theory and Practice.

Baker.F.J.; R. E.Silverton.R.E; Pallister.C.J. (2011): Introduction to Medical Laboratory Technology. 7th edition.

Srivastava.A; Koul.V; Dwivedi.S.N; Upadhyaya.A.D; Ahuja.A and Saxena.R. (2014): Performance analysis of newly developed point-of-Care hemoglobinometer (TrueHb) against an automated hematology analyzer . *Int. J. Lab. Hematology*; 37 (4) : 431-575 .

UNIT 2 BLOOD GROUP SEROLOGY (IMMUNOHAEMATOLOGY)

CONTENTS

1.0 INTRODUCTION

Blood group serologymainly involves the detection of antigens and antibodies. Each species of animals, including humans, has certain inherited antigens on the surface of the red cells which are unique for that species. These are known as isoantigens . Similarly, certain antigens the alloantigens are common to some, but not all, members of that particular species .Blood group serology involves the detection of these antigens and their antibodies

2.0 OBJECTIVES

Having gone through this course material, you will be able to:

- define blood group serology
- know how to collect blood samples
- know how to do simple blood grouping
- know various techniques /methods involved in blood grouping and serology.

3.0 MAIN CONTENT

3.1 Blood Group Serology Laboratory

Each species of animals, including humans, has certain inherited antigens on the surface of the red cells which are unique for that species. These are known as is oantigens. Similarly, certain antigens, the alloantigens are common to some, but not all, members of that particular

species .Blood group serology involves the detection of these antigens and their antibodies.

3.2 ABO Blood Group System

The ABO system is the most important blood group system in blood transfusion and organ transplantation because of two unique features:

(i) Strongly reactive antibodies are present in the serum of individuals who lack the corresponding antigens
(ii) A and B antigens are present on many tissue cells in addition to the red cells .

The ABO systems consists of four blood groups or phenotypes: A, B, AB, and O . The two antigens A and B are responsible for these four groups. If A antigen is present on the red cell, the individual is said to belong to group A, those having B antigen are group B persons. Group AB individuals have both A and B antigens while group O persons have neither A nor B antigen on their red cells. Three allelic genes, A, B and O can be inherited in the ABO system . The following combinations of alleles is possible; AA, AO, AB, BB,BO, OO, resulting in A, A, AB, B, B and O group individuals respectively. This is so because A and B genes are dominant and O gene is recessive.

3.3 ABO Grouping

Determination of ABO group is necessary both for the donors as well as the recipient, preliminary to blood transfusion. The blood group may be determined by:

(i) Detecting the antigen on the red cells
If an individual has A antigen on his red cells, he is said to belong to group A, those having AB and those who have neither A nor B antigen on their red cells belong to group O. This process of grouping is known as cell grouping.

(ii) Detecting antibodies in the serum :
According to Landsteiner's rule, corresponding antigens and antibodies cannot co-exist in the same person's blood. For example, person's who are blood group A cannot have antibodies to antigen A (anti-A) in their blood, and group B persons cannot have anti-B in their blood. However, for ABO system, natural antibodies are present in the blood of every individual. Therefore, group A person will not have anti-A in his serum, but will possess anti-B because B antigens is absent on his red cells. This process of grouping is known as serum grouping. This can be summarized thus: Blood group Antigen on red cells Antibodies in serum

	A	A
Anti-B		
	B	B
Anti-A		
	AB	AB
None		

ABO Grouping Methods
Method I: The Tile Method

Reagents:
(i) ABO grouping antisera : Anti-A, Anti-B and Anti-AB
(ii) ABO control cells (10% suspension) : A2 cells, B cells, O cells.

Specimen:
(i) 10% suspension of patient's cells in saline
(ii) Patient's serum.

3.4 Technique

(i) Mark a clean white tile with 16 wells as shown below:
(ABO Grouping-Tile Method)

Anti-A I	Anti-B I	Anti-AB I	Patient's serum I
Patient's cells	Patient's cells	Patient's cells	Patient's cells
A2 cells	A2 cells	A2 cells	A2 cells
B cells	B cells	B cells	B cells
O cells	O cells	O cells	O cells

(ii) Place one drop of Anti-A serum in all the wells of row 1, one drop of Anti-B serum in row 2, one drop of Anti-AB serum in row 3 and one drop of patient's serum in row 4.
(iii) Place one drop of 10% suspension of patient's cell in all the wells of the horizontal row 1, 10% A2 cells in row 2, 10% B cells in row 3, and 10% O cells in row 4.
(iv) Mix the cells and the serum in each well using a separate applicator stick.
(v) Allow to stand at room temperature for five minutes
(vi) Rock the Tile gently and read the results in the control and test wells macroscopically.

Observe for agglutination (clumping) as follows:
- If agglutination occur with Anti-serum A, the blood group is A
- If agglutination occurs with Anti-serum B, the blood group is B

- If agglutination occur with both Anti-serum A and Anti-serum B,

And Anti-serum AB, the blood group is AB

- If no agglutination in Anti-serum A, Anti-serum B, and Antiserum

AB, it is blood group O

- If agglutination occur with Anti-serum D (Rhesus) then the blood us Rhesus Positive, if no agglutination in Anti-D, it is Rhesus Negative.

Hence the following blood groups are possible:

(i) Blood group A +ve or A –ve
(ii) Blood group B +ve or B –ve
(iii) Blood group AB +ve or AB –ve
(iv) Blood group O +ve or O –ve

4.0 CONCLUSION

Blood group serology involves the detection of antigens and their antibodies . For this reason blood group serology laboratory is an essential and integral part of a full health set-up that will always contribute much to the diagnosis of blood group related problems.

5.0 SUMMARY

This unit has looked briefly into blood group serology laboratory. A number of things have been considered including the meaning of blood serology, ABO blood group systems; detecting antigens and their antibodies . You are advised to sit down and read through for your own good.

6.0 TUTOR-MARKED ASSIGNMENT

Discuss ABO grouping illustrating your points with diagram.

7.0 REFERENCES/FURTHER READING

Ochei.J.O.; Kolhatkar.A.A. (2008) : Medical Laboratory Science; Theory and Practice.

Baker.F.J.; R. E.Silverton.R.E; Pallister.C.J. (2011) : Introduction to Medical Laboratory Technology. 7th edition.

Srivastava.A; Koul.V; Dwivedi.S.N; Upadhyaya.A.D; Ahuja.A and Saxena.R. (2014): Performance analysis of newly developed point-of-Care hemoglobinometer (TrueHb) against an automated hematology analyzer .*Int. J. Lab. Hematology*; 37 (4) : 431-575 .

UNIT 3 CLINICAL CHEMISTRY LABORATORY

CONTENTS

1.0 INTRODUCTION

Clinical chemistry, also known as Chemical Pathology, Biochemistry or Diagnostic Chemistry, is the division of pathology that deals with observation and investigation of biochemical changes in the human body . It involves the detection and measurement of the biochemical constituents of body fluids and excretions. of The field of clinical chemistry today embraces a knowledge of Physiology, Medicine, Statistics, Electronics and other specialties necessary to provide analysis and answers to the wide variety of chemical changes in the body.

2.0 OBJECTIVE

At the end of this unit you will be able to:

- define clinical chemistry laboratory
- know some principles and techniques involves in the clinical chemistry lab.

3.0 MAIN CONTENT

3.1 Clinical Chemistry Laboratory

Clinical chemistry (also known as chemical pathology, clinical biochemistry or medical biochemistry) is the area of clinical pathology that is generally concerned with analysis of bodily fluids.

The discipline originated in the late 19th century with the use of simple chemical tests for various components of blood and urine. Subsequent to this, other techniques were applied including the use and measurement of enzyme activities, spectrophotometry, electrophoresis, and immunoassay.

Most current laboratories are now highly automated to accommodate the high workload typical of a hospital laboratory. Tests performed are closely monitored and quality controlled.

All biochemical tests come under chemical pathology. These are performed on any kind of body fluid, but mostly on serum or plasma. Serum is the yellow watery part of blood that is left after blood has been allowed to clot and all blood cells have been removed. This is most easily done by centrifugation, which packs the denser blood cells and platelets to the bottom of the centrifuge tube, leaving the liquid serum fraction resting above the packed cells. This initial step before analysis has recently been included in instruments that operate on the "integrated system" principle. Plasma is in essence the same as serum, but is obtained by centrifuging the blood *without* clotting. Plasma is obtained by centrifugation *before* clotting occurs. The type of test required dictates what type of sample is used.

A large medical laboratory will accept samples for up to about 700 different kinds of tests. Even the largest of laboratories rarely do all these tests themselves, and some must be referred to other laboratories.

3.2 Roles of Clinical Chemistry Laboratory

Chemical chemistry (also known as clinical biochemistry or chemical pathology) is the study of chemical and biochemical mechanisms of the body in relation to disease, mostly through the analysis of body fluids such as blood or urine.

In many diseases there are significant changes in the chemical composition of body fluids such as the raised blood enzymes due to their release from heart muscles after a heart attack; or a raised blood sugar in diabetes mellitus due to lack of insulin. Tests are designed to detect these changes qualitatively or quantitatively compared to results from healthy people.

Clinical chemists use a wide range of analytical techniques for example, molecular diagnostics, measurement of enzyme activities, spectrophotometry, electrophoresis, the separation of molecules based on physical characteristics and immunoassays The work involves manual techniques for which the biomedical scientist develops complex

practical and interpretive skills, through to operation and management of highly automated testing systems capable of producing thousands of results an hour. All assays that are closely monitored and quality controlled.

Tests that require examination and measurement of the cells of blood, as well as blood clotting studies, are not included as these are usually grouped under haematology.

Typical Roles
A clinical chemistry department within a hospital provides a link between front line clinical staff and the basic sciences employing analytical and interpretative skills to aid the clinician in the prevention, diagnosis and treatment of disease.

Diseases such as heart attacks, kidney failure, viral and bacterial infection, infertility, diabetics, high cholesterol, thyroid problems or measuring drug levels to make sure people are on the best dose are some of the many areas where the Clinical Chemistry Laboratory becomes involved in a persons. Some regional laboratories are involved in screening services such as phenylketonuria and cystic fibrosis in newborn babies, genetic screening and screening for illegal drug use.

3.3 Some Analytical Instruments in Clinical Chemistry Laboratory

Colorimetry
Colorimetry, in simple terms, is the measurement of colours, and is probably the most widely used method for determining the concentration of biochemical compounds. This important laboratory procedure is based on the principle that when white light passes through a coloured solution, some wavelengths are absorbed more than others. Many compounds, though, not coloured themselves, can be made to absorb light in the visible spectrum by reaction with suitable reagents. The coloured compounds absorb light at given wavelength at visible spectrum. The extent to which a solution absorbs light depends on the intensity of its colour.

Light travels in form of waves. Wavelength is the distance between two wave peaks in nanometer (nm) . Amplitude is the height of each wave. The number of waves passing a fixed point x per unit time is the frequency. The wavelength of light is expressed as:

$$\frac{\text{Velocity of light}}{\text{(Distance travelled by light/second)}}$$
$$\text{Frequency}$$

The wavelength of light determines the colour of the light seen by the naked eye. White light is a combination or mixture of light energy of different wavelengths. Any colour that is seen emits light of a particular wavelength, and is called monochromatic light.

Wavelengths between 400nm and 700nm form the visible spectrum of light which is the visible band of light of electromagnetic spectrum. Wavelengths of about 700nm are visible to the eye as red colours while those of shorter wavelength give rise in turn to colours, orange, yellow, green, blue and finally, violet, which has a wavelength of about 400nm . Wavelengths greater than 700nm have vibrations known as infrared and are not visible to the eye; wavelengths shorter than 400nm have vibrations known as ultraviolet and are also not visible.

spectrophotometer

This is an instrument used to measure absorbance at various wavelengths. It is similar to absorptiometer except that it uses diffraction gratings or glass prism to produce monochromatic light. There are two types of spectrophotometers;

(i.) Single beam spectrophotometer; It operates between 325-1000nm wavelength; using a single source of light, eg; tungsten filament lamp. It has two photocells. The light travels along only one pathway. The test solutions and blank are read in the same position.

(ii) Double beam spectrophotometer: It operates between wavelength range 185-1000nm. It has two light sources and two photocells. This instrument splits the light from the monochromatic into two beams. One beam is used for reference and the other for sample reading. It eliminates errors due to fluctuations in the light output, and the sensitivity of the detector. This is because the final reading is derived from the difference between the intensities of test and beams.

Chromatography

This is a process of separation of a mixture of solutes dissolved in a common solvent. The separation technique makes use of the differential distribution of the solutes between two phases, the mobile phase and the fixed stationary phase. The solvent is the mobile phase which carries the mixture of the solutes through the stationary phase.

Depending on the characteristics of the solute molecules, the mobile phase and the stationary phase, there is selective retardation of the solute molecules relative to the moving phase.

Under ideal conditions, the resulting different rates of migration bring about complete separation of the solutes. Chromatographic methods can be classified according to physical state of the solute carrier phase. The two main categories are Liquid chromatography in which solute phase is a liquid or solution. And Gas chromatography, in which the solute phases are in a gaseous state. Further classification is based on the form of stationary phase matrix. Liquid chromatography is subdivided into flat and column methods. In flat methods, the stationary phase is supported on a flat surface such as cellulose acetate paper or in a thin layer mechanically supported with glass or plastic. For column methods, in both liquid and gas chromatography, various types of materials such as ion-exchange resins, diatomaceous earth or internally coated fine glass capillaries may be used.

3.4 Mechanism of Separation in Chromatography

There are three mechanisms by which separation occurs in chromatography .They are adsorption, partition and exclusion chromatographs.

(i) **Adsorption chromatography;** In adsorption chromatography, the components within a sample are separated because of the differences in their attraction to the stationary phase and mobile phase. The speed of migration of a component depends on its adsorptive affinity relative to other components. The example of this technique is the thin-layer chromatography (TLC) . In ion-exchange chromatography, electrostatic forces operate between changed molecules and oppositely charged particles on the ion-exchange resin or modified cellulose or dextran. These electrostatic forces can be altered by changing the pH of the mobile phase.

(ii) **Partition chromatography;** Partition chromatography utilizes differences in the relative solubility of the solute molecules between mobile and stationary phases. The two phases may be liquid-liquid or gas-liquid. This methodology is used for gas liquid chromatography (GLC) and for high performance Liquid chromatography (HPLC).

(iii) **Exclusion chromatography (Gel-filtration or gel-permeation chromatography).**
When a mixture of small and large particles is allowed to pass over porous solid particles, the smaller molecules or ion pass through the pores of the solid particles. The pore size determines which molecules are able to enter the particle and which are excluded. The particles with large molecular size, such as proteins, are excluded more easily than other smaller particles.

Types of Chromatography in Routine Use

(i) Paper Chromatography: In paper chromatography, the stationary phase is paper, usually cellulose acetate, and mobile phase is a solvent in which the solutes in the mixture are soluble. Initially a mixture of substances; eg; carbohydrates, is spotted at one end of the porous filter paper strip. In ascending type of paper chromatography, the filter paper is hung vertically into the solvent. The solvent moves up through the paper by capillary action. As it passes through the spotted area of solutes, various fractions in the mixture move at different rates. The rate of mobility is affected by relative solubilities and polarities of the solutes and the polarity of the solvent. The paper is removed after the separation, dried and sprayed with a chemical for colour development. The spots of the solutes developed at different sites can be quantitated by the area and intensity of the colour. Paper chromatography has been employed most commonly for the separation of amino acids and carbohydrates.

(ii) Thin-layer chromatography (TLC); TLC is very similar to paper chromatography except that the paper is substituted by a thin layer(0.025mm) of very finely powered silica gel, alumina, polyacrylamide gel, starch gel or kieselguhr, bound to a glass or plastic plate . The principle of separation is similar to that of paper chromatography. The advantages of TLC over paper chromatography include easier elution of separated spot from the plate by cutting the thin layer, and faster separation of constituents.

(iii) Ion-exchange Cchromatography; Ion-exchange resins are suitable polymers which contain ionic groups as a part of their structure. Ion-exchange resins can be, cation exchangers, eg; with SO3- or COO- groups; or anion exchangers, eg; with N+(NH3)3 group . If a suitable ion-exchange resin is used for column packing, its affinities for the members of a group of ionized substances can be used for their separation. The ion-exchange resins are extensively used for the separation of amino acids. If a solution containing mixture of amino acids is applied to a column of suitable ion-exchange resin, the amino acids are linked to the resin. The degree of affinity, however, varies with the amino acids. If , after the adsorption, the column is washed or eluted by a series of buffers of varying PH, the individual amino acid can be separated and quantified with ninhydrin .

(iv) Gas chromatography: Gas chromatography is a separation process by which a mixture of compounds in a gaseous state is passed through a gas in a stationary phase. The mobile phase is usually nitrogen, helium or argon. Separation of the mixture is achieved by a difference in the partitioning of the various molecules between the two phases. If the stationary phase

consists of a thin layer of non-volatile liquid, the technique is called gas-liquid chromatography (GLC), If the stationary phase is a solid sorbent, then it is called gas-solid chromatography (GSC) . A gas chromatography has six components;

(i) Pressurized carrier gas with a flow regulator
(ii) Sample injection area
(iii) A column: gas, liquid or solid
(iv) A detector (v) A recorder (6) A thermo regulated component enclosing the injection area, column and detector.

Working at a sufficiently high temperature, the components of a mixture to be injected are in a gaseous phase. Each component of the mixture requires specific time to reach the detector after application to the column. The output of the detector is displayed on a recorder. This, retention time, may be expressed as an absolute time or as a relative retention time with reference to a standard.

High Performance Liquid Chromatography (HPLC)
In HPLC, the stationary phase is composed of uniform, ultrafine particles which greatly increase its adsorptive area. This stationary phase is packed firmly into a column. The resistance to flow in this column is high, therefore, large pressure (500-5000pounds per square inch) are required to deliver constant flow rates. Theelute from the column is monitored by a variety of detectors such as UV or redox-potential electrode detectors. HPLC is a very efficient technique for the detection and quantitation of drugs.

3.5 Electrophoresis

The movement of charged particles such as molecules or ions through an electrolyte under the influence of electric current is called Electrophoresis**.** The electric field is applied to a solution by placing two oppositely charged electrodes in the solution. Depending on the nature of charges, the molecules move through the solution towards the electrodes of the opposite charge. The positively charged particles, the cations, move towards the negatively charged electrode (cathode); and negatively charged particles, the anions, move towards the positively charged electrode (anode). At a fixed pH, the rate of migration of particles with similar charge will depend on the magnitude of charges carried on them and molecular weight; or charge/size ratio. Because of these variations in migration, it is possible to separate a complex mixture such as plasma proteins into a number of fractions or zones. This is called zone electrophoresis.

Equipment and Materials

The equipment and materials needed for electrophoresis generally consist of a buffer system, a simple applicator, a solid medium such as paper, cellulose acetate, polyacrylamide gel, starch gel, or agar gel, an electrophoresis tank and a power supply. Stains and washing solutions may be necessary for staining and elution of separated fractions.

General Technique for Electrophoresis

(i) Place a hydrated support medium such as cellulose acetate or agar gel into the electrophoresis chamber.
(ii) Place the buffer of appropriate pH in the electrode chambers of the electrophoresis tank.
(iii) Keep the support medium in contact with the buffer, for example, by using paper wicks.
(iv) Apply the sample to the support medium.
(v) Conduct electrophoresis for a determined length of time using either constant voltage or constant current.
(vi) Remove the support from the chamber immediately and dry or place it in the fixative to avoid diffusion of sample components.
(vii) Stain the components, wash the excess dye and dry or place in a cleaning agent.

4.0 CONCLUSION

The field of clinical chemistry embraces a knowledge of physiology, medicine, statistics, electronics and other specialties necessary to provide analysis and answers to wide variety of chemical changes in the body and to some extent have been discussed in this unit, for this reason I, urge you to put more effort and get the knowledge.

5.0 SUMMARY

In this unit, clinical chemistry laboratory has been defined and various methods and techniques of analysis mentioned and discussed in details; roles of clinical chemistry including some analytical instruments have also been discussed, therefore, I urge to do the needful for your own good.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define Clinical Chemistry Laboratory
2. Describe two instruments/equipment seen in the Clinical chemistry laboratory.
3. Write short notes on:
   (i) Volume of Urine
   (ii) Colour
   (iii) Appearance

7.0 REFERENCES /FURTHER READING

Burtis, Cart A; Ashwood, Edward R ; Bruns, David E(2006): Tietz textbook of Clinical Chemistry. 4th edition; Saunders .

Carson, Freida L; Christa Hladik(2009): Histotechnology. A self-instructional Text . 3rd edition. Hong; Am. Soc. For Clinical Pathology.

Robbins, Stanley (2010): Robbins and Cotran Pathology basis of disease. 8th edition, Philedelphia. Saunders /Elsevier

Cai, H; Casewell JL; Prescott JF(2014): Non culture molecular Techniques; A Diagnostic. Laboratory Perspective .

Ochei.J; Kolhatkar.A. (2008): Medical Laboratory Science; Theory and Practice .

Baker.F.J.; Silver.R.E; Palister.C.J.(2011) : Introduction to medical Laboratory Technology. 7th edition; Bounty Press Limited

UNIT 4 COLLECTION OF SPECIMEN IN CLINICAL CHEMISTRY AND TECHNIQUES

CONTENTS

1.0 INTRODUCTION

Chemistry analysis is usually performed on serum or plasma samples. To optimize results of testing, serum or plasma should be separated from cells as soon as possible after collection to minimize artifacts that occur with storage. Note that some chemistry tests can also be run on urine (e.g. protein to creatinine ratios, fractional excretion of electrolytes, urinary GGT measurement for renal injury) and ocular fluids (usually done as a post-mortem test). All samples should be kept cool.

2.0 OBJECTIVE

At the end of this unit you will be able to:

- know how to collect various types of specimens in clinical chemistry laboratory
- know to carry out some simple tests in the clinical chemistry lab.

3.0 MAIN CONTENT

3.1 Collection of Specimen in Clinical Chemistry

It should always be borne in mind that all clinical specimens are potentially pathogenic. It is therefore important that all necessary safety precautions must be observed when collecting specimens. In addition, proper identification of the patient must be made before collecting specimen from him/her. A properly filled request form must accompany each specimen.

Blood Specimens
In case of blood specimens, specimen containers used for blood for biochemical investigation must be leak proof and chemically clean. Syringes and needles for collecting the blood samples must also be chemically clean, dry and sterile.

Venepuncture is the accepted method of blood collection. Some factors that affect the correctness of test results do originate from blood collection. These factors include;

(i) Wrong venipuncture technique
(ii) Haemolysis of red blood cells
(iii) Use of wrong containers
(iv) Instability of some substances in blood

The use of disposable needles and syringes minimizes the risk of infections due to serum hepatitis virus and human immunodeficiency virus (HIV) . For the same reasons, plastic tubes and bottles are preferred to glass ones.

Venepuncture Technique
It is easier to enter a vein which can be felt than the one that is only seen. The tourniquet should not be applied too tightly nor too long in order not to cause venous stasis which can lead to concentration of certain blood substances such as haemoglobin, plasma proteins and calcium . It is wrong to collect blood for chemical analysis from the arm into which an intravenous infusion is already being given.

Haemolysis of Red Blood Cells
Red blood cells are not often used for biochemical investigations as the rupture of the red blood cells can lead to unreliable results. This is because haemolysis causes substances from the cells to be released into the serum or plasma thereby giving a false increase in the concentration of the substances being tested.

To avoid haemolysis:

(i) Use good, clean, sterile and dry syringe and needle.
(ii) Withdraw blood slowly and steadly.
(iii) Allow sufficient time for the blood to clot and retraction of the clot to occur.
(iv) Avoid prolonged centrifuging; 3 to 5 minutes at 700rpm (700g) is adequate.
(v) Do not shake blood samples; instead, mix gently if the container contains an anticoagulant.
(vi) Do not freeze whole blood samples.

3.2 Specimen Containers

Most biochemical analysis requiresserum. Blood is therefore collected into dry, cleancontainers. There are some tests, however, that require plasma. To obtain plasma, anticoagulants are used to prevent the blood from clotting. Whenever possible, using plasma instead of serum is more advantageous because the yield of plasma from a given volume of blood is greater, and the tests can be performed immediately. Lithium heparin is the most ideal anticoagulant as it does not affect chemical reactions; it helps to reduce the action of haemolysis and lacks sodium or potassium salts which might interfere when electrolytes are to be measured. But due to its high cost , other anticoagulants such as EDTA(ethylene diamine tetra acetic acid) and fluoride-oxalates are widely used even though they contain sodium and potassium salts . Fluoride is an enzyme inhibitor which prevents glycolysis (breakdown of glucose to lactic acid by enzyme action). Therefore, it must not be used where enzyme activity is to be estimated.

3.3 Preservation of Blood

Chemical substances in blood are present in both plasma and cells in varying quantities. Prompt separation of plasma or serum from cells is important in order to prevent chemical changes which can occur in whole blood. Refrigeration of blood specimen prior to separation of plasma can lead to false potassium level. Glycolysis can occur if fluoride-oxalate anticoagulant is not used. Bilirubin will be decomposed on prolonged exposure to light.

It is essential to refrigerate serum or plasma at 4oC or freeze at -20oC until analysed. As much as possible, blood specimen should be handled aseptically to prevent and minimize chances of bacterial contamination.

3.4 Urine Specimens

Urine is an important specimen for clinical analysis. A single specimen of urine may be adequate for analysis depending on the type of examination required.
The container of urine collection should be clean, dry, leak-proof and sufficiently wide mouthed for the patient to use. The container must also be free from all traces of disinfectants and other chemicals. Collection of urine can be early morning, timed or random.

(i) **Early Morning Urine Specimen**
The first urine voided on waking up in the morning is referred to as early morning specimen. This specimen usually contains the highest concentration of substances than others passed later in the day. It is therefore, more suitable for easy detection of abnormalities.

(ii) **Timed Specimen of Urine**
It may be necessary to collect a timed specimen of urine for certain quantitative analysis of substances such as hormones, calcium, protein, phosphates and steroids. This is because the concentration of these substances in urine varies from sample to sample; and so, a pooled specimen over a period of time; eg; 24hours, will give a more accurate result.

A successful collection of 24hour urine specimen is as follows:
(a) The patient is made to empty his/her bladder and the time is noted, eg at 07.00 hours. This urine is discarded and should not form part of the collection.
(b) T he patient is given a 2 litre capacity container preferably with a suitable preservative or stabilizer.
(c) The patient is instructed to collect into the container all the urine he/she passes over the next 24 hours, up to and including the urine passed at 07.00 hours the following day.

(iii) Random Specimen of Urine
When quantitative estimation of a chemical substance in the urine is not required, random urine sample can be collected to detect the presence of that substance; eg, blood or bilirubin in urine.

Preservation of Urine
Chemical changes take place in urine on keeping at room temperature over a period of time. Some of these changes are:

(a) Breakdown of urea to ammonia by the action of bacteria resulting in a rise in the pH of the urine.
(b) Oxidation of urobilinogen to urobilin

(c) Breakdown of glucose by bacteria
(d) Precipitation of urates in acidic urine .

All these chemical changes can be arrested or slowed down by refrigerating the 24 hour specimen.

Chemical Preservatives

(i) Concentrated hydrochloric acid: 10ml of conc. HCL is used to preserve 24 hour specimen of urine. It should be noted that urine for protein or urate estimation must never be acidified.
(ii) Thymol and other chemicals: A few crystals of thymol are used to preserve 24 hour specimen of urine required for total protein and creatinine estimation. Chemical substances such as chloroform, toluene and formalin are used to preserve urine for chemical analysis while boric acid is ideal for urine which may require bacterial culture.

Storage of a large container of 24 hour specimen of urine usually presents a problem. It is convenient to keep small representative portions rather than the whole collection. To do this, mix the urine thoroughly and carefully measure and note down the total volume. Place small volume in about 4 small universal bottles labeled with patient's details, date and time of collection.

Cerebrospinal Fluid (CSF):
CSF is usually sent to the Clinical chemistry laboratory for the estimation of glucose, total proteins and a few other investigations. For glucose estimation, the specimen is preferably collected into a clean, dry container with fluoride-oxalate preservative to prevent glycolysis. It can also be collected into an ordinary clean container and kept in the refrigerator if there is any delay in estimation.

Collection of CSF from the lumbar vertebrae is a delicate operation and should always deserve emergency treatment. Usually the sample is shared between microbiology and biochemistry laboratories, and so it is that there is cooperation between the two departments in the interest of the patient.

3.5 Urine Analysis

The physical, chemical and microscopic examination of urine is known as urinalysis or routine urine analysis. Urinalysis is an important part of the initial examination of a patient and the results provide a valuable picture of the patient's general health pattern. Generally, urinalysis will indicate:

(i) **The state of the kidneys and the urinary tract**
Chemical tests for the presence of protein or blood; together with the physical properties and microscopic examination for casts, cells and certain crystals are very helpful in assessing and treating renal and urinary tract disease.

(ii) Information about metabolic and systemic (non-renal) abnormalities –Tests for glucose, ketone bodies, bilirubin and urobilinogen are useful parameters in the diagnosis of metabolic and systemic disorders such as diabetes and jaundice .

Composition of Urine

Urine is the fluid containing water soluble waste products excreted from the blood via the kidneys. Urine is mainly composed of 95% water and the rest being made up of urea, uric acid, creatinine, sodium, potassium, chloride, calcium, phosphates, etc. The composition of urine varies a great deal and is affected by three factors:

(i) Nutritional status of the individual
(ii) State of metabolic processes
(iii) Ability of the kidneys to selectively handle the material presented to them.

Properties of Normal Urine

pH----------4.6-8.0 (mean:6.1)
Specific gravity
Neonates----------1.012
Infants----------1.002-1.006
Adults----------1.003-1.030
Volume per day
Neonates----------30-60ml
Children----------400-1400ml
Adults----------600-2500ml
Organic constituents/24 hours
Urea----------15-30g
Creatinine----------1.0-1.8g
Uric acid----------0.3-0.6g
Protein (albumin)----------0.0-0.1g
Glucose----------<0.5g

Collection and preservation of Urine

Urine sample for analysis should be collected in a clean, dry container and examined as soon as possible. In most cases, the first specimen freely voided in the morning is preferred although specimen collected 2 to 3 hours after eating is suitable when testing for glucose. On standing, red blood cells and white blood cells are destroyed; casts are decomposed; glucose is lost and bacterial contamination of urine takes

place causing alkalization of the sample. Therefore, the specimen must be examined when fresh, ideally within 30 minutes of collection. If there is any delay in processing, it should be suitably preserved.

Routine Tests for Urine Analysis

The routine tests for urine analysis are grouped in three parts namely, physical, chemical and microscopic examinations. Certain tests are graded according to their degree of accuracy: screening or qualitative tests; semi-quantitative tests and quantitative tests. The results of qualitative tests are reported as positive or negative; or present or absent. For semi-quantitative, the results are graded as negative, trace, 1+, 2+. 3+ or 4+; or sometimes results are given as small, moderate, or large quantities. The quantitative tests, on the other hand, determine accurately the amount of substance that they detect .

Physical Examination

Physical examination of urine is also referred to as macroscopic examination and this involves the assessment of physical properties such as volume, colour, appearance, odour, foam, specific gravity and pH.

(**Volume:** Under normal conditions, a direct relationship exists between urine volume and water intake of a person. Thus, if water intake is increased, the kidney will eliminate a larger volume of urine than normal. Likewise, if a water intake is decreased, the kidney will eliminate a smaller volume of urine.

Certain situations, however, give rise to abnormal urine volumes . Here are some terms to describe such volumes:

Polyuria: This term refers to consistent elimination of an abnormally large volume of urine (>2000mls) in 24 hours.

Causes: Diabetes mellitus
Neurotic polydipsia (abnormal thirst)
Chronic renal failure

Diuresis: This is a term that describes any increase in urine volume, even if the increase is only temporary.

Causes: Excessive fluid intake
Diuretic drugs
Intravenous saline/glucose

Oliguria: The term refers to the excretion of an abnormally small volume of urine (<200mls) in 24 hours.

Causes: Dehydration due to vomiting, diarrhea,
Renal ischemia
Acute renal tubular necrosis
Acute glomerulonephritis
Obstruction to urine flow

Anuria: This is the complete absence of urine formation

Causes: Same as for oliguria

Nocturia: The excretion, by an adult, of urine more than 500mls at night.

Cause: Chronic glomerulonephritis

(b) **Colour:** The colour of normal urine varies greatly, even in one person, in a single day . In general, the normal urine colour is described as straw, yellow or amber. This colour of normal urine is due to the presence of three pigments: trichrome(a yellow pigment present in large concentrations) ; uroerythrin(a by-product of red blood cell degradation) and urobilin(another pigment resulting from red blood degradation).

(c) **Appearance/Transparency:** Normally urine is clear when freshly voided; but it often becomes cloudy when allowed to stand for a certain length of time . This cloudiness is usually due to precipitation of chemical substances (crystals, or amorphous phosphates and urates) in urine or due to growth of bacteria . However, cloudiness in freshly voided urine is usually of clinical significance and may be due to the presence of mucus, bacterial, white blood cells or red blood cells etc. The degree of transparency of a urine specimen is expressed as clear, hazy, cloudy or turbid.

(d) **Odour:** Normal, freshly voided urine has a characteristic faintly aromatic odour due to the presence of certain volatile acids. Bacterial action occurs when the urine is allowed to stand and this produces ammoniacal odour. The presence of ketone bodies is indicated by the so-called fruity or sweet odour of urine.

(e) **Foam:** Normal urine will produce a moderate amount of white foam when shaken. A urine sample with a high protein concentration produces a large amount of white foam on shaking, for example, in nephritic syndrome. Characteristic yellow coloured foam is seen in urine samples containing bile pigments.

(f) **Specific Gravity:** Specific gravity (sp.gr.) of urine is a measure of the amount of dissolved substances in the urine. It is the

weight of the urine compared to the weight of an equal volume of distilled water at a constant temperature.

Eg; Sp.gr. =Weight of urine

Weight of same volume of
Distilled water.

Urine of low specific gravity are called **hyposthenuric** (<1.007), and those of fixed specific gravity of about 1.010 are called **isothenuric.**

(i) pHRegulation of acidity in extracellular fluid is one of the important functions of the kidney . By checking the pH of the urine, this aspect of the KIDNEY function can be determined. The pH of blood is normally maintained at 7.4 . A blood pH of less than 6.8 or greater than 7.8 can be fatal. A high degree of acidity is produced due to carbon dioxide formed during normal metabolism. This acidity is eliminated from the body by the kidneys and the lungs.

3.6 Chemical Examinations

Clinical laboratories use commercially prepared test strips to perform chemical examination on urine. These are plastic strips that hold small square shape test pads. Each pad is for different test, the reagent of which is impregnated in it. When a strip is briefly and completely dipped into a urine sample, each test pad absorbs the urine and a chemical reaction changes the colour pad within seconds to minutes. The colour changes for each reaction pad is compared with a colour chart provided with the test strips (usually on the container of the strips). Automated instrument are also available for the colour comparison. The most frequently performed chemical tests on urine are the following: Glucose, Protein, pH, Blood, Ketones, Specific gravity, Leucocytes, Nitrite, Bilirubin and Urobilinogen.

Procedure for Urinalysis using Reagent Test Strips

(i) Dip the reagent pad areas of the strip completely into the urine.
(ii) Remove immediately
(iii) Tap the strip against the edge of the urine container (to remove Excess urine on pads)
(iv) Compare the resulted colour on each pad with the colour chart Provided at the appropriate time stipulated for each test pad (Found on the colour chart provided).

3.7 Microscopic Examination

Microscopic examination of urine is performed on urine sediment. That is, urine that has been centrifuged to concentrate the substances in it at the bottom of the tube. In practice, urine microscopy is not usually performed as part of routine urinalysis, but usually Requested separately. When urine is microscopically examined, the following substances are usually sought for: white blood cells, red blood cells, epithelia cells, bacteria, yeast, egg of schistosoma haematobium, trichomonas, casts and crystals.

Procedure for Urine Microscopy

(1) Mix the urine (in the specimen container) gently
(2) Pour about 2-4mls into a centrifuge tube.
(3) Centrifuge the sample at low speed (2500g) for 5 minutes
(4) Decant the supernatant and mix the sediment at the bottom
(5) Using a Pasteur pipette, add a drop of the sediment on a slide
(6) Apply a cover slip
(7) Observe first under 10X objective, then
(8) Examine the observed objects using 40X objective
(9) Report (record) your findings

3.8 Urine Pregnancy Test using Pregnancy Test Strips

This test is based on detection of a hormone called human Chorionic Gonadotrophin (hCG) in urine of suspected pregnant woman.
Early morning urine is the specimen of choice. Usually, the sample is

Collected after 5 days of missed menses. The sample is collected in a Clean urine specimen container.

Material Required

Pregnancy test strips (commercially available)

Procedure

(i) Pour an aliquot of the urine sample into the cover of thecontainer.
(ii) Dip the test end of the strip into the aliquot of urine ensuring that the urine level is not above the demarcated mark on the strip.
(iii) Leave for 2 minutes.
(iv) Observe for the followings and report appropriately:
- If two red lines appear: Pregnancy test is positive
- If only one red line appear: Pregnancy test is negative
- If no line appears at all: Discard the result and repeat, the test is invalid.

3.9 Measurement of Fasting Blood Sugar using Glucose Oxidase Method

This test is used in the diagnosis and management of diabetes mellitus. The patient is instructed to have an overnight fast of 10-14hrs prior to sample collection in the morning. The sample is collected into fluorideoxalate specimen bottle. Glucose oxidase method is one of the most common methods of measuring blood glucose. While the oxalate prevents the blood from clotting, fluoride preventsglycolysis, so that the glucose level of the specimen is maintained after sample collection. Most clinical laboratories use. Commercially prepared glucose reagent.

Procedure

(i) Centrifuge the blood at low speed (2000-3000g) for 5 minutes
(ii) Select three clean test tubes and label as test, standard and blank rrespectively
(iii) Pipette 20 microliter of the plasma and 20 microlitre of glucose Standard solution into tubes labeled test and standard respectively.

* Add 3mls of working glucose reagent into the three tubes.
* Incubate in water bath at 37oC for 10 minutes.
* Select wavelength of 520 nm on the spectrophotometer
* Use the to set the spectrophotometer to zero.
* Read the absorbance of both the test and standard on the spectrophotometer

Calculation: Plasma glucose level = Absorbance of test x Conc of standard / Absorbance of Plasma in mmol/L.

4.0 CONCLUSION

Sample collections are the starting points of quality control and various ways of sample collections have been detailed. The techniques of processing some of the samples have also been detailed, for this reason I, urge you to put more effort and get the knowledge.

5.0 SUMMARY

In this unit, sample collections and techniques in clinical chemistry have been discussed. Containers, preservation of specimens as well as urinalysis, microscopy, pregnancy test and Fasting blood sugar estimations have been detailed. Please you are advised to go through them for your own good.

6.0 TUTOR-MARKED ASSIGNMENT

1. Write short notes on:
   (i) Volume of Urine
   (ii) Colour
   (iii) Appearance
2. Discuss the health implication of Urine pH

7.0 REFERENCES /FURTHER READING

Burtis,Cart A; Ashwood, Edward R ; Bruns, David E(2006): Tietz textbook of Clinical Chemistry. 4th edition; Saunders .

Carson, Freida L; Christa Hladik(2009): Histotechnology. A self-instructional Text . 3rd edition. Hong; Am. Soc. For Clinical Pathology.

Robbins, Stanley (2010): Robbins and Cotran Pathology basis of disease. 8th edition, Philedelphia. Saunders /Elsevier

Cai, H; Casewell JL; Prescott JF(2014): Non culture molecular Techniques; A Diagnostic. Laboratory Perspective .

Ochei.J; Kolhatkar.A. (2008): Medical Laboratory Science; Theory and Practice .

Baker.F.J.; Silver.R.E; Palister.C.J.(2011) : Introduction to medical Laboratory Technology. 7th edition; Bounty Press Limited