



**NATIONAL OPEN UNIVERSITY OF NIGERIA**

**SCHOOL OF SCIENCE AND TECHNOLOGY**

**COURSE CODE: CHS301**

**COURSE TITLE: INTRODUCTION TO CLINICAL LABORATORY  
TECHNIQUES**



**CHS301**  
**INTRODUCTION TO CLINICAL LABORATORY**  
**TECHNIQUES**

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## **Introduction**

Introduction to Clinical Laboratory Techniques (CHS301) is a second semester two-credit unit course available to students of Bachelor of Science, (B.Sc.) Community Health in the National Open University of Nigeria (NOUN).

Clinical laboratory techniques entail the concepts, principles, procedures and equipment used in a professional clinical laboratory.

This course provides the fundamental knowledge of the clinical laboratory practice.

## **Course Aims**

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

## **Course Objectives**

Each unit has 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 unit. Reading the objective 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:

- distinguish between the functions of the different clinical laboratory divisions (departments)
- explain the basic approach to the laboratory in the laboratory diagnosis of disease
- collect clinical specimens of acceptable quality suitable for laboratory testing for public health intervention or for epidemiological studies
- comprehend the general principle and techniques employed in clinical laboratory practice
- have the basic knowledge required to perform some common and simple laboratory procedures
- 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 textbook “Introduction to Medical Laboratory Technology” by Baker & Silverton

(Sixth or Seventh Edition), Butterworth: Heinemann publishing Co.. and also, the Manual of Basic Techniques for a Health Laboratory (2<sup>nd</sup> Edition). World Health Organisation, Geneva could be found useful.

The course should take a total of 10-12 weeks, at the end of which shall be an examination. This course is divided into five study units. There is Tutor-Marked Assignment at the end of each unit, you are advised to answer the assignment 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 units 3 and 4 respectively. The reports of the visits must be submitted to the facilitator for assessment. The report is expected to show the procedure of activity learnt during the visits.

### **The Course Materials**

The components of the course are:

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

### **Study Units**

The study units in this course are as follows:

Unit 1	The Clinical Laboratory and Diagnostic Skills in Health Practice
Unit 2	Specimen Collection for Medical Laboratory Testing
Unit 3	Principles and Techniques of Clinical Laboratory Test
Unit 4	Conducting Simple Laboratory Tests
Unit 5	Laboratory Management

Unit 1 focuses on the clinical laboratory and its four major specialty divisions, clinical and laboratory diagnosis of diseases.

The main focus of unit 2 is the critical issues that affect the quality of specimen presented for laboratory testing. Appropriate collected samples, sample handling or transportation being the main issues.

Unit 3 will acquaint you with the basic major techniques employed in the laboratory and also explain the relationship between Scientific Principles and Techniques of the common analytical methods. One or two visits to a neighbouring clinical laboratory, so as to be more acquainted with the basic instrument are expected. You are expected to give a brief report/account of the visits to your facilitator (e.g. activity learnt, diagram of instrument seen).

Unit 4 focuses on the laboratory procedures of some commonly performed tests. You are expected to study the procedures with emphasis on urinalysis, urine microscopy, stool microscopy and blood group determination. Two visits to a neighbouring clinical laboratory are beneficial to facilitate the learning of these procedures. A report of the visits (showing the activities learnt or participated) is expected to be submitted to your facilitator for assessment.

Unit 5 deals with the rudiments of management as applicable to clinical laboratory.

An average of 2 ½ weeks is assigned for the study of each unit.

### **Assessment**

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

1. Report of a minimum of 4 visits to a clinical laboratory recommended in the course material. (15%)
2. Tutor-Marked Assignments. (20%)
3. Final Examination. (65%)

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

- Collection of blood sample by venipuncture
- Diagram of any 2 specific equipment seen in the laboratory visited (Discussed in unit 3)
- Report of test procedures learnt during visits. (Stool microscopy, blood group by tile method, urinalysis, occult blood test etc) as done in the laboratory visited.

There are 5 Tutor-Marked Assignments to be submitted, which will total up to 20%.

The end of course exam will be of 2 ½ hours and it has a value of 65% of the total course work.

<b>Assessment</b>	<b>Marks</b>
Assignment 1-5	20%
Report of visits to the laboratory ( minimal 4 visits)	15%
End of course exam	65%
<b>Total</b>	<b>100%</b>

### **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.

Wishing you success in the course.



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**MODULE 1**

Unit 1	The Clinical Laboratory and Diagnostic Skills in Health Practice
Unit 2	Specimen Collection for Medical Laboratory Testing
Unit 3	Principles and Techniques of Clinical Laboratory Test
Unit 4	Conducting Simple Laboratory Tests
Unit 5	Laboratory Management

**UNIT 1 THE CLINICAL LABORATORY AND  
DIAGNOSTIC SKILLS IN HEALTH PRACTICE****CONTENTS**

1.0	Introduction
2.0	Objectives
3.0	Main Content
3.1	The Clinical Laboratory
3.2	Universal Precautionary Measure in Clinical Laboratory
3.3	Medical Diagnosis
3.4	Key Elements of Diagnostic Skills
3.5	Laboratory Diagnosis of Diseases
4.0	Conclusion
5.0	Summary
6.0	Tutor-Marked Assignment
7.0	References/Further Reading

**1.0 INTRODUCTION**

This unit discusses the clinical laboratory and its four operational departments (functional divisions). It also discusses major techniques of clinical/medical diagnosis of disease and the role of the clinical laboratory in diagnosis of diseases.

**2.0 OBJECTIVES**

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

- distinguish between the divisional functions of the different clinical laboratory departments
- explain the key elements of diagnostic skills
- explain the basic approach of the laboratory in diagnosis of diseases.

### 3.0 MAIN CONTENT

#### 3.1 The Clinical Laboratory

Clinical laboratory (also called medical laboratory) is a facility that provides controlled conditions in which tests are done on clinical specimens 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 clinical laboratory consists of four major divisions (or departments). These are described below:

**Medical Microbiology and Parasitological Laboratory:** This laboratory deals with the study of human pathogens. Pathogens are biological agents that cause diseases to their hosts.

They include microorganisms (bacteria, viruses and fungi) and parasites (e.g. intestinal worms, lice and malaria parasites) of medical importance.

In bigger health centres or research institutes, medical microbiology and parasitological laboratory is usually split into sub-unit like bacteriology, parasitology and virology laboratories.

**Haematology:** This laboratory is involved in the performance of relevant tests (on blood) in the diagnosis of blood diseases, (e.g. Anaemia, Haemoglobinopathies, Leukaemia etc) and blood transfusion services e.g. blood group, blood cross matching.

**Chemical Pathology Laboratory (Clinical Chemistry or Clinical Biochemistry Laboratory):** This division of laboratory is concerned with the performance of quantitative and qualitative tests on clinical specimens to investigate the state of various body chemistries.

Such clinical specimens include body fluids (e.g. whole blood, plasma, serum, urine, sweat, cerebrospinal fluid) and occasionally faeces, tissue, hair e.t.c.

**Histopathology Laboratory:** This is the laboratory where tissues (or cells) are processed for microscopic examination in order to investigate or study disease manifestations on the tissue (or cells), structure, for diagnostic purposes e.g. Cancer diagnosis.

In the laboratory, tissue samples are processed onto glass slides from which effects of diseases on the histological architecture of tissues can

be microscopically examined and hence diagnostic inferences are made e.g. Cancer diagnosis.

### 3.2 Universal Precautionary Measure 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.

Universal basic precautions assume that all clinical specimens contain infectious agents and should therefore be handled as such. This approach eliminates the need to identify infected patients or specimen from Human Immunodeficiency Virus (HIV) or other blood borne pathogen infected patients. The followings are the laboratory universal safety precautions.

1. Universal precautions should apply to blood and all body fluid containing visible blood, semen, vaginal secretions, tissues, cerebrospinal fluid, peritoneal fluid, pericardial fluid, synovial fluid and amniotic fluid.
2. Laboratory workers should use protective barriers appropriate for the laboratory procedure and the type and extent of exposure expected. All persons processing blood should wear gloves and laboratory coats; and these should be removed before leaving the laboratory. Biological safety barriers should be used wherever necessary.
3. Hands should be washed immediately when contaminated with blood or other body fluids, after removing gloves and after completing laboratory activities.
4. Use of needles and syringes should be minimised. They should be used in situations in which there is no alternative. If used, needles should not be recapped or bent or broken by hand. After use, needles and other sharp instruments should be placed in a 'sharp-safe' puncture-resistant container for disposal.
5. Specimens of blood should be placed in strong-leak-proof containers during transport.
6. Mouth pipetting must not be performed in the laboratory. Mechanical devices should be used.
7. Contaminated materials used in the laboratory should be decontaminated appropriately before reprocessing or disposal.
8. Laboratory work surfaces should be cleaned and decontaminated with appropriate disinfectant after a blood or body fluid spill and at the end of day's work.

### 3.3 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

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.

- (bi) 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:

- 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.
- 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.
- 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.

(bii) Medical Tests

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

- Medical Imaging
- Medical Laboratory Tests
- 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:

- many signs and symptoms may not be specific for any particular disease
- some diseases may be asymptomatic (present no symptom)
- patients often forget or mispresent past symptoms
- symptoms 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.

### **3.4 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 recognise 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

### 3.5 Laboratory Diagnosis of Diseases

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

**Communicable diseases:** These are diseases caused by pathogenic microbial agents, transmissible directly or indirectly from one person to another. (Dorland illustrated medical, they are also called infectious diseases dictionary 2004)

**Non-communicable diseases:** These are not transmissible from one person to the other. They are systemic diseases which might be genetic, lifestyle origin and sometimes unknown.

Laboratory diagnosis of diseases generally entails qualitative and quantitative analyses of clinical specimens (body fluids, tissues and other solids 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:

- microscopic visualisation of the pathogenic agents in the specimen (Harrison)

- growing the causative microorganisms on or in a medium that support such growth (culture media)
- 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 viewed under 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 concerned with such tests. Methods employed by these laboratories in the diagnosis of non-communicable diseases involve the following:

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

### **SELF ASSESSMENT EXERCISE**

1. Mention 5 examples of infectious diseases.
2. List 5 examples of non- communicable diseases.

## **4.0 CONCLUSION**

In this unit, the four major divisions (or departments) into which clinical laboratory is divided with their various functions is discussed together with the diagnostic skills and methods involved in the laboratory diagnosis of communicable and non-communicable diseases.

## **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. The clinical laboratory is functionally divided into four units or departments.

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:

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

## 6.0 TUTOR-MARKED ASSIGNMENT

1. In a tabular form, write the four major functional divisions of the clinical laboratory and their functions.
- 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

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## **UNIT 2 SPECIMEN COLLECTION FOR MEDICAL LABORATORY TESTING**

### **CONTENTS**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
  - 3.1 Clinical Specimens
  - 3.2 Patients Preparation Prior to Specimen Collection
  - 3.3 Specimen Containers
  - 3.4 Collection of Blood Samples
  - 3.5 Transportation
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

### **1.0 INTRODUCTION**

Appropriate specimen collection (or quality of specimen collection) is very pivotal to the generation of reliable results of clinical laboratory testing.

The quality of the specimen to be tested depends so much on:

- The patient being in the correct state required for such tests
- Use of appropriate specimen containers
- Correct handling of the sample
- Collection of the appropriate sample for the investigation needed.

These are the focus of this unit and they form the very first critical issues in reliability or clinical usefulness of medical laboratory test results.

### **2.0 OBJECTIVES**

By 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**

#### **3.1 Clinical Specimens**

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

- Blood
- Urine
- Faeces
- Body Tissues
- Other body fluids/secretion e.g. cerebrospinal fluids, sweat, saliva, sputum, aspirate or ascitic tap and seminal fluid
- 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.

#### **3.2 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 in blood sugar 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 overnight 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 3-7 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 this 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 urine of choice for urinalysis and microscopic analysis.
- (b) **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 midway 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.
- (c) **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 litres keg.) until *x* a.m the next day. This is called 24 hr urine specimen.
- (d) **Random urine specimen:** This is a urine specimen collected without reference to any form of time. 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 not the specimen of choice for either. However, it is satisfactory and hence, generally acceptable. There are no specific guidelines for random urine specimen collection.

### 3.3 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 Diaminetetraacetic acid (EDTA), Fluoride-oxalate mixture and citrate.

Most of the specimens for haematology tests are collected inside EDTA bottle; however, citrate anticoagulated 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.

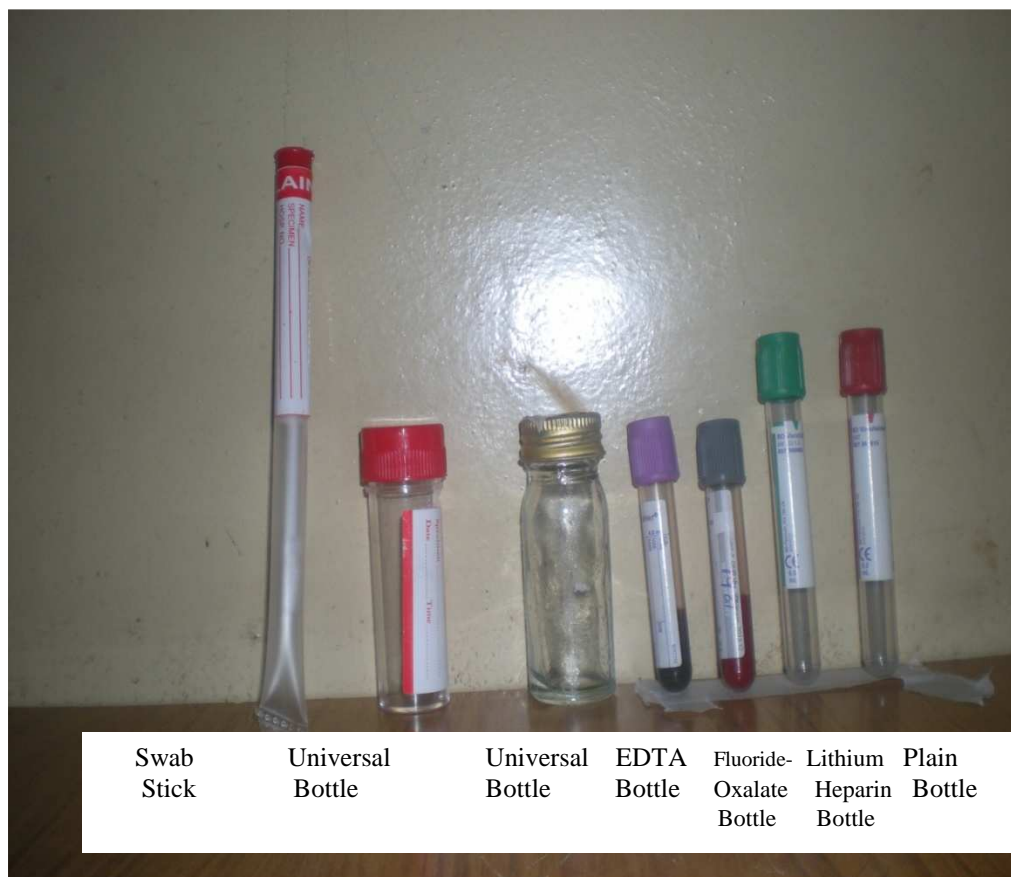
Specimen meant for blood glucose measurement is specifically collected inside a fluoride-oxalate bottle.

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



**Fig. 1: Colour Codes for Blood Specimen Containers for Laboratory Tests**

### **3.4 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 are the basic information expected on the sample label and the laboratory request form:

- Full name of patient
- Age
- Sex
- Occupation (on form only)
- Date and time of sample collection
- Clinical details (on form only)
- Requested tests.

### **3.5 Transportation of Clinical Specimens**

Specimens to be sent to the laboratory require special attention for safe packing of the material. The packing 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 primary container which has the specimen and it must be leakproof and well covered. It should be kept in upright position.
- 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.
- 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.

## **4.0 CONCLUSION**

In this unit, you have learnt what clinical specimens are, and also acquainted with the importance of patient preparation before sample collection you should be able therefore able to prepare patients when necessary, for clinical samples collection. Using the international colour code for blood specimen container cover (top) you should be able to identify the appropriate blood specimen containers for specific tests. A visit to a clinical laboratory (public or private) would intimate you the procedure of collecting blood specimens.

Moreover, you also learnt how clinical specimens are packaged for transportation.

## 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 in the reliability or clinical usefulness of laboratory results.

Collections of clinical specimens that will generate clinically useful results include the following:

- patient preparations which encompasses all instructions, guidance or tutelage given to patient to ensure that the right sample are appropriately collected
- 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
- appropriate transportation of clinical specimen accompanied with approximately filled request form

## 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. In a tabular form, show the colour identification of blood specimen container name and test(s) for which the container is used.

## 7.0 REFERENCES/FURTHER READING

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## **UNIT 3      PRINCIPLES AND TECHNIQUES OF CLINICAL LABORATORY TESTING**

### **CONTENTS**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
  - 3.1 Principle of Tests
  - 3.2 Laboratory Techniques
  - 3.3 Atomic Emission Spectrophotometric Techniques
  - 3.4 Atomic Absorption Spectrophotometric Techniques
  - 3.5 Electrophoretic Techniques
  - 3.6 Chromatographic Techniques
  - 3.7 Centrifugation
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

### **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 and biology) are employed in development of analytical methods (or techniques). 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**

By 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.

- Occult blood test detects the presence of blood or blood product that cannot be seen physically in faeces or urine.
- Stool microscopy detects or reveals the presence of parasite in faeces.
- 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.

- Full blood count: A test which among others reveals the relative blood cellular compositions.
- 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 the reacting 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 ( $\text{H}_2\text{O}_2$ ) 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:

- Glucose oxidase (an enzyme) specifically oxidizes glucose, to give a product from which oxygen evolved.
- The oxygen evolved oxidizes a chromogen to give a coloured compound, the intensity of which is directly proportional to the amount of glucose present in 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. The 'Wikipedia' defined laboratory techniques as the sum of procedures used on natural sciences as chemistry, biology, and physics in order to conduct an experiment. According to 'wikipedia', all of such procedures follow scientific method, while some of them involves the use of complex laboratory equipment, others do not require such specific or expensive supply. 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.



**Fig. 2: 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 substance being measured interact with an 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)

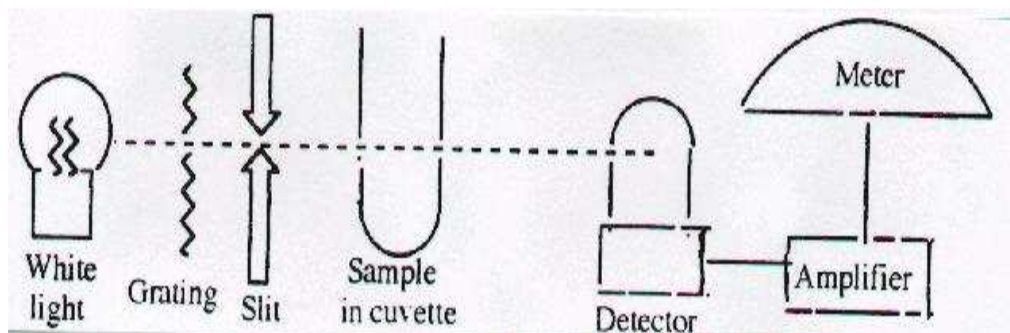
3. Purple (or pinkish)

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.



**Fig 3: Schematic Diagram of a Spectrophotometer**



**Fig. 4: Picture of a Spectrophotometer****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 wavelength that is characteristic of the element (e.g. sodium emit primarily a wavelength of 589 nm with a yellow colour while potassium emits primarily, a wavelength of 400 nm and 767 nm with a violet or lilac 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.



**Fig. 5: Picture of an Atomic Emission Flame Photometer**

### **6.1 Atomic Absorption Spectrophotometric Technique**

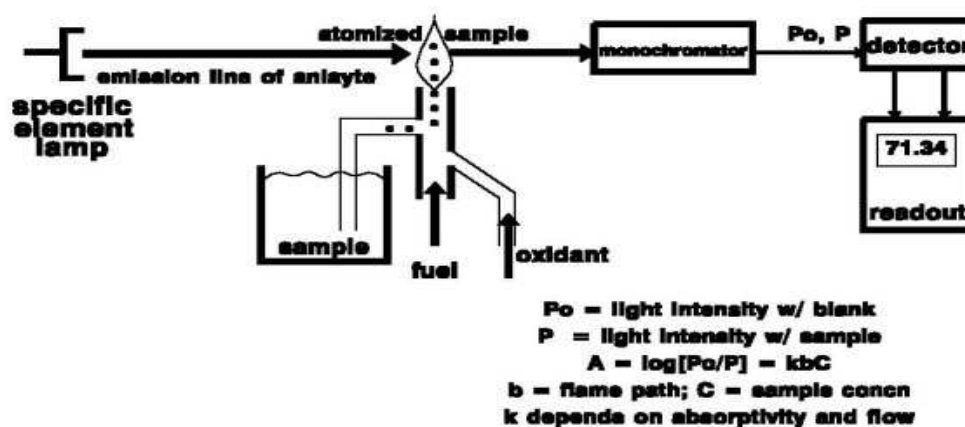
Atomic absorption spectrophotometric technique is employed in the measurement of trace elements in body fluids.

The principle by which this quantitative technique is based states that an atom 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 absorbs light 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.

## **Atomic Spectroscopy with Flames**

### **Atomic Absorption Spectroscopy**



**Fig. 6: Schematic Diagram of Atomic Absorption Spectrophotometer**



**Fig. 7: Picture of Atomic Absorption Spectrophotometer**

Source: [www.sjsu.edu/faculty/chem55/55aovw.htm](http://www.sjsu.edu/faculty/chem55/55aovw.htm)

### **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.

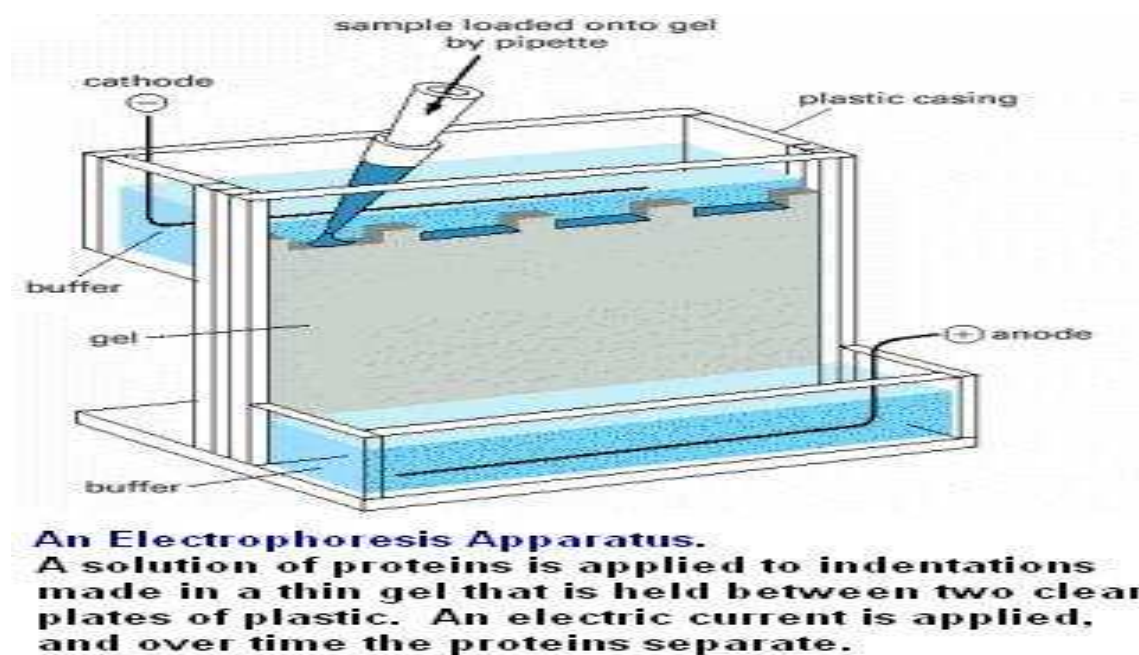


Electrophoretic technique is typically used in the clinical laboratory for the separation of proteins. It is primarily a qualitative method of analysis, but it can be adopted for quantitative analysis.

### Principle of Electrophoretic Separation of Proteins

Proteins in serum vary in their iso-electric points. Iso-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 iso-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 iso-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.



**Fig. 8: An Electrophoresis Apparatus**

**Source:**<http://www.molecular detective.org/TutorialProteomics/GelElectrophoresis02.JPG>



**Fig. 9: Picture of an Electrophoretic Apparatus**

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 utilises 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:

- Thin layer chromatography
- Molecular sieve chromatography
- 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 major use 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



**Fig. 10: Picture of a Microhaematocrit Centrifuge**



**Fig. 11: Picture of a Bench 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:

- quantitative analysis which is any test that measures the level of a specific substance in a sample (e.g. blood glucose measurement)
- 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.

- Optical techniques make use of light either of a specific wavelength 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 quantify the substance of interest in the sample
- 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 tool
- 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
- 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.

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## **UNIT 4      CONDUCTING SIMPLE LABORATORY TESTS**

### **CONTENTS**

- 1.0 Introduction
- 2.0 Objective
- 3.0 Main Content
  - 3.1 Blood Group Determination by Tile Method
  - 3.2 Urine Pregnancy Test using Pregnancy Test Strips
  - 3.3 Detection of Occult Blood using Okokit Method
  - 3.4 Measurement of Fasting Blood Glucose using Glucose Oxidase Method
  - 3.5 Urinalysis
  - 3.6 Parasitological Examination of Stool
- 3.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

### **1.0 INTRODUCTION**

In performance of clinical laboratory testing, Standard Operating Procedure (SOP) is followed. This unit presents some common and simple important laboratory procedures.

### **2.0 OBJECTIVE**

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

- state and discuss some clinical laboratory procedures.

### **3.0 MAIN CONTENT**

#### **3.1 Blood Group Determination by Tile Method**

The method of determining blood group is based on antigen-antibody agglutination reaction.

#### **Material Required**

- Tile (e.g. Wall tile)
- Antisera (Anti-serum A, Anti-serum B, Anti-serum AB and Anti-serum D which are commercially available)

**Procedure**

- Add one drop of Anti-serum A, Anti-serum B, Anti-serum AB and Anti-serum D respectively on a clean tile.
- Add one drop of blood to each of them.
- Mix gently and rock the tile for 1 minute. (Do not allow the respective points to mix with another)
- Observe for agglutination (clumping) as follows:
  - If agglutination occur with Anti-serum A, the blood group is A
  - If agglutination occur 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 Anti-serum AB, it is blood group O
  - If agglutination occur with Anti-serum D (Rhesus) then the blood is Rhesus Positive, if no agglutination in Anti-D, it is Rhesus Negative. Hence the following blood groups are possible:
    - Blood group A +ve or A –ve
    - Blood group B +ve or B –ve
    - Blood group AB +ve or AB –ve
    - Blood group O +ve or O –ve

**3.2 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**

- Pour an aliquot of the urine sample into the cover of the container.
- 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.
- Leave for 2 minutes.
- 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.

### 3.3 Detection of Occult Blood using Okokit Method

Occult blood refers to blood that cannot be physically seen (“hidden”). Detection of Occult Blood in faeces is used in the diagnosis of gastrointestinal Carcinoma or Ulcer. Many chemical methods are available, all 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

- Make a 1:40,000 dilution of whole blood in distill water
- Prepare a thin smear of the faeces at the center of the test paper
- Prepare a similar smear of the diluted blood
- Place one Okokit tablet on each of the smears
- Add two to three drops of diluent on to the tablets
- Add two more drops after 2 minutes
- 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 reported.

### 3.4 Measurement of Fasting Blood Glucose 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 fluoride-

oxalate 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 prevents glycolysis, so that the glucose level of the specimen is maintained after sample collection.

Most clinical laboratories use commercially prepared glucose reagent.

### Procedure

- Centrifuge the blood at low speed (2000-3000g) for 5 minutes
- Select three clean test tubes and label as test, standard and blank respectively
- Pipette 20 microlitre of the plasma and 20 microlitre of glucose standard solution into tubes labeled test and standard respectively
- Add 3 mls of working glucose reagent into the three test tubes
- Incubate in water bath at 37°C for 10 minutes
- Select wavelength of 520 nm on the spectrophotometer
- Use the blank to set the spectrophotometer to zero
- Read the absorbance of both the test and standard on the spectrophotometer.

Calculation:

$$\text{Plasma Glucose Level} = \left\{ \frac{\text{Absorbance of test}}{\text{Absorbance of plasma}} \times \text{conc. of standard} \right\} \text{mmol/l}$$

### 3.5 Urinalysis (Urine Analysis)

Urinalysis is an array of essentially qualitative analysis performed on urine for screening and diagnostic purposes. A random urine specimen is acceptable; however, first morning urine is the specimen of choice. A complete urinalysis includes visual (physical), chemical and microscopic examinations of a urine sample. Urine sample for urinalysis must be done within 2hrs of sample collection.

#### Visual Examinations

This is physical examination of the urine where by the following physical properties of the urine is observed and recorded:

- **Colour:** A normal urine is amber or yellow colour, or colourless. Unusual or abnormal urine colour can be the result of a disease process, medications or from certain foods.

- **Clarity:** This refers to how clear the urine is. Normally, urine is expected to be clear. Turbid or cloudy urine may contain pus cells, epithelia cells, bacteria, prostatic fluid, sperm etc.

Urine colour and clarity can be a sign of presence of some substances in urine. However, confirmation of suspected substances in urine is obtained during the chemical and microscopic examinations.

### **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**

- Dip the reagent pad areas of the strip completely into the urine.
- Remove immediately
- Tap the strip against the edge of the urine container (to remove excess urine on pads)
- 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).

### **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**

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

### **3.6 Parasitological Examination of Stool**

#### **Sample Collection**

Faeces sample for parasitological tests are collected into a clean, wide mouth container with tight cover to avoid leakage. Contamination with urine should be avoided.

#### **Macroscopic Examination**

Faeces are usually examined and the following properties of the sample are recorded.

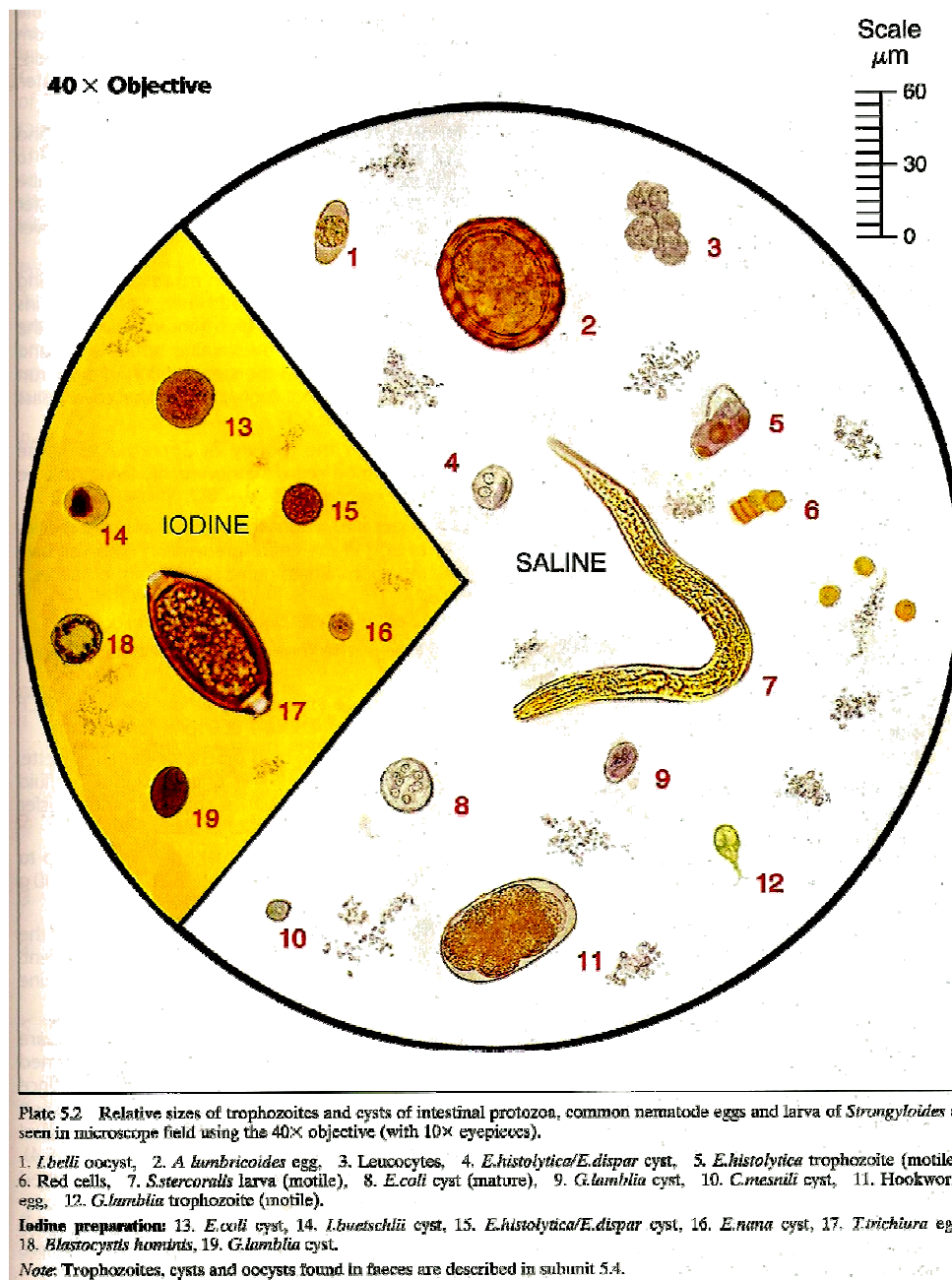
- Colour (Normal stool is brown in colour)
- Consistency (e.g. formed stool, semi-formed, watery)
- The presence of blood must be reported
- Presence of worm or worm segments must also be reported.

#### **Microscopic Examination**

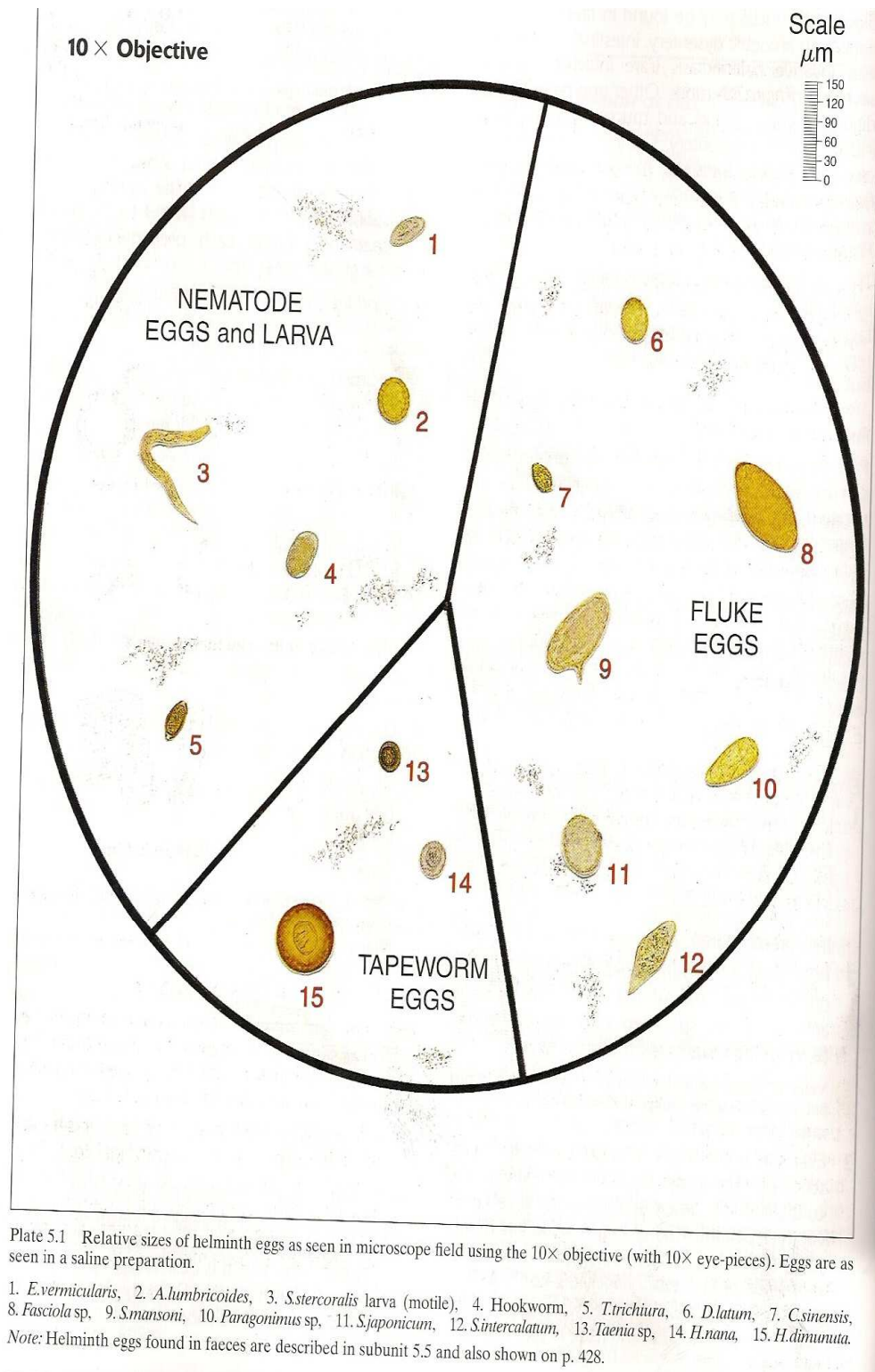
Wet preparation of the faeces samples are prepared using normal saline and iodine as follows:

- Place a drop of normal saline on one end of a slide
- Place a drop of iodine solution on the other end
- Using a wire loop or a piece of applicator stick, mix a small amount of the faeces specimen (about 2mg or matchstick head size) with the saline and a similar amount with the iodine solution
- Examine the saline preparation under the microscope using 10X objective and 40X objective for parasites (larvae, ciliates, ova, cysts, or trophozoites of parasites)
- Examine the iodine preparation the same way to assist in the identification of ova, cysts, larvae or trophozoites of parasites.

See the below atlas for identification of parasites ova, larvae or cyst as they would appear under the microscope.



**Fig. 12: Relative Size of Trophozoites and Cysts of Intestinal Protozoa**



**Fig. 13: Relative Size of Helminth Eggs**

## 4.0 CONCLUSION

Some simple medical laboratory tests were discussed and their procedures were laid out. It is worthy of note that one procedure, more often than not, differs significantly from the other. Hence, it is imperative that a clinical laboratory is visited in order to have prior physical contact, especially of

## 5.0 SUMMARY

Clinical laboratory testing procedures discussed in this unit include the following:

- blood group determination which is done by appropriately mixing Anti-sera (A, B, AB, Anti-serum D) with a drop of blood respectively and observe for agglutination. The resulting blood group is determined by specific anti serum with which it agglutinate and whether it also agglutinate with Anti-serum D or not
- urine Pregnancy test by strip method involves dipping the test area of the strip into a sample of early morning urine of the person being observed. Presence of pregnancy is detected by the appearance of two red coloured line (positive) and appearance of a single red line denotes absence (negative) of pregnancy
- urinalysis which is an important diagnostic test of renal, live dysfunctions which is carry out by briefly dipping the urinary strip completely into the urine and allow the test pads to contact with the sample and removing the excess urine on pad by taping the strip against the edge of the container
- the resulted colour that depict the presence or otherwise of various diagnostic chemical substances in urine is matched with the colour chart supplied with the strips for appropriate reporting and interpretation.
- faecal Occult blood test, which is employ in the diagnosis of gastrointestinal Carcinoma or ulcer, can be done using Okokit method by placing the tablet on a smear of the stool made on the special mat supplied with the reagent kit, and add two drops of diluent and another two drops after 2 minutes. The blue colour change observed after 5 minutes depicts the presence (positive) of occult blood and no colour change denote the absence (negative).
- stool microscopy, in this form of test, saline and iodine wet preparations are made. The iodine preparation stains ova or cyst of parasites providing contrast which aid the identification of the parasite ova

- fasting Blood Sugar by Glucose Oxidase method: The reaction in this enzymatic method yields a colour derivative product and the concentration of glucose is measured as a function of the colour intensity when compared with a standard solution similarly treated.

## **6.0 TUTOR-MARKED ASSIGNMENT**

1. Briefly describe the principle of glucose oxidase method.
2. State three other clinical laboratory testing procedures.

## **7.0 REFERENCES/ FURTHER READING**

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## **UNIT 5      LABORATORY MANAGEMENT**

### **CONTENTS**

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
  - 3.1 Fundamental of Management
  - 3.2 Management Functions
  - 3.3 Human Resources Management
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  - 3.5 Control and Improvement of the Laboratory Processes
  - 3.6 Financial Management
  - 3.7 Management of Equipment and Supplies
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- 6.0 Tutor-Marked Assignment
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### **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**

By 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 Fundamental of Management**

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

- Management functions
- Human resources management
- Planning and execution of goals
- The control and improvement of the laboratory processes
- Management of finances and supplies
- Management of equipment

### 3.2 Management Functions

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

- **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.
- **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.
- **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.
- **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.
- **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.

### 3.3 Human Resources Management

Generally in management, people are regarded as the most valuable assets of any organisation. So, goal fulfilling oriented organisation 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 and informed employees. Essentials of building a positive culture include:

- **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.
- **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.
- **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.
- **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 non-productive environment. Problem resolution should be handled in a manner that preserves personal dignity for all concerned.
- **Provision an environment of open communication:** This can be effected by holding monthly operational meetings

## B. Management Tools

The common management tools are:

- Meeting
- Memoranda
- Letters
- 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.

## 3.4 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 organisation (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:

- **The business plan:** This deals with the laboratory's mission, vision, objectives and their goals.

- **The marketing plan:** This describes the customers, type or nature of tests and services to be rendered.
- **The operation plan:** It describes the facilities and equipment, and the staffing of the laboratory
- **The financial plan:** It describes needs for capital, financial resources and budget.
- Strategies for implementation.

### 3.5 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 the three phases of laboratory testing. These phases are:

- **Pre-Analytical Phase:** This includes guidelines and information about specimen collection and transportation.
- **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.
- **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.

### 3.6 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:

- Budgeting
  - Test Cost Accounting
  - Capital Expenditure
  - Expense Report
  - 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.

### **3.7 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:

- management functions (planning, organising, staffing, directing and controlling)
- 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
- 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

- 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
- 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
- management of equipment and supplies requires the laboratory to have a management policy for their equipment and supplies.

## **6.0 TUTOR-MARKED ASSIGNMENT**

1. List the fundamental elements of management applicable to laboratory management.
2. Briefly discuss the management function.
3. Planning is the road map to achieving a desired goal; briefly discuss this in relation to clinical laboratory management.

## **7.0 REFERENCES/FURTHER READING**

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