Course Code: BIO 303
Course Title: BIOLOGICAL TECHNIQUES
Number of Units: 2 UNITS
Course Duration: 3 hours per week

COURSE DETAILS:

Course Coordinator: Sammy O. Sam-Wobo (B.Sc., M.Sc., PhD)
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Office Location: B201, COLNAS Building
Other Lecturers: Prof. A. B. Idowu and Dr. Gabriel Dedeke

COURSE CONTENT:

Experimental design
Microscope, preparation of microscope slides,
Photometry, colorimetry, chromatography, conductometry,
Specialized preservation techniques

COURSE REQUIREMENTS:

The course is a compulsory course for 300L Biological Sciences and Microbiology students.
Students are expected to participate in all course activities and have a minimum of 75%
attendance to be able to write the final examination

READING LIST:

LECTURE NOTES

High points

Experimental Designs
Types of Research
The design is the pattern, which the data collection will take. In other words, the tools and
how they will be used for data collection. The research design will depend on:

      a.   Project objectives
      b.   Type of research

At this stage the researcher must keep in mind the type of statistics that will be used and
the type of tables that will be necessary. Too often many researchers embark on data
collection without a thought about how the data will be collected and analysed.

Generally research can be classified into either subject/discipline area such as Bio-medical
Sciences Research, Social Research, and Educational Research. Although disciplines differ,
the entire body of research can be classified into three broad categories: historical,
descriptive and experimental.

Historical or Retrospective Research

It is that area of investigation which deals with the collection of information on past events and situations using objectives tools. The design also includes the organisation of the information obtained in order to appreciate the internal consistency in the past and thus generalize to present and future events.

The main sources of historical research are:
a.   Oral evidence, physical evidence, artifacts, pictures autobiographies, records, minutes, letters, memoirs, witness account. These are directly obtained and are called primary sources of information for this reason.
b.   Secondary sources are data obtained from a source, which also obtained it from some other source e.g. biographies, implied evidence, story retold by someone.

Descriptive Research

Descriptive research is a term used to qualify a wide range of other types of research. Irrespective of the type, descriptive research collects information about a defined situation, condition or environment and the people. It is a commonly encountered type of research in Social Sciences, field research, epidemiological research and biomedical research (applied).

Surveys are especially popular and will be given special attention as a typical type of descriptive research. Surveys involve the collection and analysis of data of a small number of the population and generalizing it to the entire population. The commonly used tools for collection of information are questionnaires, interviews and observations. Epidemiological investigations, social research and similar studies, controls may not be necessary since the variables can hardly be manipulated.

Experimental Research

Experimental research is a controlled investigation where certain variables are manipulated while certain variables are kept constant. The control group is the Standard with which the Experimental group will be compared in order to assess the role of the variable factor on the experimental group.

Research Design

This is not the same as experimental design and should not be seen as such.
The research design is the framework (or the plan) for data collection in the most objective and logical manner in order to test the research objectives.
The research framework must be designed before data is collected.

Guideline for developing Research Design
The following steps will assist the development of a good design
1.   State the objectives
2.   List the required information (variables) which will be collected from each objectives.
3.   Identify the instrument for data collection i.e. the tool for collecting information about the variables.
4.   Identify the population and the sampling frame.
5.   Specify the likely (statistical) analysis to be done.
6.   Sketch (dummy) tables that would be required for presenting the information.

There is a wide range of designs that could be used depending on the objectives.

Sampling

Data is a term used to describe all the pieces of information upon which decisions will be made after arranging them in an intelligible form.

Population

It is not practical to look at every object in the situation being investigated. The investigator must therefore take a sample form the population (entire set of objects) because:

- the size of the population may be too large to handle
- time constraint may not permit
- limited resources for a large survey may not permit

The sample is a portion of the entire set of observable events which is carefully selected to represent the latter.

Sample Size Determination

The method used to select a representative sample is to first establish a sampling frame i.e. to define the number of observable events (e.g. census figures, or electoral registers). There are general rules about determination of sample size:

Systematic sampling is commonly used when objects that are arranged in particular series make up the population (e.g. Houses).

Stratification is a method of selection which ensures that every segment (stratum) of the population is identified and its proportion of the populating determined.

Self-selection. Many researchers sometimes request community members to assemble at a central point and to include all those who show up is included in the sample. This method is widely used in health surveys. It has several disadvantages and is not recommended in research.

(i)   It is not much different from using hospital records of attendance.
(ii)   The members in the sample are self-selected; hence every member foes not have an equal chance of being in the sample.
(iii)   Those who think they have the problem under investigation will be more in the sample than those who think they do not have the problem.

Instruments of Data Collection

Research Instruments are tools for obtaining the required data from the population in order to realize the objectives of the research.

General Guide in Selection of Research Tools

- List the objectives
- Identify the variables of interest, i.e. those factors which when measured will provide information on the objectives.
- Identify the tool that will be used to measure (collect information about) the listed factors.

MICROSCOPY

Microscopy is the principal tool in biology. Its development underlines the development of cytology, histology, parasitology and many other branches.

TYPES OF MICROSCOPE
- COMPOUND MICROSCOPES (LIGHT MICOSCOPE)
- PHASE CONTRAT MICROSCOPES
- FLUORESCENCE MICROSCOPE
- TRANSMISSION ELECTRON MICROSCOPE
- SCANNING ELECTRON MICROSCOPE

BASIC COMPONENTS OF COMPOUND MICROSCOPE
Beginning from below:
- Light Source
- Condenser
- Object Stage
- Objective lenses
- Body tube
- Eyepiece

N.B.: The components and Instrumentation of different types of Microscopes vary from one type to the other. Though they are all built on the same general pattern.

CHROMATOGRAPHY – (From two Greek words – chroma-colour and graphcin to write).
- It is a collective term for a set of laboratory techniques for the separation of mixtures.
- It involves passing a mixture dissolved in a MOBILE PHASE through a STATIONARY PHASE, which separates the ANALYTE to be measured from other molecules in the mixture, based on differential partitioning between the mobile and stationary phases.

CHROMATOGRAPHY
(a)   PREPARATIVE (a form of purification)
(b)   ANALYTICAL (These two are not mutually exclusive)

GROUPING OF CHROMATOGRAPHY BY TECHNIQUES
(A)   TECHNIQUES BY CHROMATOGRAPHIC BOO SHAPE
- Column Chromatography – Stationary phase is in column.
- Planar Chromatography – Stationary phase is a plane such paper, glass plate.
    (i)   Paper chromatography
    (ii)   Their layer chromatography
(B)   DISPLACEMENT CHROMATOGRAPHY
(c)   TECHNIQUES BY PHYSICAL STATE OF MOBILE PHASE
(D)   AFFINITY CHROMATOGRAPHY

COLORIMETRY:
COLOURIMETRY is a technique used to determine the concentration of coloured compound in solution.
COLORIMETER: A device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light.

CALIBRATION:
1.   Prepare different solutions plans a control (usually a mixture of distilled water and another solution).
2.   Fill the control unto a *cuvette* an place inside a colorimeter to calibrate the machine.

USE
- After calibration, then the machine can be used to find the densities and/or concentrations of the other solutions.
- Calibration must be repeated after each determination.

FILTER
The colour and size of the filter used are extremely important. For example if liquid/solution to be measured is blue the filter must be set to red.
N.B.: Colourimeric assays use reagent that undergo considerable and measurable colour change in the presence of the analyte from which density or concentration or presence of a substance is determined.

SPECTRO PHOTOMETRY
SPECROPHOTOMETRY is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.
-       It deals with visible light, near-ultraviolet and near-infrared rays (between 250nm-2500nm).
-       It involves the use of a SPECTROPHOTOMETER popularly referred to simply as SPEC.

SPECTROPHOTOMETER
-       It is a photometer (a device for measuring light intensity).
-       It is most commonly used for the measurement of transmittance or reflectance in a solution or transparent material, the polished glass.
-       Depending on the wavelength of the photometric determination, calibrations are needed on the machine using standards that vary on type.

USES
Span various fields, such as, physics chemistry biochemistry and molecular biology. Ultimately a spectrophometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths.

CLASSES:
There are two major classes of the devices:
(i) Single Beam
(ii) Double Beam

SEQUENCE OF EVENT IN A SPECTROPHOTOMETER:
1.   Light source shines through a monochromator
2.   An output wavelength is selected and beamed at the sample.
3.   A fraction of the monochromatic light is transmitted through the sample and to the photodetector.

CALIBRATION – The procedure for calibration is known as ZEROING

1. Absorbancy of a reference substance is set as a baseline value.
2. The absorbancies of all other substances are recorded relative to the initial "zeroed" substance.
3. The spectrophotometer then displays percentage (%) absorbancy (amount of light absorbed relative to the initial substance).

TYPES OF SPECTROPHOTOMETRY
1. UV – Visible spectrophotometry – (visible region 400 – 700nm) spectrophotometry – used in colorimetry science). (UV = Ultraviolet).
2. IR – Spectrophotometry – (designed for main infrared regions).
3. Spectroradiometers – (designed to measure spectro density of illuminants).
4. Atomic Absorption Spectrophotometry.