EDO UNIVERSITY IYAMHO
Department of Microbiology
MCB 313 Microbiological Techniques

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Lectures: Wednesday, 11am – 12 pm, NLT3: Faculty of Science building

Practical: Wednesday, 1pm-3pm, Microbiology Laboratory, Faculty of Science building

Office hours: Wednesday, 9am to 10am (just before class) Office: Faculty building, Floor1, Rm 7

Description: This course is intended to give the students a thorough knowledge of microbiological techniques and tools for conventional and modern best practices. This course covers cutting-edge topics such as microscopy, cultivation and enumeration of microorganisms and identification of cellular, colonial and biochemical characteristics of microorganisms.

Prerequisites: Students should have some advance knowledge of systemic and diagnostic microbiology. They should be acquainted with the concepts in theory of general characteristics of microorganisms (*e.g.,* cultural and genetic characteristics); isolation of microorganisms (*e.g.,* role of microorganisms in food, water, air); sterilization and disinfection and identification of microorganisms. Students should also be familiar with basic concepts of microbial taxonomy such as kingdom, order, family, class, genus, and species for micro-organisms.

Assignments: We expect to have 5 individual homework assignments throughout the course in addition to a Mid-Term Test and a Final Exam. Home works are due at the time stated. Home works are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams. There will also be 1 individual practical project in this class. The goal of this project is to have the students experiment with very practical aspects of microbiology.

Grading: We will assign 10% of this class grade to home works, 10% for the practical, 10% for the mid-term test and 70% for the final exam. The Final exam is comprehensive.

Textbook: The recommended textbook for this class are as stated:

Title: ***Basic Medical Microbiology***

Authors: Patrick Murray

Publisher: Elsevier, 1st edition

ISBN-9780323478533

Year: 2017

Title: ***Microbiology Practical Manual***

Compiled by: Ehis-Eriakha C.B. (Ph.D), Ezeanya C.C., Omenai D.I.

Publisher: Edo University Iyamho, Edo State, Nigeria

Year: 2018

Title: ***District Laboratory Practice in Tropical Countries****, Part 1, 2nd Edition*

Author: Monica Cheesbrough

Title: ***District Laboratory Practice in Tropical Countries****, Part 2, 2nd Edition*

Author: Monica Cheesbrough

Title: Microbiology: Laboratory Theory and Application, 3rd Edition

Authors: Michael J. Leboffe and Burton E. Pierce

Lectures: Below is a description of the contents. We may adjust the directive to accommodate the materials you need for the projects.

Microscopy

Microscopy deals with the use of the microscope. Microscopes are instruments used to view microorganisms. They magnify these tiny organisms that are too small to be seen with the unaided eye. Microscopes differs by their resolving power. There are basically two main classes of microscope: The Light Microscope and The Electron Microscope. Under the light microscope, there are different types typically used in microbiology. They are: Bright-field microscope, Phase-Contrast Microscope, Dark-field Microscope, Fluorescence Microscope and Differential Interference Contrast (DIC) microscope. The electron microscope have remarkably high resolving power and have been explored extensively by microbiologist to observe detailed structures of microorganisms. There are two main types of electron

microscope: Transmission electron microscope and Scanning electron microscope. In electron microscopy, there are some significant techniques. For example; the application of ultrathin section of embedded materials. In microscopy, there have been other microscopes that have been explored like: Confocal Scanning Laser Microscope, Scanning Probe Microscope etc.

LIGHT MICROSCOPE

The light microscope is known to possess a distinct resolving power which is approximately half the wavelength of light.

TYPES OF LIGHT MICROSCOPE

Bright field Microscope

This is the widely available and used microscope in the field of microbiology across several countries. It possess two main lenses: objective and ocular lens. Specimens are viewed owing to the contrast established between the specimen and the field (bright). Dyes are introduced to specimens to improve their contrast.

Phase Contrast Microscope

The invention of the microscope resulted from the need to improve the contrast difference between specimens and the field (surrounding medium). Consequently, microbial cells are viewed life without staining.

Dark-field Microscope

In dark-field microscopy, there is an improvement in the lighting system in such a manner that light penetrates from the sides of the viewed sample. Thus, a dark field is created in contrast to the brighten sides of the specimen.

Fluorescence Microscope

Here, fluoresce specimens are readily visualized using this microscope. Fluoresce is described as the absorption of short wave-lengths of light with the reflection of long wavelengths of light.

ELECTRON MICROSCOPE

One unique feature of the electron microscope is the high resolving power. Owing to that, very detailed structures of microbial cells have been examined. Compared to the light microscope, the resolution of electron microscope is due to the short wavelengths of electrons compared to the protons of white light. One significant principle of operation of an electron microscope is the "application of shadowing".

There are of two types:

Transmission Electron Microscope (TEM): The concept behind the operation of this type of microscope is the application of beam of electrons protruding from the electron gun following focusing by an electromagnetic condenser lens.

Scanning Electron Microscope (SEM): It employs three-dimensional imaging of the surface of microbial cells.

Cultivation of microorganisms and Types of growth medium

In the cultivation of microorganisms, it is crucial that the suitable growth media is used. In microbiology, culture medium is used for the cultivation of bacteria and fungi. Culture medium is an artificial formulation which could be in solid or liquid form used for the growth, transport and storage of microorganisms. The medium consist of specific required growth factors (e.g.; carbon source-carbohydrates; some other growth factors include: vitamins in minute quantity). Culture media are of different types which encompasses of general-purpose and specific-purpose medium. In specific-purpose medium, there are different types of culture medium. They are: Differential, Selective, Enrichment medium etc.

DYES AND STAINING TECHNIQUES FOR DIFFERENTIATION OF MICROORGANISMS

Dyes are members of the chromofoam group with double bond which attributes to their distinct colour. Dyes bind with cells by ionic co-valent or hydrophobic bond (eg; positively charged dyes will bind to negatively charged structure on the cell). There are basically two kinds of dyes: Acidic (eg; pepsin) and Basic (eg; methylene blue). The acidic dyes binds to positively charged cell structure whereas the basic dyes binds to negatively charged cell structure.

There are different types of staining in microbiology. They include: Gram stain, Giemsa stain, Acid-fast stain etc. Basically, staining of bacterial cell is done to reveal the shape, size and arrangement of the cells. Consequently, enhances easy distinction of among different bacterial species on the basis of the colour they retain.

GRAM STAIN

In bacterial taxonomy, gram stain provides a clear demonstration of their characteristics. It is a fundamental staining technique. The technique employs the application of a basic dye (crystal violet). The mainstay of the differentiation by gram staining is a factorial of the bacterial cell wall.

ACID-FAST STAIN

Bacteria are described as acid-fast when they retain a basic dye (carbol-fuchsin) that have been dissolved in a phenol-alcohol-water mixture. Preceding, discoloration with acid-alcohol is a contrasting counterstain which appears as blue or green.

GEIMSA STAIN

This stain is mostly applied on parasites and some bacteria like Chlamydia to improve contrast thus making them more visible under the light microscope.

FLAGELLA STAIN

Here, staining with basic fuchsin enhances the visibility of the flagella under a light microscope as flagella are too tiny to be visible under the light microscope.

CAPSULE STAIN

The most common capsule stain that is employed in microbiology is the Welch method. This method involves the treatment with a hot basic dye (crystal violet) and subsequently washing with copper sulfate solution.

NEGATIVE STAIN

This employs the use of an acidic stain to stain the background thereby allowing the microbial cells to contrast in their colourless form. This is specifically for difficult-to-stain cells.

Techniques for enumeration of microorganisms

In enumeration of microorganisms, different methods are applied. Here, irrespective of the methods, it involves the determination of the number of viable and culture-able colony forming units (CFU) in a sample. Between 20 and 200 CFU can be counted on a typical Petri plate. Colonies above 200 are presented as "too numerous to count" (TNC). Two methods for enumeration of microorganisms that are commonly applied in microbiology: POUR–PLATE METHOD, and SPREAD–PLATE METHOD.

Preparation of media for microbial growth

In the preparation of microbial growth media, the sterile state of the media is subject to the amount of aseptic techniques and sterilization done during preparation. Sterilization is mostly done by moist heat method (eg; autoclave). Autoclaving typically used for sterilization of growth media is done at 121^0 c for 15minutes and later allowed to cool to 45^0C. The procedure for preparation of culture media is subject to the manufacturer's instruction for a specific culture medium. However, in a preparation the basic steps involves measurement of an adequate agar powder and dilution into appropriate quantity of deionized water in a flask.

PURE CULTURE TECHNIQUES AND STERILIZATION

Pure culture techniques are procedures geared to ensure isolation of a given microorganism from a mixed culture. Furthermore, the culture is protected from subsequent contamination. Sterilization involves absolute destruction, inactivation of microorganisms. Sterilization can be achieved using any TWO physical methods: Dry Heat and Moist heat sterilization. The different methods of sterilization will be explored which include: Dry heat, Red heat, Incineration, Flaming, Hot air, Infra-red radiation, moist heat, Tyndallisation, Pasteurization, Ethylene oxide gas and Ionization radiation etc.

DRY HEAT STERILIZATION

There are many methods employed in dry heat sterilization. They include:

Red heat:

Instruments such as inoculating wire-loops and needles are sterilized in flame until red hot prior to use.

Incineration:

This involves the use of electrically heated gas-forced device called an Incinerator. This method is most useful in sterilizing pathological materials.

Flaming:

This involves the use of flame to sterilize materials in the laboratory.

Hot air:

This is one of the simplest and commonest method of sterilization till date. Materials like: glassware and other laboratory items in an oven.

RADIATION

Infra-red radiation:

This method involves the use of infra-red rays for sterilization which are directed on the objects.

MOIST HEAT STERILIZATION

Moist heat sterilization involves sterilization by steam under pressure.

Tyndallisation:

This method involves the sterilization of solutions via heating for consecutive days.

Pasteurization:

This method is employed in the destruction of microbes. Pasteurization involves two processes:

- The Holder Process.
- The Flash process.

The Holder Process

The elevated temperature that is reached usually in milk sterilization, the temperature is $62^{O}C$ for 30 minutes.

The Flash Process

An accelerated temperature kept at a very short duration (20 seconds).

Sub-culturing procedures

Sub-culturing involves the aseptic transfer of microbial colonies from its original growth media to another sterile growth medium. Sub-culturing is done in microbiology for several reasons but most importantly it is done to obtain pure culture. The simplest and commonest technique of sub-culturing is the streaking methods. This methods involves the use of a sterile inoculating loop or needle to aseptically transfer microbial colonies by making streaking on the agar plate. The most appropriate streaking method is the quadrant streak method.

Identification of microorganisms

Methods for identification of microorganisms include: studying the shape of the microorganism and arrangement of the cells (microscopic), colonial morphology (macroscopic) and biochemical analysis. In identification of microorganisms, staining techniques are employed in microscopic examination for enhancement of cell details. Such staining techniques used in the identification of microorganisms includes: Simple stain, Gram stain, Giemsa stain, Acid-fast stain etc.

Maintenance and preservation of cultures

Maintenance and preservation of cultures involves periodic sub-culturing technique to ensure the live organisms remains alive although with the risk of contamination and mutation. Culture organisms are preserve for the purpose of generating a stock culture of standard strains which imperatively serves as control or for other studies. There are different ways of maintaining and preserving culture organisms. They include: Culture plate, culture tube, freeze drying (eg; lyophilisation). Serial sub-culturing techniques are employed to keep fast growing microorganisms alive. Some of the useful medium used for preserving bacteria are: Robertson's cooked meat medium while for Fungi is Sabouraud dextrose medium.