- Course Code: MCB 202
- Course Title: General Microbiology II
- Number of Units: 3 Units
- Course Duration: Three hours per week

## COURSE DETAILS:


- Course Coordinator: Prof (Mrs) M.O. Bankole *PhD*
- Email: aadmokole@yahoo.com
- Office Location: Room A212, COLNAS
- Other Lecturers: Dr (Mrs) Oluwafemi
  Dr (Mrs) Odedara


## COURSE CONTENT:

**1. Principles and Methods of Microbiology** Microscopy and Microscopic techniques, Staining techniques for differentiation of microorganisms
**2. Culturing of microbes:** Preparation of culture media for microbial growth, enrichment and cultivation of microbes, Isolation of pure cultures, Factors affecting the growth of microorganisms, Conditions for Growth, Enumeration of microorganisms, Maintenance of cultures
**3. Identification of microorganisms:** Colonial and cellular morphology, biochemical and molecular identification procedures.
**4. Principles of Sterilization and Disinfection**

## COURSE REQUIREMENTS:

This is a compulsory course for Microbiology students

## READING LIST:


1. Pelczar, M.J., Reid, R.D. and Chan, E.C.S. *Microbiology.* McGraw-Hill Book Company.
   New York. Fourth Edition. 1977.
2. Pyatkin, K. and Krivoshein, Y. *Microbiology*. Mir Publishers, Moscow. 1980
3. Pelczar, M.J., Krieg, N. and Chan, E.C.S. *Microbiology: Concepts and Applications*. McGraw-Hill, New York. International Edition. 1993

4. Willey, J.M., Sherwood, L.M. and Woolverton, C.J. *Prescott, Harley, and Klein's Microbiology*. International Edition. McGraw-Hill, New York. 2008

## LECTURE NOTES

- **Principles and Methods of Microbiology**
  - Microscopy and Microscopic techniques
  - Contributions of Zechariah Jensen, Robert Hooke, Antonie van Leeuwenhoek etc

- **Types of Microscopes**
  - Light/Compound Microscope: Function of the parts of compound microscope
  - Bright-field microscopy
  - Dark –field microscopy
  - Phase contrast microscopy
  - Fluorescent microscopy
  - Electron Microscopy
  - Scanning Electron Microscopy

STAINING TECHNIQUES FOR DIFFERENTIATION OF MICROORGANISMS

- Stains (Dyes) are generally salts in which one of the ions is coloured (chromophore). A salt is a compound composed of a positively charged ion and a negatively charged ion.
- For example, the dye, methylene blue, is actually the salt, methylene blue chloride, which will dissociate in water into a positively charged methylene blue ion which is blue in colour and a negatively charged chloride ion which is colourless.
- The specificity of stains are as determined by their chemical structure.
- **Basic stains** are stains that are cationic (positively charged) and will therefore react with material that is negatively charged and bind to negatively charged cell structures in the microbial cytoplasm. They work best at neutral or alkaline pH.

- The cytoplasm of all bacterial cells have a slight negative charge when growing in a medium of near neutral pH and will therefore attract and bind with basic dyes. The cationic or basic dye has an affinity for nucleus and ribosome that exist in the cytoplasm of the cell with a net negative charge and are termed basophilic structures.

- Some examples of basic dyes are Crystal Violet, Safranin, Basic Fuchsin and Methylene Blue. [dye]+ OH-
- **Acid stains** have negatively charged chromophores and are repelled by the bacterial surface forming a deposit around the organism.
- They are repelled by the negatively charged cytoplasm and gather around the cells, leaving the cells clear, transparent and unstained. When used, the

background is stained but the bacteria remain colourless. They are used for negative staining techniques.

- The anionic (negatively charged) or acid dye has an affinity for positively charged cytoplasm and other components like mitochondria and cilia, which are then termed acidophilic structures.
- Nigrosin and Congo red are examples of acid dyes.
  [dye]- H+

- **Neutral stains** are mixtures of acid and basic stains and have a coloured cation and a coloured anion.

- They are used to stain both nucleus and cytoplasm.

- Examples are Leishman's stain and India ink. Leishman's stain, which is made by mixing the acid stain eosin with the basic stain methylene blue in alcohol.

- Cell materials are said to be neutrophilic if receptive to neutral dyes.

- Principles of Gram Staining

- Principles of Acid Fast Staining

- Principles of Endospore staining

- Principles of capsule staining

- Principles of flagella staining

CULTURING OF MICROORGANISMS

- **Preparation of culture media for microbial growth, enrichment and cultivation of microbes:** Weighing of appropriate quantity of the appropriate medium, mixing with distilled water, autoclaving, sterilization of Petri dishes, aseptic techniques of pouring the molten medium into the Petri dishes.

- **Isolation of pure cultures**: Sterilization of inoculating loop, aseptic techniques, streaking, incubation, subculturing.

**FACTORS AFFECTING THE GROWTH OF MICROORGANISMS**

- **Nutritional Requirements**

All forms of life, from microorganisms to human beings, share certain nutritional requirements, in terms of the chemicals necessary for their growth and normal functioning.

- Source of Energy:
  Microorganisms can be classified as
  - ✓ Phototrophs: utilising radiant energy (sunlight) as sole source of energy

- ✓ Chemotrophs: depend on oxidation of chemical compounds for their energy.

- Source of Carbon
  - ✓ Autotrophs: Carbondioxide is their sole carbon source
  - ✓ Heterotrophs: organic form of carbon is the sole source of carbon

- Other nutrients: Water, Vitamins

- Other elements: Nitrogen, Sulphur, Phosphorus, Sodium, Potassium, Calcium, Magnesium, Manganese, Iron, Zinc, Copper, Cobalt.

**CONDITIONS FOR MICROBIAL GROWTH**

- Temperature: Microorganisms could be classified as:
  - ✓ Psychrophiles: they are able to grow at 0˚C or lower, though they grow at higher temperatures (15˚-20˚C).
  - ✓ Mesophiles: they grow within temperature range of approximately 25˚-40˚C.
  - ✓ Thermophiles: they grow at temperatures between 45˚ – 60˚C. There are some extreme thermophiles that can withstand temperatures about 100˚C e.g *Thermus aquaticus.*

Optimum temperature is the temperature of incubation which allows for most rapid growth during a short period of time (12 to 24 h).

- pH:
  - ✓ Acidophiles: they grow at acidic pH
  - ✓ Neutrophiles: they grow at neutral pH
  - ✓ Alkalophiles: they grow at basic/alkaline pH

For most bacteria, the optimum pH for growth ranges from 6.5 – 7.5.

- Oxygen Requirement:
  - ✓ Aerobes: grow in the presence of free oxygen
  - ✓ Anaerobes: grow in the absence of free oxygen
  - ✓ Facultative anaerobes: grow either in the presence or absence of free oxygen.
  - ✓ Microaerophiles: grow in the presence of minute quantities of free oxygen.

**CONDITIONS FOR MICROBIAL GROWTH**

- Additional requirements:
- ✓ Halophiles: They grow only in medium with unusually high concentration of salt (10-15%) found in brines, salt packs, ocean water and certain foods.

- Growth Curve of microorganisms

**ENUMERATION OF MICROORGANISMS**

- ✓ Direct microscopy
- ✓ Plate Count
- ✓ Membrane Filter Count

- ✓ Determination of cell density by turbidimetric method
- ✓ Determination of cell concentration by measurement of Nitrogen Content

**MAINTENANCE OF CULTURES**

- ✓ Periodic transfer unto fresh medium
- ✓ Rapid Drying in a frozen state (Lyophilisation)
- ✓ Overlaying with mineral oil
- ✓ Storage at very low temperatures

**IDENTIFICATION OF MICROORGANISMS**

- ❑ **Morphological Identification**

➢ **Shape and Arrangement**

✓ Spherical Cells

They are ellipsoidal cells called cocci (singular: coccus). The particular pattern is characteristic of particular bacteria.

- Diploccocci are arranged in pairs.
- Streptococci are arranged in chains.
- Tetracocci are arranged in two planes characteristic group of four cells.
- Staphylococci have an irregular pattern and are arranged in bunches.
- Sarcinae are in a regular pattern producing a cubiodal arrangement.

- Cylindrical cells: They are rodlike (singular: bacillus; plural; bacilli).
- Diplobacilli arranged in pairs
- Streptobacilli arranged in chains, palisade arrangement grouped like matchsticks.

✓ Spiral-shaped bacteria (singular: spirillium, plural: spirilla) .

They occur as unattached cells with characteristic spiral shape and rigid cell wall.

Short incomplete spirals are called comma bacteria or vibrio.

MOLECULAR IDENTIFICATION OF MICROORGANISMS

This classification is based on DNA and RNA relatedness between organisms.

- ➢ % G+C: This determines whether the organisms are similar or differ. If two organisms have different % G+C, then they are likely to be different.
- ➢ DNA-DNA Hybridization/DNA Homology: If DNA from two organisms are similar, pairing will occur in the DNA strands when mixed, if not, they are not of the same species.
- ➢ 16S rRNA Sequencing: Ribosomal RNA homology and ribosomal RNA cataloguing determine the molecular characteristics and can be used to demonstrate the degree of relatedness.

BIOCHEMICAL IDENTIFICATION OF MICROORGANISMS

- ➢ Biochemical reactions

- ✓ Gram reaction: The response of bacteria cell wall to gram staining is able to classify the organism as either Gram positive or Gram negative.

- ✓ Assimilation of sugars

PRINCIPLES OF STERILIZATION AND DISINFECTION

- Microorganisms can be removed, inhibited, or killed by physical agents, physical processes or chemical agents.

- Sterilization is the process of destroying all forms of microbial life while disinfection only kills the growing cells but not necessarily the resistant spores of pathogenic organisms.
- Steam under Pressure (Autoclaving): This is achieved at 121˚C and 1 atmospheric pressure using an autoclave.
- Tyndallisation: This is also called fractional sterilisation. It involves heating a material at 100˚C (temperature of free flowing steam) on three successive days with periods of incubation in between the days. Steam Arnold is an instrument used for this.
- Boiling water:

- Pasteurization: This process uses controlled temperature for a particular period of time.

It could be achieved at 62.8˚C for 30 minutes (as for milk) which is called Low-temperature holding (LTH) method, or 71.7˚C for 15 seconds in the High Temperature Short time (HTST) method.

- Dry Heat Sterilization: This is used for laboratory glasswares, oils, powders etc. It is achieved with an hot-air oven. For glasswares, at 160˚C for 2 hours.
- Incineration: This is the destruction of microorganisms on a material by burning. Eg for inoculating loop in Bunsen burner.

- Freezing: This makes the organisms dormant and not necessary kills them.
- Radiation: Ionizing radiation, UV radiation, X-rays, Gamma rays
- Filtration: This is used to sterilise heat labile materials such as enzymes, vitamins and antibiotics. Membrane filters sterilise biological fluids. High-efficiency particulate filters (HEPA) filters, also called Fibreglass filters, are used to disinfect the air and are used in designing of laminar air flow.
- Ultrasonicator: For decontaminating delicate cleaning instruments.

DISINFECTION METHODS

- Phenols and phenolic compounds
- Alcohols eg ethanol, methanol
- Halogens e.g chlorine, iodine, hypochlorites
- Heavy metals and their compounds e.g mercury chloride, silver nitrate and copper sulphate
- Dyes e.g malachite green, brilliant green and crystal violet
- Detergents e.g surfactants

- Quaternary ammonium compounds e.g cetylpyridinium
- Acids and Alkali
- Glutaraldehyde
- Gaseous chemosterilisers eg ethylene oxide, formaldehyde.