COVENANT UNIVERSITY

OMEGA SEMESTER TUTORIAL KIT
(VOL. 2)

PROGRAMME: MICROBIOLOGY

100 LEVEL

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LIST OF COURSES

MCB121: Laboratory Techniques in Microbiology

***Not included**

COVENANT UNIVERSITY
CANNANLAND, KM 10 IDIROKO ROAD
P.M.B. 1023, OTA, OGUN STATE, NIGERIA

TITLE OF EXAMINATION: B.SC EXAMINATION
COLLEGE: SCIENCE & TECHNOLOGY
SCHOOL: NATURAL & APPLIED SCIENCES
DEPARTMENT: BIOLOGICAL SCIENCES **PROGRAMME**: MICROBIOLOGY
SESSION: 2015/2016 **SEMESTER:** OMEGA
COURSE CODE: MCB 121 **CREDIT UNIT:** 3
COURSE TITLE: LABORATORY TECHNIQUES IN MICROBIOLOGY
INSTRUCTION: Answer FOUR questions **TIME:** 3 HOURS

Section A

1a. Define the following terms and give an example for each term as it relates to the study of Microbiology: (a) Stains (b) Simple stain (c) Differential stain…… ……6mks
1b. List any 5 staining technique you know, give the staining procedure of any one of them …. …….10mks
1c. Draw a typical fungi (*Muc*or *or Aspergillus*) as will be seen on a wet mount…….1.5 mks

2a. What are the ingredients in: (i) Gram staining reagents (ii) Albert staining reagents…...10mks
2b. Give reasons why bacteria cells are hard to see and give solutions to seeing them....3.5 mks
2c. List 8 types of microscopes that can be used in the study of bacterial cells……...4mks
3a. List and draw five bacterial cell shapes……..7.5mks
b. Enumerate 10 foods for which microorganisms are responsible for their production….5mks
c. Name five each of bacteria and fungi species associated with man or his environment...5mks

SECTION B

4) a. Differentiate between a liquid medium and a solid medium (7.5 Marks)

b. Discuss the differences between the following:

i. A pure culture (5 Marks)

ii. A mixed culture (5 Marks)

5) a. Write short notes on the different culturing techniques used in the microbiology laboratory for culturing microorganisms (10 Marks)

b. Write short notes on the characteristics of Agar agar (7.5 Marks)

6) a. Discuss the major types of media used in the microbiology laboratory. Give examples of the different types (10 Marks)

b. Write short notes on the steps involved in the culturing of microorganisms (7.5 Marks)

COVENANTUNIVERSITY

CANAANLAND,KM10,IDIROKOROAD P.M.B
1023, OTA, OGUN STATE, NIGERIA

TITLE OF EXAMINATION: B.Sc DEGREE EXAMINATION
COLLEGE: College of Science and Technology
DEPARTMENT: Biological Sciences **PROGRAMME**: Microbiology
SESSION: 2015/2016 **SEMESTER:** OMEGA
COURSE CODE: MCB 121 **CREDIT UNIT:** 3
COURSE TITLE: Laboratory Techniques in Microbiology
COURSE COORDINATOR: Prof. A. A. Ajayi
COURSE LECTURERS: Prof. A.A. Ajayi; Prof. S.U. Oranusi

MARKING GUIDE

SECTION A

1) a. Differentiate between a liquid medium and a solid medium (5 Marks)

` b. Discuss the differences between the following:

i. A pure culture (5 Marks)

ii. A mixed culture (5 Marks)

iii. A stock culture (5 Marks)

1a. The difference between a liquid medium and solid medium is basically on whether or not a solidifying agent is added

- **Liquid medium** - This is just water to which the appropriate kinds and concentrations of nutrients have been added.
- **Solid Medium** - This is a liquid medium to which a solidifying agent is added. The solidifying agent is agar. It is also called agar agar

1b. The pure culture Technique: This is a term used to describe the technique employed by microbiologist to isolate, grow and maintain organisms of interest. A pure culture is therefore the organism of interest.

The pure culture technique used in the microbiology laboratory consists of the following steps.

- ❖ Preparation of a suitable medium for the organisms. This is so essential because many microorganisms grow in some laboratory basic media while others require specific and special media for their growth and reproduction.
- ❖ Sterilization of a medium is to remove or destroy all living organisms already present Isolation of a pure strain of the organism
- ❖ Isolation of a pure strain of the organism
- ❖ Inoculation of sterile medium with the pure strain. Care should be taken to work strictly under aseptic conditions as contaminations particularly with similar organisms are not easily detected and in liquid cultures contaminations are hardly detected.
- ❖ Incubation of the inoculated plates

A mixed culture: In Microbiology, a mixed culture refers to a laboratory culture that contains more than one species of organism that are grown in a medium. Most pure cultures are usually derived from mixed cultures by methods of separating individual cells so that, when they multiply, each will form an individually distinct colony.

2) a. Write short notes on the different culturing techniques used in the microbiology laboratory for culturing microorganisms (10 Marks)

b. Write short notes on the characteristics of Agar agar (10 Marks)

2a. Microorganisms are cultured in various ways, especially bacteria and therefore there are the following types of culture techniques.

- ✓ Broth Cultures
- ✓ Slant Cultures
- ✓ Stab Cultures
- ✓ Shake Culture
- ✓ Plate Culture
- ✓ Pour Plates
- ✓ Streak Plates

Broth Cultures: Broth cultures are also called liquid cultures. These are cultures or organisms grown in water to which nutrients have been added.

Slant (Agar Slope) Cultures: These consist of test tubes or small bottles to which molten solid medium (e.g. nutrient agar) has been added and allowed to set in a slanting or slopping position. The inoculum may be spread or streaked over the surface of the slope.

Stab Cultures: This is similar to the slant cultures except that the tubes containing solid media are allowed to cool at an upright (vertical) position. It is inoculated by plunging a long straight wire (carrying the inoculum) vertically into the middle of the tube.

Shake Cultures: Test tubes or bottles containing a solid medium are employed. The medium is warmed to 45°C, inoculated and mixed thoroughly by rotating the tube between the hands. The tube is then allowed to cool in an upright position.

Plate cultures are of two types

- Pour Plate
- Streak Plate
- For pour plate - The inoculum is added to a tube or flask of molten medium (45°C), mixed well and it is then aseptically poured into sterile plates.
- Streak Plate - The molten solid medium is poured into sterile plate and allowed to set. The surface of the medium is then streaked.

2b. **Agar or Agar agar** - Agar agar is a polysaccharide extract derived from seaweed, and it has gelling property. It has a molecular weight of between 11,000 KD and 100,000kD. Agar can remain solid at temperatures as high as 100°C but when molten it resolidifies below 45°C. It is added to media at a final concentration of between 1.2% and 2%. There are other gelling agents. Gelatin is another example. The advantage of agar over gelatin is that gelatin readily becomes liquid at 28°C

3) a. Discuss the major types of media used in the microbiology laboratory. Give examples of the different types (10 Marks)

b. Write short notes on the steps involved in the culturing of microorganisms (10 Marks)

3a. The Major groups of media are the following:

- Routine Laboratory Medium
- Differential Medium
- Selective Medium

- Enriched Medium
- Enrichment Medium

Routine Laboratory Medium

- This medium is used for the cultivation of common microorganisms' e.g. nutrient agar or broth, peptone water, meat and yeast extract

Differential Medium

- Contains substances which enable a particular organism to differ visually from other organisms based on certain physiological reactions. Example is the Deoxycholate –citrate medium which contains neutral red indicator. On this medium, colonies of lactose fermenting organisms are red while those of non-lactose fermenters appear colorless

Selective Medium

- This medium consists of substance which inhibits the growth of certain organisms but not of others. Almost all media are selective because no single culture medium favors and supports the growth of a wide range of microbial groups.
- The MacConkey medium is used mainly for gram -negative and enteric bacteria because the bile salt and crystal violet in it inhibits the growth of Gram –positive bacteria and it is therefore selective for Gram –negative and enteric bacteria. The azide- dextrose broth is also selective for streptococci

Enriched Medium

- An enriched medium has certain substances such as blood and serum which is necessary for the growth of some fastidious organisms.
- Example is the fildes' broth. It is used for the cultivation of organisms requiring easily assimilated X and V factors, and they are present in blood contained in the medium.

Enrichment Medium

- This consist of substances which are capable of inhibiting the growth of one kind of organism while allowing unrestricted, growth of the desired organism which may be present in small numbers.
- These substances are therefore said to be enriching the population e.g. Salmonella and some strains of shigella will grow but most of the coliform and other bacteria which are inhibited by selenite will not grow in selenite broth.
- It is thus used in the enrichment and subsequent isolation of the organism from faeces or other sources suspected of faecal contamination.

3b. The pure culture technique used in the microbiology laboratory consists of the following steps.

- ❖ Preparation of a suitable medium for the organisms. This is so essential because many microorganisms grow in some laboratory basic media while others require specific and special media for their growth and reproduction.
- ❖ Sterilization of a medium is to remove or destroy all living organisms already present Isolation of a pure strain of the organism
- ❖ Isolation of a pure strain of the organism
- ❖ Inoculation of sterile medium with the pure strain. Care should be taken to work strictly under aseptic conditions as contaminations particularly with similar organisms are not easily detected and in liquid cultures contaminations are hardly detected.
- ❖ Incubation of the inoculated plates

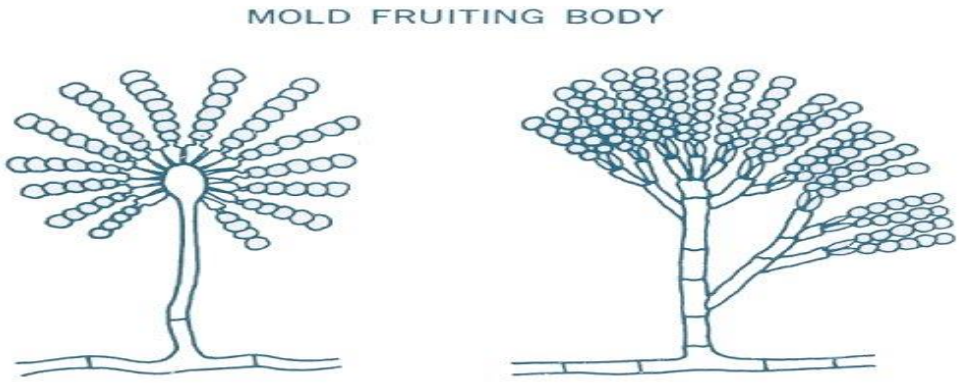
4a. Define the following terms and give an example for each term as it relates to the study of Microbiology: (a) Stains (b) Simple stain (c) Differential stain..... .....6mks

a. **stain** is a substance that adheres to a cell, giving the cell color e.g Methylene blue
b. **Simple stain** is a stain using only a single dye that does not differentiate between different types of organisms. There typically is only a single staining step and everything that stains is stained the same color e.g Methylene blue
c. **Differential stain** is a stain that uses more than one dye and stains different kinds of organisms different colors e.g. Gram stain; Ziehl-Neelsen acid fast stain

b. List any 5 staining technique you know, give the staining procedure of any one of them 10mks

i) Methylene blue staining technique (ii) Gram staining technique (iii) Ziehl-Neelsen acid fast staining technique (iv) Albert staining technique (v) Spore staining technique (vi) Flagella staining technique (vii) Iodine staining technique etc

c. Draw a typical fungi (*Muc*or *or Aspergillus*) as will be seen on a wet mount.....1.5 mks



5a. What are the ingredients in: (i) Gram staining reagents (ii) Albert staining reagents.....10mks

(i) Ingredients in Gram staining reagents include: (1). **Crystal violet** (***Solution A:-*** Crystal violet 2.0 gm; Ethanol, 95% 20 ml; ***Solution B:-*** Ammonium oxalate 0.8 gm; Distilled

water 80 ml. Mix solutions A and B. Store for 24 hours before use).(2). ***Gram iodine:-*** Iodine crystals 1.0 gm, Potassium iodide 2.0 gm, Distilled water 300 ml.

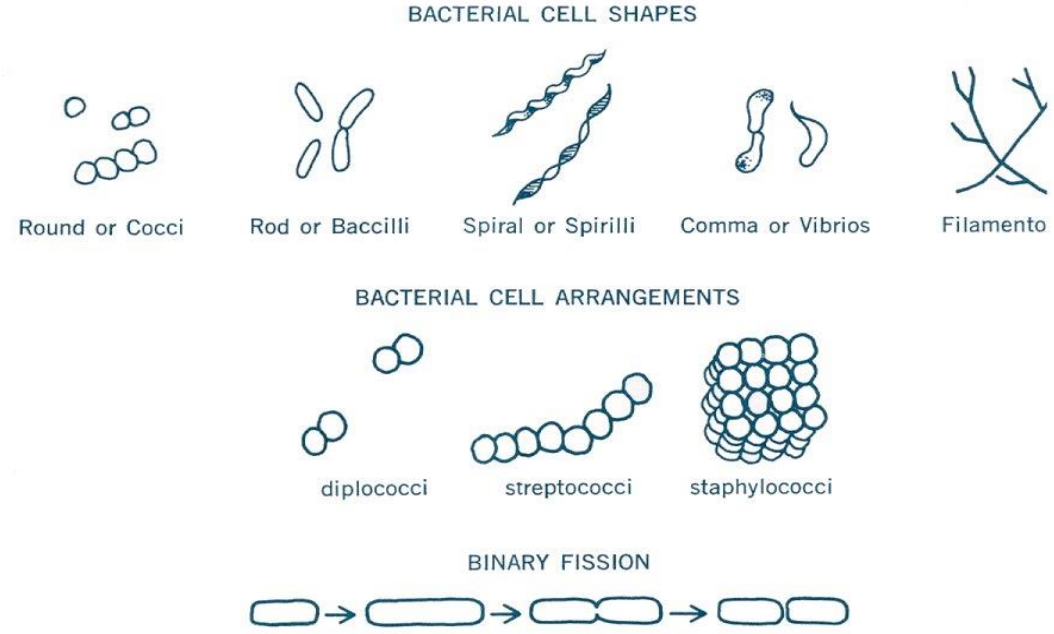
(ii) Ingredients in Albert staining reagents include: 1. ***Albert stain I:-*** Toluidine blue 0.15 gm; Malachite green 0.20 gm; Glacial acetic acid 1.0 ml; Alcohol (95%) 2.0 ml; Distilled water 100 ml. Grind and dissolve the dyes in alcohol, add water and then add acetic acid. Let the mixture stand for 24 hours and then filter.

2. ***Albert stain II:-*** Iodine 2.0 gm; Potassium iodide 3.0 gm; Distilled water 300 ml. Dissolve iodine and potassium iodide in water by grinding in a mortar with a pestle. Filter through a filter paper.

b. Give reasons why bacteria cells are hard to see and give solutions to seeing them....3.5 mks
Bacterial cells are hard to see because: (i) they are small (ii) they are almost transparent
Solutions that can be employed to see bacteria cells include: (i) magnification under a microscope (ii) staining techniques (iii) cultural techniques to form visible colonies from invisible cells

c. List 8 types of microscopes that can be used in the study of bacterial cells.....4mks
(i) Light microscopy (ii) Compound light microscopy (iii) Bright-field microscopy
(iv) Dark-field microscopy (v) Phase-contrast microscopy (vi) Electron microscopy (EM)
(vii) Transmission electron microscopy (TEM) (viii) Scanning Electron Microscopy (SEM)

Q3a. List and draw five bacterial cell shapes.....7.5mks



b. Enumerate 10 foods for which microorganisms are responsible for their production....5mks

Buttermilk	Cheeses	Cocoa	Bread	Gari	Akamu/Eko	Wine
Coffee	Pickles	Sauerkraut	Fufu	Iru	Beer	Pito

Vanilla	Vinegar	Yogurt	Lafun	Ogiri	Burukutu	Oyopo

c. Name five each of bacteria and fungi species associated with man or his environment..5mks

Bacteria: *Escherichia coli, Salmonella, Shigella, and other Enterobacteriaceae, Pseudomonas, Moraxella, Helicobacter, Bdellovibrio, acetic acid bacteria, Legionella, cyanobacteria, spirochaetes, green sulfur, green non-sulfur bacteria, Neisseria gonorrhoeae, Neisseria meningitidis, Moraxella catarrhalis, Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila,, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens, Acinetobacter baumannii, Corynebacterium, Mycobacterium, Nocardia, Streptomyces, Staphylococcus, Streptococcus, Enterococcus, Bacillus, Clostridium, Mycoplasma etc*.....**Any five**

Fungi: *Aspergillus*, *Penicillium, Fusarium, Rhizopus, Mucor* , *Candida albicans, Absidia, Claviceps, Gibberella, Alternaria, Myrothecium*, *Phomopsis, Stachybotrys*, *Trichoderma*, *Trichothecium,* mushrooms, *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Cryptococcus neoformans etc*.....**Any five**