Sri Lakshmi Narayana Institute of Medical Sciences

Date: 09.12.2020

From

Dr. G. Somasundaram
Principal of Allied Health Sciences,
Sri Lakshmi Narayana Institute of Medical Sciences
Bharath Institute of Higher Education and Research,
Chennai.

To

The Dean,
Sri Lakshmi Narayana Institute of Medical College
Bharath Institute of Higher Education and Research,
Chennai.

Sub: Permission to conduct value-added course: CLINICAL MICROBIOLOGY

Dear Sir,

With reference to the subject mentioned above, the department proposes to conduct a value-added course titled **CLINICAL MICROBIOLOGY** from January to February 2020. We solicit your kind permission for the same.

Kind Regards

Dr.G. Somasundaram

FOR THE USE OF DEANS OFFICE

Names of Committee members for evaluating the course:

The Dean: **Dr. Balagurunathan. K**

The HOD: **Dr. Somasundram. G**

The Expert: **Dr. Arvind.**

The committee has discussed about the course and is approved.

Dean	Subject Expert	HOD
(Sign & Seal)	(Sign & Seal)	(Sign & Seal)

DEAN
Prof.K.BALAGURUNATHAN,M.S
(General surgeon)
SRI LAKSHMI NARAYANA
INSTITUTE OF MEDICAL SCIENCES
OSUDU PONDICHERRY

PRINCIPAL
Allied Health Sciences
Sri Lakshmi Narayana Institute of Allied Health Sciences
Osudu, Agaram Post, Puducherry - 605 502.

OFFICE OF THE DEAN

Sri Lakshmi Narayana Institute of Medical Sciences

OSUDU, AGARAM VILLAGE, VILLIANUR COMMUNE, KUDAPAKKAM POST,
PUDUCHERRY - 605 502.
[Recognised by Medical Council of India, Ministry of Health letter No. U/12012/249/2005-ME (P -II) dt. 11/07/2011]
[Affliated to Bharath University, Chennai - TN]

Circular

23.12.2020

Sub: Organizing Value-added Course: CLINICAL MICROBIOLOGY .reg

With reference to the above-mentioned subject, it is to bring to your notice that Sri Lakshmi Narayana Institute of Medical Sciences, **Bharath Institute of Higher Education and Research** is organizing "**CLINICAL MICROBIOLOGY**". The course content and registration form is enclosed below."

The application must reach the institution along with all the necessary documents as mentioned. The hard copy of the application should be sent to the institution by registered/ speed post only so as to reach on or before January to February 2020. Applications received after the mentioned date shall not be entertained under any circumstances.

Dean

Encl: Copy of Course content

<u>**VALUE ADDED COURSE**</u>

1. Name of the programme & Code

“CLINICAL MICROBIOLOGY”. & VAC05/AHS/2020-06/01

2. Duration & Period

30 hrs. & January to February 2020

3. Information Brochure and Course Content of Value-Added Courses

Enclosed as Annexure- I

4. List of students enrolled

Enclosed as Annexure- II

5. Assessment procedures:

Assessment - *Enclosed as Annexure- III*

6. Certificate model

Enclosed as Annexure- IV

7. No. of times offered during the same year:

1-time January to February 2020

8. Year of discontinuation: 2021

9. Summary report of each program year-wise

Value Added Course- January to February 2020					
Sl. No	**Course Code**	**Course Name**	**Resource Persons**	**Target Students**	**Strength & Year**
1	VAC05/AHS/2020-06/01	“CLINICAL MICROBIOLOGY”	**Dr. Arvind**	AHS	30 students June to August 2020

10. Course Feed Back

Enclosed as Annexure- V

RESOURCE PERSON

COORDINATOR
Dr.G.Somasundram

PRINCIPAL
Allied Health Sciences
Sri Lakshmi Narayana Institute of Allied Health Sciences
Osudu, Agaram Post, Puducherry - 605 502.

Course Proposal

Course Title: "CLINICAL MICROBIOLOGY"

Course Objective:

1. To enhance the performance skill in Clinical Microbiology.
2. To assess the objectives and protocols in Clinical Microbiology.
3. To assess the reaction of target allied Health students towards the Clinical Microbiology getting their feedback.

Course Outcome: Improvement in the "CLINICAL MICROBIOLOGY"

Course Audience: Students of AHS Batch 20
Course Coordinator: Dr. G. Somasundaram
Course Faculties with Qualification and Designation:
1.Course Curriculum/Topics with schedule (Min of 30 hours)

SlNo	Date	Topic	Time	Hours
1.	06.01.2020	Introduction to Clinical microbiology	4-5p.m	1
2.	07.01.2020	Taxonomic classification of organisms	2-3p.m	1
3.	08.01.2020	binomial nomenclature	4-5p.m	1
4.	09.01.2020	Bacterial species	4-5p.m	1
5.	10.01.2020	eukaryotic cell and prokaryotic cell	4-5p.m	1
6.	11.01.2020	Bacterial Cell and Bacterial structure	4-5p.m	1
7.	13.01.2020	Bacterial structure and Cell envelope proper	4-5P.M	1
8.	17.01.2020	Components of cell wall of Gram-negative bacteria	4-5p.m	1
9.	18.01.2020	Functions of cell wall	4-5p.m	1
10.	20.01.2020	Cell membrane and Function of cell membrane	4-5p.m	1
11.	21.01.2020	Polyamines and Function of polyamines	4-5p.m	2
12.	22.01.2020	Cytoplasmic granules and Nuclear apparatus	4-5p.m	1
13.	23.01.2020	Flagellum and Flagellar arrangements	2-3p.m	1
14.	24.01.2020	Spores and Arrangements of spores	4-5p.m	1
15.	25.01.2020	Morphology of bacteria	4-5p.m	1

16.	27.01.2020	Staining of bacteria	3-5p.m	2
17.	28.01.2020	Why are stains not taken up by every microorganism? and Why are stains not taken up by every microorganism?	3-5p.m	2
18.	30.01.2020	General methods of staining	4-5p.m	1
19.	31.01.2020	Differentiation (decolorization)	4-5p.m	1
20.	01.02.2020	Types of microbiological stains and staining methods	3-5p.m	2
21.	03.02.2020	Ziehl-Neelson staining method and Procedure for Ziehl-Neelson staining method	4-5p.m	1
22.	04.02.2020	Cultivation of bacteria in culture media	4-5p.m	1
23.	05.02.2020	Assessment procedure and giving feedback in weaker areas	1-5p.m	4
		Total		**30hrs**

REFERENCE BOOKS:

1. *Thomson, R. B.; Wilson, M. L.; Weinstein, M. P. (2010). "The Clinical Microbiology Laboratory Director in the United States Hospital Setting". Journal of Clinical Microbiology. **48** (10): 3465–3469. doi:10.1128/JCM.01575-10. PMC 2953135. PMID 20739497.*
2. *Frank N. Egerton (2006). "A History of the Ecological Sciences, Part 19: Leeuwenhoek's Microscopic Natural History". Bulletin of the Ecological Society of America. **87**: 47–58. doi:10.1890/0012-9623(2006)87[47: AHOTES]2.0.CO;2.*
3. *Madigan M; Martinko J, eds. (2006). Brock Biology of Microorganisms (13th ed.). Pearson Education. p. 1096. ISBN 978-0-321-73551-5.*
4. *Brock TD (1999). Robert Koch: a life in medicine and bacteriology. Washington DC: American Society of Microbiology Press. ISBN 978-1-55581-143-3.*
5. Jump up to :[a] [b] *Willey, Joanne; Sandman, Kathleen; Wood, Dorothy (2020). Prescott's Microbiology. 2 Penn Plaza, New York, NY 10121: McGraw-Hill Education. p. 188. ISBN 978-1-260-21188-7.*

CLINICAL MICROBIOLOGY

INTRODUCTION TO MICROBIOLOGY

Microbiology is a subject which deals with living organisms that are Individually too small to be seen with the naked eye. It considers the microscopic forms of life and deals about their Reproduction, physiology, and participation in the process of nature, Helpful and harmful relationship with other living things, and Significance in science and industry. Subdivision of microbiology Bacteriology deals about bacteria. Mycology deals about fungi. Virology deals about viruses.

THE MICROBIAL WORLD

TAXONOMIC CLASSIFICATION OF ORGANISMS

TAXONOMY is the science of organismal classification. Classification is the assignment of organisms (species) into unorganized scheme of naming. ideally these schemes are based on evolutionary relationships (i.e the more similar the name, the closer the evolutionary relationships). Thus, classification is concerned with: -

The establishment of criteria for identifying organisms & assignment to groups (what belongs where)

The arrangement of organisms into groups of organisms of organism (e.g. At what level of diversity should a single species be split in to two or more species?).

Consideration of how evolution resulted in the formation these groups.

TAXON: -

A group or category of related organisms.

Two key characteristics of taxa are:

-Members of lower level taxa (e.g. Species) are more similar to each other than are members of higher-level taxa (e.g. Kingdom or domain).

-Member of specific taxa are more similar to each other than any are to members of different specific taxa found at the same hierarchical level (e.g. Humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, Escherichia coli). Thus, once you know that two individuals are member of the same taxon, you can inter certain similarities between the two organisms. NOTE that taxa are dynamic, changing as our knowledge of organism and evolutionary relationships change

BINOMIAL NOMENCLATURE

Organisms are named using binomial nomenclature (viruses are exceptions)

Binomial nomenclature employs the names of the two-level taxa, genus and species, to name a specie.

Binomial nomenclature includes:

Genus comes before species (e.g., Escherichia coli)

Genus name is always capitalized (e.g., Escherichia)

Species name is never capitalized (e.g., coli)

Both names are always either italicized or underlined

(e.g. Escherichia coli)

The genus name may be used alone, but not the species name (i.e saying or writing "Escherichia "alone is legitimate while saying or writing "coli" is not) Strain

A strain in some ways is equivalent to a breed or

subspecies among plants or animal. Strain is the level

below the species

Two members of the same strain are more similar to each other than either is to an individual that is a member of a different strain, even if all three organisms are members of the same species

Bacterial species

- A bacterial species is defined by the similarities found among its members. Properties such as biochemical reactions, chemical composition, cellular structures, genetic characteristics, and immunological features are used in defining a bacterial species. Identifying a species and determining its limits presents the most challenging aspects of biological classification for any type of organism. A formal means of species is by employing a dichotomous key to guide the selection of test used to efficiently determine those bacterial properties most relevant to bacterial identification the five-kingdom system the five-kingdom system was first proposed in 1969 and is showing its age

The five kingdoms include:

- Plantae (the plants)
- Fungi (the fungi)
- Animalia (the animals)
- Protista (the unicellular eukaryotes)
- Monera (the prokaryotes)
- Kingdom of Monera

Three categories:

- Eubacteria Are our common, everyday bacteria, some of which are disease – causing; also, the taxon from which mitochondria originated.
- Cyanobacteria

Are photosynthetic eubacteria, the taxon from which chloroplast

Archaebacterial

Are distinctive in their adaptation to extreme environments (e.g., very hot, salty, or acidic) though not all archaebacterial live in extreme environments. These distinctions are more phenotypic than they are evolutionary

(i.e., a cyanobacteria are eubacteria, and neither is an

archaebacterial).

Kingdom Protista

Protista like Monera consist mostly of unicellular organisms. Distinctively, however, the members of Kingdom Protista are all eukaryotic while the members of kingdom Monera are all prokaryotic. Some members of protista are multicellular, however Kingdom protista represents a grab bag, essentially the place where the species are classified when they are not classified as either fungi, animals or plants.

Kingdom Fungi

Unlike protests, the eukaryotic fungi are typically non – aquatic species. They traditionally are nutrients absorbers plus have additional distinctive features. They do exist unicellular fungi, which we call yeast

DOMAIN

The domain is a taxonomic category that, depending on point of view, is either above the level of kingdom or supercedes the kingdom. The domain system contains three members

¾ Eukaryotes (domain Eukarya)

¾ Eubacteria (domain Bacteria)

¾ Archaebacteria (domain Archaea)

Viral classification

Classification of viruses is not nearly as well developed as the classification of cellular organisms. Today viruses tend to be classified by their chemical, morphological and physiological attributes (e.g. genome = DNA vs RNA, virion particle = enveloped vs non-enveloped and myriad details of their intracellular infection cycles). Binomial nomenclature is not employed to name viruses;

instead viruses are named by their common names (e.g., Human Immunodeficiency Virus a.m. HIV) Dichotomous key A means of assigning an organism to a specific taxonomic category typically involves the use of specific criteria that may posed as questions (e.g. What does the organism look like etc.). Relevant criteria may be arranged as a dichotomous key. In a dichotomous

key questions are arranging hierarchically with more general questions are asked first, with questions becoming more specific asked subsequently

EUKARYOTIC CELL

Eu- true

Karyote- nucleus

The eukaryotic cell has a true membrane bound nucleus, usually containing multiple chromosomes, a mitotic apparatus, a well-defined endoplasmic reticulum and mitochondria.

PROKARYOTIC CELL

Pro- primitive

Karyote- nucleus

The prokaryotic cell possesses naked DNA with out associated basic proteins, divides a mitotically by binary fission and bounded by a semi rigid cell wall.

Bacterial Cell

General property:

- Typical prokaryotic cell
- Contain both DNA and RNA
- Most grow in artificial media
- Replicate by binary fission
- Almost all contain rigid cell wall
- Sensitive to antimicrobial agent

STRUCTURE OF BACTERIA

Bacterial structure is considered at three levels.

1. Cell envelope proper: Cell wall and cell membrane.
2. Cellular element enclosed with in the cell envelope: Mesosomes, ribosomes, nuclear apparatus, polyamines and cytoplasmic granules.
3. Cellular element external to the cell envelope: Flagellum, Pilus and Glycocalyx.

Cell envelope proper

A. Cell wall Multi layered structure and constitutes about 20% of the bacterial dry weight. Average thickness is 0.15-0.5 am. Young and rapidly growing bacteria has thin cell wall but old and slowly dividing bacteria has thick cell wall.

It is composed of N-acetyl Muramic acid and N-acetyl Glucosamine back bones cross linked with peptide chain and pentaglycine bridge.

Components of cell wall of Gram-negative bacteria

1. Peptidoglycan
2. Lipoprotein
3. Phospholipid

4. Lipopolysaccharide

Components of cell wall of Gram-positive bacteria

1. Peptidoglycan
2. Teichoic acid

Functions of cell wall

1. Provides shape to the bacterium
2. Gives rigidity to the organism
3. Protects from environment
4. Provides staining characteristics to the bacterium
5. Contains receptor sites for phages/complements
6. Site of action of antibody and colicin
7. Contains toxic components to host Bacteria with defective cell walls Bacteria with out cell wall can be induced by growth in the presence of antibiotics and a hypertonic environment to prevent lysis.

They are of three types:

1. Protoplasts: Derived from Gram-positive bacteria and totally lacking cell walls; unstable and osmotically
fragile; produced artificially by lysozyme and hypertonic medium: require hypertonic conditions for maintenance.

2. Spherules: Derived from Gram-negative bacteria;
retain some residual but non-functional cell wall material; osmotically fragile; produced by growth with penicillin and must be maintained in hypertonic medium.

L- forms: Cell wall-deficient forms of bacteria usually produced in the laboratory but sometimes spontaneously formed in the body of patients treated with penicillin; more stable than protoplasts or spherulites, they can replicate in ordinary media.

B. Cell membrane

Also named as cell membrane or cytoplasmic membrane

It is a delicate trilaminar unit membrane.

It accounts for 30% of the dry weight of bacterial cell.

It is composed of 60% protein, 20-30% lipids and 10-20% carbohydrate.

Function of cell membrane

1. Regulates the transport of nutrients and waste products into and out of the cell.
2. Synthesis of cell wall components
3. Assists DNA replication
4. Secrets proteins
5. Carries on electron transport system
6. Captures energy in the form of ATP
7. Cellular element enclosed with in the cell envelope

A. Mesosomes

Convoluted invagination of cytoplasmic membrane often at sites of septum formation.

It is involved in DNA segregation during cell division and respiratory enzyme activity.

B. Ribosomes

Cytoplasmic particles which are the sites of protein synthesis.

It is composed of RNA (70%) and proteins (30%) and constitutes 90% of the RNA and 40% of the total protein.

The ribosome monomer is 70s with two subunits, 30s and 50s.

Polyamines

They are of three types

- Putrescin
- Spermidine
- Spermine

It is found in association with bacterial DNA, ribosomes and cell membrane.

Function of polyamines

1. Antimutagenic
2. Prevent dissociation of 70s ribosome into subunits.
3. Increase resistance of protoplast lysis.

Cytoplasmic granules

represent accumulated food reserves.

- Nature of granules
- Glycogen
- Poly-beta hydroxy butyrate
- Babes-Ernst (Volutid)

Nuclear apparatus

Well defined nucleus and nuclear membrane, discrete chromosome and mitotic apparatus are not present in bacteria; so nuclear region of bacteria is named as nuclear body, nuclear apparatus and nucleoid.

Bacterial genome consists of single molecule of double stranded DNA arranged in a circular form.

Besides nuclear apparatus, bacteria may have extra chromosomal
genetic material named as plasmids. Plasmids do not play any role in the normal function of the bacterial cell but may confer certain additional properties (E.g. Virulence, drug

resistance) which may facilitate survival and propagation of the
micro- organism.

3. Cellular element external to the cell envelope

A. Glycocalyx (capsule and slime layer) Capsule is gel firmly adherent to cell envelope.

Slime is gel easily washed off from cell envelope.

All bacteria have at least a thin slime layer.

Capsule is composed of polysaccharide and protein (D-Glutamate of Bacillus anthracis)

Features of capsule

1. Usually weakly antigenic.
2. Not necessary for viability.
3. Endows virulence.
4. Protects from phagocytosis.
5. Capsulated strains are invariably non-motile.
6. Visualized by negative staining and capsule staining.
7. Detected by quelling phenomenon.

B. Flagellum

It is the organ of locomotion in bacterial cell and consists of thee parts. These are. The filament

- The hook
- The basal body

The basal body and hook are embedded in the cell surface while the filament is free on the surface of bacterial cell.

Their presence in bacterial cell is detected by

- Hanging drop preparation
- Swarming phenomenon on surface of plate agar
- Motility media
- Special staining methods
- Silver impregnation methods
- Dark –field microscopy
- Electron microscopy

Size: 3-20µm in length and 0.01-0.013µm in diameter.

It is composed of protein named as flagellin.

The flagellar antigen in motile bacterium is named as H (Haunch) antigen.

Flagellar arrangements

1. Atrichias: Bacteria with no flagellum.
2. Monoicous: Bacteria with single polar flagellum.
3. Ophiotrichids: Bacteria with bunch of flagella at one pole.
4. Amphitricha: Bacteria with flagella at both poles.
5. Peritrichous: Bacteria with flagella all over their surface.

End flagella (axial filament)

It is the organ of motility found in periplasmic space of spirochetes.

C. Pili (fimbriae)

It is hair like structure composed of protein (pilin)

Two types (Based on function)

- . Common pili: The structure for adherence to cell surface.
- Sex pili: The structure for transfer of genetic material from the

donor to the recipient during the process of conjugation.

D. Spores

Resting cells which are capable of surviving under adverse environmental conditions like heat, drying, freezing, action of toxic chemicals and radiation. Bacterial spore is smooth walled and oval or spherical in shape. It does not take up ordinary stains. It looks like areas of high refractivity under light microscope.

It is significant in spread of disease and indicator of sterility of materials.

Spores are detected by

- . Simple staining methods
- Special staining methods

Arrangements of spores

1. No bulging of cell wall

- . Oval central
- . Oval sub terminal
- Spherical central

2. Bulging of cell wall

- Oval sub terminal
- Oval terminal
- Spherical terminal
- Free spore

Classification of bacteria

Bacterial classification depends on the following characteristics.

1. Morphology and arrangement
2. Staining
3. Cultural characteristics

4. Biochemical reactions

5. Antigenic structure

6. Base composition of bacterial DNA

Morphology and staining of bacteria are the commonly used characteristics to classify bacteria.

1. Morphology of bacteria

When bacteria are visualized under light microscope, the following morphology is seen.

1. Cocci (singular coccus): Round or oval bacteria measuring about 0.5-1.0μmb in diameter. They are found in single, pairs, chains or clusters.

2. Bacilli (singular bacillus): Stick-like bacteria with rounded, tapered, square or swollen ends; with a size measuring 1-10μm in length by 0.3-1.0μm in width.

3. Coccobacilli (singular coccobacillus): Short rods.

4. Spiral: Spiral shaped bacteria with regular or irregular distance between twisting.

E.g. Spirilla and spirochetes

Staining of bacteria

Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes). The principle of staining is to identify microorganisms selectively by using dyes, fluorescence and radioisotope emission. Staining reactions are made possible because of the physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or dyes by cells of microorganisms. Individual variation in the cell wall constituents among different groups of bacteria will consequently produce variations in

colors during microscopic examination. Nucleus is acidic in character and hence, it has greater affinity for basic dyes. Whereas, cytoplasm is basic in character and
has greater affinity for acidic dyes.

There are many types of affinity explaining this attraction force:

1. hydrophobic bonding
2. reagent-cell interaction
3. reagent-reagent interaction
4. ionic bonding
5. hydrogen bonding
6. covalent bonding

Why are stains not taken up by every microorganism?

Factors controlling selectivity of microbial cells are:

1. number and affinity of binding sites
2. rate of reagent uptake
3. rate of reaction
4. rate of reagent loss (differentiation or regressive staining)

Properties of dyes

Why dyes color microbial cells?

Because dyes absorb radiation energy in visible region of electromagnetic spectrum i.e., “light” (wave length 400-650). And absorption is anything outside this range it is colorless.
E.g., acid
fuchsin absorbs blue green and transmit red.

General methods of staining

1. Direct staining

Is the process by which microorganisms are stained with simple?
dyes. E.g., methylene blue

2. Indirect staining – is the process which needs mordants. A mordant is the substance which, when taken up by the microbial cells helps make dye in return, serving as a link or bridge to make the staining recline possible. It combines with a dye to form a colored "lake", which in turn combines with the microbial cell to form a "cell-mordant-dye complex". It is an integral part of the staining reaction itself, without which no staining could possibly occur. E.g., iodine.

A mordant may be applied before the stain or it may be included as part of the staining technique, or it may be added to the dye solution itself.

An accentuator, on the other hand is not essential to the chemical union of the microbial cells and the dye. It does not participate in the staining reaction, but merely accelerate or hasten the speed of the staining reaction by increasing the staining power and selectivity of the dye. Progressive staining - is the process whereby microbial cells are stained in a definite sequence, in order that a satisfactory differential coloration of the cell may be achieved at the end of the correct time with the staining solution. Regressive staining - with this technique, the microbial cell is first over stained to obliterate the cellular desires, and the excess stain is removed or decolorized from unwanted part. **Differentiation (decolorization)**

- is the selective removal of excess stain from the tissue from microbial cells during regressive staining in order that a specific substance may be stained differentially from the surrounding cell. Differentiation is usually controlled visually by examination under the microscope

Uses

1. To observe the morphology, size, and arrangement of bacteria.
2. To differentiate one group of bacteria from the other group.

Biological stains are dyes used to stain micro-organisms.

Types of microbiological stains

- Basic stains
- Acidic stains
- Neutral stains

NB: This classification is not based on PH
of stains.

Basic stains are stains in which the coloring substance is contained in the base part of the stain. The acidic part is colorless. E.g. Acidic stains are stains in which the coloring substance is contained in the acidic part of the stain. The base part is colorless. It is not commonly used in microbiology laboratory.

E.g. Eosin stain

Neutral stains are stains in which the acidic and basic components of stain are colored. Neutral dyes stain both nucleic acid and cytoplasm. E.g. Giemsa stain

Types of staining methods

1. Simple staining method
2. Differential staining method
3. Special staining method

1. Simple staining method

It is type of staining method in which only a single dye is used. Usually used to demonstrate bacterial morphology and arrangement

Two kinds of simple stains

1. Positive staining: The bacteria or its parts are stained by the
dye.

E.g. Carbol fuchsin stain

Methylene blue stain

Crystal violet stain

Procedure:

- . Make a smear and label it.
- . Allow the smear to dry in air.
- . Fix the smear over a flame.
- . Apply a few drops of positive simple stain like 1% methylene blue, 1% carbolfuchsin or 1% gentian violet for 1 minute.
- Wash off the stain with water.
- Air-dry and examine under the oil immersion objective.

2. Negative staining: The dye stains the background and the
bacteria remain unstained. E.g. Indian ink stain Negros in stain

Differential staining method Multiple stains are used in differential staining method to distinguish different cell structures and/or cell types. E.g. Gram stain and ZiehlNeelson stain

A. Gram staining method

Developed by Christian Gram. Most bacteria are differentiated by their gram reaction due to differences in their cell wall structure. Gram-positive bacteria are bacteria that stain purple with crystal violet after decolorizing with acetone-alcohol. Gram-negative bacteria

are bacteria that stain pink with the counter stain (safranin) after losing the primary stain (crystal violet) when treated with acetone-alcohol.

Required reagents:

- Gram's Iodine
- Acetone-Alcohol
- Safranin

Procedure:

1. Prepare the smear from the culture or from the specimen.
2. Allow the smear to air-dry completely.
3. Rapidly pass the slide (smear upper most) three times through the flame.
4. Cover the fixed smear with crystal violet for 1 minute and wash with distilled water.
5. Tip off the water and cover the smear with gram's iodine for 1 minute.
6. Wash off the iodine with clean water.
7. Decolorize rapidly with acetone-alcohol for 30 seconds.
8. Wash off the acetone-alcohol with clean water.
9. Cover the smear with safranin for 1 minute.
10. Wash off the stain wipe the back of the slide. Let the smear to air-dry.
11. Examine the smear with oil immersion objective to look for bacteria.

Interpretation:

- Gram-positive bacterium ……………Purple
- Gram-negative bacterium …………. Pink

B. Ziehl-Neelson staining method

Developed by Paul Ehrlichin1882, and modified by Ziehl and Nelson Ziehl-Neelson stain (Acid-fast stain) is used for staining Mycobacteria which are hardly stained by gram staining method. Once the Mycobacteria is stained with primary stain it can not be decolorized with acid, so named as acid-fast bacteria.

Reagents required:

- Carbol-fuchsin
- Acid-Alcohol
- Methylene blue/Malachite green

Procedure for Ziehl-Neelson staining method

1. Prepare the smear from the primary specimen and fix it by passing through the flame and label clearly
2. Place fixed slide on a staining rack and cover each slide with concentrated carbol fuchsin solution.
3. Heat the slide from underneath with sprit lamp until vapor rises (do not boil it) and wait for 3-5 minutes.
4. Wash off the stain with clean water.
5. Cover the smear with 3% acid-alcohol solution until all color is removed (two minutes).
6. Wash off the stain and cover the slide with 1% methylene blue. For one minute.
7. Wash off the stain with clean water and let it air-dry.
8. Examine the smear under the oil immersion objective to look for acid fast bailli.

Interpretation:

Acid fast bacilli………… Red

Back ground………………Blue

Reporting system

0 AFB/100 field …………………No AFB seen

1-2 AFB/ 300 field……………… Scanty

1-10 AFB/100 field………………1+

11-100AFB/100 field.……………2+

1-10 AFB/field…… ……………3+

>10 AFB/field……………… ….4+

NB: AFB means number of acid-fast bacilli seen.

3. Special stains

a. Spore staining method

b. Capsule staining method

Procedure:

1. Prepare smear of the spore-forming bacteria and fix in flame.
2. Cover the smear with 5% malachite green solution and heat over steaming water bath for 2-3 minutes.
3. Wash with clean water.
4. Apply 1% safranin for 30 seconds.
5. Wash with clean water.
6. Dry and examine under the oil immersion objective.

b. Capsule staining method: Welch method

Procedure:

1. Prepare smear of capsulated bacteria.
2. Allow smear to air-dry; do not fix the smear.
3. Cover the smear with 1% aqueous crystal violet for 1 minute over steaming water bath.
4. Wash with 20% copper sulfate solution. Do not use water.
5. examine under the oil immersion objective.

CULTIVATION OF BACTERIA IN CULTURE MEDIA

Culture media

It is the media containing the required nutrients for bacterial growth.

Uses: Isolation and identification of micro-organisms

- Performing anti-microbial sensitivity tests Common ingredients of culture media
- Peptone
- Meat extract
- Yeast extract
- Mineral salts
- Carbohydrates
- Agar
- Water

Peptone: Hydrolyzed product of animal and plant proteins: Free amino acids, peptides and proteases (large sized peptides).

It provides nitrogen; as well carbohydrates, nucleic acid fractions, minerals and vitamins.

Meat extract: supply amino acids, vitamins and mineral salts.

Yeast extract: It is bacterial growth stimulants.

Mineral salts: these are: Sulfates as a source of sulfur.

- Phosphates as a source of phosphorus.
- Sodium chloride
- Other elements

Carbohydrates: Simple and complex sugars are a source of carbon and energy.

- Assist in the differentiation of bacteria.

E.g. Sucrose in TCBS agar differentiates vibrio species.

Lactose in MacConkey agar differentiates enterobacteria.

Agar: It is an inert polysaccharide of seaweed.

It is not metabolized by micro-organism.

Property

It has high gelling strength

high melting temperature (90-95 o

c) . low gelling temperature

- It forms firm gel at 1.5% W/V concentration.
- It forms semisolid gel at 0.4-0.5% W/V concentration.

Uses:

Solidify culture media

May provide calcium and organic ions to inoculated bacteria. Water Deionized or distilled water must be used in the preparation of culture media.

Types of culture media

- Basic /Simple /. To prepare enriched media
- . To maintain stock cultures of control bacterial strains
- . To subculture pathogenic All-purpose media

It is a media that supports the growth of micro-organisms that do not require special nutrients.

Uses:

bacteria from selective/differential medium prior to performing biochemical or serological tests.

E.g. Nutrient Broth, Nutrient Agar

2. Enriched media

Media that are enriched with whole blood, lazed blood, serum, special extracts or vitamins to support the growth of pathogenic bacteria.

E.g. Blood Agar, Chocolate Agar

3. Enrichment media

Fluid media that increases the numbers of a pathogen by containing enrichments and/or substances that discourage the multiplication of unwanted bacteria.

E.g. Selenite F broth media Alkaline peptone water

4. Selective media

Media which contain substances (E.g. Antibiotics) that prevent or slow down the growth of bacteria other than pathogens for which the media are intended.

E.g. Modified Thayer –Martin Agar

Salmonella-Shigella (SS) agar

1. Differential media

Media to which indicator substances are added to differentiate bacteria.

E.g. TCBS Agar differentiates sucrose fermenting yellow colonies of Vibrio cholerae to non-sucrose fermenting blue colonies other Vibrio species.

NB: Most differential media distinguish between bacteria by an indicator which changes color when acid is produced following carbohydrate fermentation.

2. Transport media

Media containing ingredients to prevent the overgrowth of commensals and ensure the survival of pathogenic bacteria when specimens cannot be cultured soon after collection.

3. Enrichment media

Fluid media that increases the numbers of a pathogen by containing enrichments and/or substances that discourage the multiplication of unwanted bacteria.

E.g. Selenite F broth media Alkaline peptone water

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Media which contain substances (E.g. Antibiotics) that prevent or slow down the growth of bacteria other than pathogens for which the media are intended.

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Choice of culture media

The selection culture media will depend on:

1. The major pathogens to be isolated, their growth requirements and the features by which they are recognized.

2. Whether the specimens being cultured are from sterile sites or from sites having normal microbial flora.

3. The cost, availability and stability of media.

4. The training and experience of laboratory staff in preparing, using and controlling culture media.

Forms of culture media

1. solid culture media

2. semisolid culture media

3. Fluid culture media

1. solid culture media

. Plate cultures in petri dishes

. stab/slope cultures in tubes and bottles

Uses: Description of bacterial colonies

- size: diameter in mm
- Out line: circular, entire, wavy, indented

  Elevation: flat, raised, low convex and dome shaped.
- Transparency: transparent, opaque, and translucent.
- Surface: smooth (mucoid) and shiny, rough and dull.
- Color: colorless, white, pink, and pigmented
- changes in medium

E.g. Hemolysis in Blood Agar

Blackening of medium due to hydrogen sulfide production.

2. Semisolid culture media

Uses:

. as an enrichment media

. as motility media

3. Fluid culture media

Bacterial growth in fluid media is shown by a turbidity in the medium.

Uses:

. as an enrichment media

. as biochemical testing media

. as blood culture media

Preparation of culture media

Culture media contains essential ingredients for microbial growth requirements. For successful isolation of pathogens, culture media must be prepared carefully.

Most culture media are available commercially in ready –made dehydrated form.

The major processes during preparation of culture media

- Weighing and dissolving of culture media ingredients
- Sterilization and sterility testing
- Addition of heat-sensitive ingredients
- Dispensing of culture media
- pH testing of culture media
- Quality assurance of culture media
- Storage of culture media

1. Weighing and dissolving of culture media ingredients

Apply the following while weighing and dissolving of culture media ingredients

- Use ingredients suitable for microbiological use.
- Use clean glass ware, plastic or stainless-steel equipment.
- Use distilled water from a glass still.

- Do not open new containers of media before finishing previous ones.
- Weigh in a cool, clean, dry and draught-free atmosphere.
- Weigh accurately using a balance.
- Wear a facemask and glove while weighing and dissolving toxic chemicals.
- Do not delay in making up the medium after weighing.
- Add powdered ingredients to distilled water and mix by rotating or stirring the flask.
- Stir while heating if heating is required to dissolve the medium.
- Autoclave the medium when the ingredients are dissolved.

2. Sterilization and sterility testing

Always sterilize a medium at the correct temperature and for the correct length of time as instructed in the method of preparation.

Methods used to sterilize culture media

A) . Autoclaving

B) . Steaming to 100 OC

C) . Filtration

A) Autoclaving

Autoclaving is used to sterilize most agar and fluid culture media.

B) Steaming at 100 OC

It is used to sterilize media containing ingredients that would be inactivated at temperature over 100 OC and re-melt previously bottled sterile agar media.

C) Filtration

It is used to sterilize additives that are heat-sensitive and cannot be autoclaved.

Sterility testing

The simplest way to test for contamination is to incubate the prepared sample media At 35-37 OC for 24 hours. Turbidity in fluid media and microbial growth in solid media confirm contamination.

3. Addition of heat-sensitive ingredients Refrigerated-heat sensitive ingredients should be warmed at room temperature before added to a molten agar medium. Using an aseptic technique, the ingredients should be added when the medium has cooled to 50 OC, and should be distributed immediately unless further heating is required.

4. pH testing

The pH of most culture media is near neutral, and can be tested using pH papers or pH meter.

5. Dispensing of culture media

Media should be dispensed in a clean draught-free room using aseptic technique and sterile container.

Dispensing agar media in Petridis

- Lay out the sterile Petri dishes on a level surface.
- Mix the medium gently by rotating the flask or bottle.
- Flame sterilize the neck of flask or bottle.
- Pour 15 ml of medium in each Petridis.
- Stack the plates after the medium has gelled or cooled.

Store the plates in a refrigerator.

NB: Agar plates should be of an even depth and of a firm gel.

The surface of the medium should be smooth and free from bubbles.

6. Quality control

• Inoculate quarter plates of the medium with a five hours broth culture for each control organism.

• Use a straight wire to inoculate and wire loop to spread the inoculum.

• Depending on the species, incubate aerobically, CO2-enriched atmosphere and anaerobically at 35-37 OC for 24 hours.

• Examine for the degree of growth, morphology and other characteristics of microbial colonies.

• Record the result of each control species and compare to your standard reading.

Storage of culture media

• Dehydrated culture media and dry ingredients should be stored at an even temperature in a cool dry place away from direct light.

• Plates of culture media, and additives like serum, blood and antimicrobials in solid form require storage at 2-8 OC.

• Antimicrobials in solution form should be stored at –20 OC.

• All culture media and additives should be labeled with the name and date of preparation.

Inoculation of culture media

When inoculating culture media, an aseptic technique must be used to prevent contamination of specimens and culture media, and laboratory worker and the environment.

Aseptic technique during inoculation of culture media

• Decontaminate the workbench before and after the work of the day.

• Use facemask and gloves during handling highly infectious specimens.

• Flame sterilize wire loops, straight wires, and metal forceps before and after use.

• Flame the neck of specimen and culture bottles, and tubes after removing and before replacing caps and plugs.

Inoculation of media in Petri dishes

The inoculation of media in Petri dishes is named as ‘plating out’ or ‘looping out’. Before inoculating a plate of culture media, dry the surface of the media by incubating at 37 OC for 30 minutes. To inoculate a plate, apply the inoculum to a small area of the plate (‘the well’) using sterile wire loop and then spread and thin out the inoculum to ensure single colony growth. Inoculation of butt and slant media to inoculate butt and slant media, use a sterile straight wire to stab into the butt and then streak the slant in a zigzag pattern.

Inoculation of slant media

To inoculate slant media, use a straight wire to streak the inoculum down the center of the slant and then spread the inoculum in a zigzag pattern.

Inoculation of stab media

To inoculate stab media, use a straight wire to stab through the center of the medium and withdraw the wire along the line of inoculum. Inoculation of fluid media to inoculate fluid media, use straight wire or wire loops.

Incubation of cultures

Inoculated media should be incubated as soon as possible.

Optimal temperature, humidity and gaseous atmosphere should be provided for microorganisms to grow best. The temperature selected for routine culturing is 35-37 OC. Some pathogens require CO_2-enriched atmosphere to grow in culture media, and the simplest way to provide CO_2-enriched atmosphere is to enclose a lighted candle in an airtight jar which provides 3-5% CO_2 by the time the candle is extinguished. Anaerobic atmosphere is essential for the growth of strict anaerobes, and the techniques for obtaining anaerobic conditions are the following:

. Anaerobic jar with a gas generating kit.

. Reducing agents in culture media.

BACTERIAL NUTRITION

Bacteria, like all cells, require nutrients for the maintenance of their metabolism and for cell division. Bacterial structural components and the macromolecules for the metabolism are synthesized from the elements. The four most important elements of bacteria are carbon, hydrogen, oxygen and nitrogen. Carbon Organisms require a source of carbon for the synthesis of numerous organic compounds that comprise protoplast.

Depending on their requirements, bacteria can be classified as

1. Autotrophs: Free-living, non-parasitic bacteria which use carbon dioxide as carbon source.

The energy needed for their metabolism can be obtained from: Sun Light-Photoautotrophs

. Inorganic compounds by oxidation-Chemoautotrophs

3. Heterotrophs: Parasitic bacteria require more complex organic compounds as their source of carbon and energy. Human pathogenic bacteria are heterotrophs. The principal source of carbon is carbohydrate which are degraded either by oxidation, in the presence of oxygen, or by fermentation, in the absence of oxygen, to provide energy in the form of ATP.

Hydrogen and oxygen

- Obtained from water.
- Essential for the growth and maintenance of cell.

Nitrogen

- Constitutes 10% of dry weight of bacterial cell.
- Obtained from organic molecules like proteins and inorganic molecules like ammonium salts and nitrates.

NB: Main source of nitrogen is ammonia, in the form of ammonium salt.

Growth factors

Growth factors are organic compounds that are required by microorganisms in small amounts which the cell cannot synthesize from other carbon source. These are amino acids, purines and pyrimidines, and vitamins.

Prototroph: Wild-type bacteria with normal growth requirements.

Auxotroph's: Mutant bacteria, which require an additional growth factor not needed by the parental or wild type strain.

SRI LAKSHMI NARAYANA INSTITUTE OF ALLIED HEALTH SCIENCE

MEDICAL EDUCATIONAL PROJECT

CLINICAL MICROBIOLOGY- VAC05/AHS/2020-06/01

S.No.	Name of the Students	University Register Number	Signature
1	PAVITHRA S	UAH1901114	
2	POOVARASAN P	UAH1901115	
3	PRAVEEN KUMAR E	UAH1901116	
4	RAMYA R	UAH1901117	
5	SANJAI B	UAH1901118	
6	SELVAKUMAR R	UAH1901119	
7	SENTHIL RAJA D	UAH1901120	
8	SHALINI R	UAH1901121	
9	SHURUTHY S	UAH1901122	
10	SINDHUJA P	UAH1901123	
11	SHIVA JOTHI S	UAH1901124	
12	SOUNDARYA M	UAH1901125	
13	SRIRAM A	UAH1901126	
14	THIRUMURUGAN A	UAH1901127	
15	TIOLEODOSS A	UAH1901128	
16	UMAMAHESWARI S	UAH1901129	
17	YOGASRI V	UAH1901130	
18	AMARNATH K	UAH1906101	
19	CHANDRA SEKAR M	UAH1906102	
20	EZHILARASAN M	UAH1906103	
21	JEEVA J	UAH1906104	
22	PAVITHRA P	UAH1906105	
23	PRAVEEN KUMAR R	UAH1906106	
24	SANTHIYA S	UAH1906107	
25	SELVAPRIYA S	UAH1906108	
26	SHAJAKHAN	UAH1906109	
27	SOORIYA A	UAH1906110	
28	SUJITH G	UAH1906111	
29	THULASIRAMAN	UAH1906112	
30	VIGNESH S	UAH1906113	

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Annexure - III

Assessment Form

Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry

COURSE CODE: VAC05/AHS/2020-06/01

Multiple Choice Question ***15x2=30***

1. Which of these bacterial components is least likely to contain useful antigens?

a. Cell wall
b. Flagella
c. Ribosomes
d. Capsule

2. Which of the following contains structures composed of N-acetylmuramic acid and N-acetylglucosamine?

a. Mycoplasmas
b. Amoeba
c. E.coli
d. Spheroplast

3. The association of endotoxin in gram-negative bacteria is due to the presence of

a. Steroids
b. Peptidoglycan
c. Lipopolysaccharides
d. Polypeptide

4. The prokaryotic cell membrane

a. Contains metabolic enzymes
b. Is selectively permeable
c. Regulates the entry and exit of materials
d. Contains proteins and phospholipids

5. Which of the statements regarding gram staining is wrong?

a. *Mycobacterium tuberculosis* stains blue because of the thick lipid layer
b. *Streptococcus pyogenes* stains blue because of a thick peptidoglycan layer
c. *Escherichia coli* stains pink because of a thin peptidoglycan layer
d. *Mycoplasma pneumoniae* is not visible in the Gram's stain because it has no cell wall

6. Which of the following is not a recognised cause of diarrhoea?

a. *Vibrio cholerae*
b. *Escherichia coli*
c. *Clostridium perfringens*
d. *Enterococcus faecalis*

7. Which of the following is a gram-positive eubacteria?

a. *Actinomyces*
b. *Clostridium*
c. *Rhizobium*
d. *Clostridium, Actinomyces*

8. Which of the following microorganisms is not responsible for urinary tract infection?

a. *Proteus mirabilis*
b. *Escherichia coli*
c. *Klebsiella pneumoniae*
d. *Bacteroides fragilis*

9. Which of the following is diagnosed by serologic means?

a. Actinomycosis
b. Q-fever
c. Pulmonary tuberculosis
d. Gonorrhoea

10. Diarrhoea is not caused by

a. *Shigella dysenteries*
b. *Streptococcus pyogenes*
c. *Clostridium difficile*
d. *Salmonella enteritis*

11. The coagulase is done to differentiate

a. *Staphylococcus aureus* from *Staphylococcus epidermidis*
b. *Staphylococcus epidermidis* from *Neisseria meningitidis*
c. *Streptococcus pyogenes* from *Enterococcus faecalis*
d. *Streptococcus pyogenes* from *Staphylococcus aureus*

12. Prokaryotic cells are more resistant to osmotic shock than eukaryotic cells because

a. Their cell wall is composed of peptidoglycan
b. They are selectively permeable
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d. They block water molecules from entering the cell

13. The bacterial genus where sterols are present in the cell membrane is

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14. The bacterium that infects other gram-negative bacteria is

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c. *Bdellovibrio*
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15. Which phage is used for phage display technique?

a. T7
b. M13
c. ƛ-phage
d. ϕ6

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RAMYA .R. UAH1901117

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Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry

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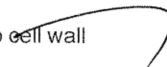

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SELVAKUMAR . K . UAH1901119

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Sri Lakshmi Narayana Institute of Medical Sciences

Affiliated to Bharath Institute of Higher Education & Research

(Deemed to be University under section 3 of the UGC Act 1956)

CERTIFICATE OF MERIT

This is to certify that **Poovarasan P(UAH1901115)** has actively participated in the Value Added Course on **CLINICAL MICROBIOLOGY(VAC05/AHS/2020-06/01)** held during January 2020 – February 2020 Organized by Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry- 605 502, India.

RESOURCE PERSON

Dr. G.Somasundram

COORDINATOR

Sri Lakshmi Narayana Institute of Medical Sciences

Affiliated to Bharath Institute of Higher Education & Research

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CERTIFICATE OF MERIT

This is to certify that **PAVITHRA S(UAH1901114)** has actively participated in the Value Added Course on **CLINICAL MICROBIOLOGY(VAC05/AHS/2020-06/01)** held during January 2020 – February 2020 Organized by Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry- 605 502, India.

RESOURCE PERSON

Dr. G.Somasundram

COORDINATOR

Annexure- V

Student Feedback Form

Course Name: “CLINICAL MICROBIOLOGY”

Subject Code: VAC05/AHS/2020-06/01

Name of Student: ____ Roll No.: ______________

We are constantly looking to improve our classes and deliver the best training to you. Your evaluations, comments and suggestions will help us to improve our performance

Feedback Form

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. The course met my expectations.	○	○	○	○	○
2.I will be able to apply the knowledge learned.	○	○	○	○	○
3.The course objectives for each topic were identified and followed.	○	○	○	○	○
4.The content was organised and easy to follow.	○	○	○	○	○
5.The quality of instruction was good.	○	○	○	○	○
6.Class participation and interaction were encouraged.	○	○	○	○	○
7.Adequate time was provided for questions and discussion.	○	○	○	○	○

8.How do you rate the course overall?

- ○ Excellent
- ○ Good
- ○ Average
- ○ Poor
- ○ Very poor

9. The aspects of the course could be improved?

10. Other comments?

Annexure- V

Student Feedback Form

Course Name: "CLINICAL MICROBIOLOGY"

Subject Code: VAC05/AHS/2020-06/01

Name of Student: R. Selvakumar Roll No.: UAH1901119.

We are constantly looking to improve our classes and deliver the best training to you. Your evaluations, comments and suggestions will help us to improve our performance

Feedback Form

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. The course met my expectations.	✓	o	o	o	o
2.I will be able to apply the knowledge learned.	o	✓	o	o	o
3.The course objectives for each topic were identified and followed.	✓	o	o	o	o
4.The content was organised and easy to follow.	o	✓	o	o	o
5.The quality of instruction was good.	✓	o	o	o	o
6.Class participation and interaction were encouraged.	o	o	✓	o	o
7.Adequate time was provided for questions and discussion.	o	o	✓	o	o

8.How do you rate the course overall?

- o Excellent
- ✓ Good
- o Average
- o Poor
- o Very poor

9. The aspects of the course could be improved? Good.

10. Other comments? –

Signature of the student: Selvk R.

Date: 5/2/2020

Annexure- V

Student Feedback Form

Course Name: "CLINICAL MICROBIOLOGY"

Subject Code: VAC05/AHS/2020-06/01

Name of Student: S. SANTHIYA Roll No.: UAH1906107.

We are constantly looking to improve our classes and deliver the best training to you. Your evaluations, comments and suggestions will help us to improve our performance

Feedback Form

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. The course met my expectations.	o	✓	o	o	o
2.I will be able to apply the knowledge learned.	✓	o	o	o	o
3.The course objectives for each topic were identified and followed.	o	✓	o	o	o
4.The content was organised and easy to follow.	✓	o	o	o	o
5.The quality of instruction was good.	o	✓	o	o	o
6.Class participation and interaction were encouraged.	o	o	✓	o	o
7.Adequate time was provided for questions and discussion.	o	o	✓	o	o

8.How do you rate the course overall?

- o Excellent
- ✓ Good
- o Average
- o Poor
- o Very poor

9. The aspects of the course could be improved? improved my knowledge.

10. Other comments? Good.

Signature of the student: Santhiya

Date: 5/02/2020

Date: 05.02.2020

From

Dr.G.Somasundram
Department of Pharmacology,
Sri Lakshmi Narayana Institute of Medical Sciences
Bharath Institute of Higher Education and Research,
Chennai.

Through Proper Channel

To

The Dean,
Sri Lakshmi Narayana Institute of Medical Sciences
Bharath Institute of Higher Education and Research,
Chennai.

Sub: Completion of value-added course: CLINICAL MICROBIOLOGY

Dear Sir,

With reference to the subject mentioned above, the department has conducted the value-added course titled: **CLINICAL MICROBIOLOGY** January to February 2020 for 30 AHS Students. We solicit your kind action to send certificates for the participants that is attached with this letter. Also, I am attaching the photographs captured during the conduct of the course.

Kind Regards,

Dr.G.Somasundram

Encl: Certificates

Photographs