

Model Answer-AS-2524- Pharmaceutical Microbiology
B. Pharm. Vth Semester

Section A –Answ. 1.

- I. i) Monotrichous ii) lophotrichous iii) amphitrichous iv) peritrichous
- II. Image are focused by light beam whereas in electron microscope image is focused by electromagnetic field. In light microscope low power lens is used whereas beam of electron provide very high resolution in electron microscope.
- III. Thermolabile injection can be sterilized by filtration
- IV. Robert Koch discovered technique of staining and fixation.
- V. Cocci or Coccus Bacilli or bacillus, spiral, vibrio, Spirochetes
- VI. An antigen-presenting cell (APC) or accessory cell is a cell that displays foreign antigens complexed with major histocompatibility complexes (MHC's) on their surfaces. T-cells may recognize these complexes using their T-cell receptors (TCRs). These cells process antigens and present them to T-cells.
- VII. Sterilization is defined as method of completely destroying or eliminating the microorganism by any suitable method.
Disinfection is destruction of pathogenic organism by disinfecting agents.
- VIII. Aseptic technique means controlled process or condition in which the level of microbes is reduced to extent that microbes can be excluded from a product during processing.
- IX. Minimum concentration of antibiotic or other agents required to inhibit growth of microorganism.
- X. Agents which destruct or kill the microorganism are called disinfectants and when these agents are used on live object (such as human body) for the purpose of killing microbes are called antiseptics.
- XI. Surgical instruments, scissors, needles, glass, syringes, media, injections, cotton, etc.
- XII. Antigen interacts with no. of cells and activate them. These cells start complex cellular interaction and initiate immune response.

Section B

Ans. 2. : Detailed information should cover following key points.

- General characteristics of viruses- Six characterizes
 - o Single DNA or RNA nucleic acid
 - o Contains a protein coat
 - o Multiply inside living cells by using synthesizing machinery of cell
 - o Can transfer viral nucleic acid to other cells

- Easily transmitted , not affected by antibiotics
- Unable to grow by binary fusion
- virus are classification on basis of their capsid structure as follows –each subcategory to be elaborated
 - Helical viruses
 - Polyhedral viruses
 - Enveloped viruses
 - Complex viruses

Five steps of virus life cycle should be discussed

- Attachment or adsorption
- Penetration
- Biosynthesis
- Maturation and
- Release

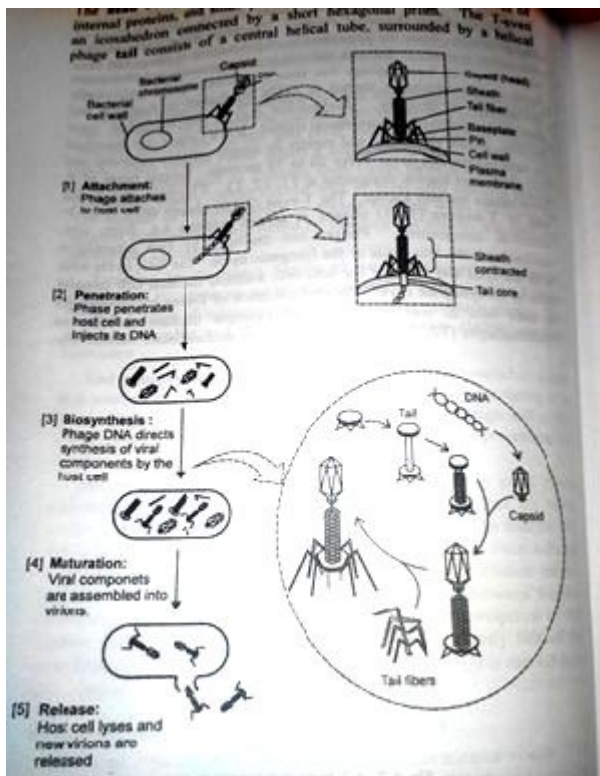


Figure showing T lytic cycle of a T even bacteriophage describing above steps.

Ans. 3. : Liquid disinfectives are generally evaluated by suspension test. Suspension test are essential test for sterility upon bacterial suspensions performed after treatment with the disinfectant for a prescribed time and under controlled conditions. Suspension tests include phenol coefficient type test (Rideal-Walker test and chick martin test) or dilution test and Kelsey –Skyles test.

Phenol is an established disinfectant and the effectiveness of any antiseptic and disinfectant is evaluated against this standard chemical disinfectants.

Rideal-Walker test : formula for determination of Rideal-Walker test (RW) coefficient should be covered

- Detailed method discussing, media, temperature, time , standard test organism and dilutions should be discussed
- As phenol is used as standard hence, This test is also known as phenol coefficient test
- Also, drawback of the test should be included

Chick martin test: Detailed method discussing, media, temperature, time and dilutions should be discussed

Table; phenol coefficient of some common disinfectant

Disinfectant/Antiseptic	<i>S. typhi</i>	<i>S. aureus</i>
Hydrogen Peroxide	0.01	—
Formalin	0.7	0.3
Phenol	1.0	1.0
Lysol	3.2	5.0
Tincture Iodine	5.8	6.3
Ethyl Alcohol	6.3	6.3
Chloramine	100.0	133.0
Mercury Chloride	143.0	100.0

Ans. 4. : Bacteria differ widely in their nutritional requirements. Some bacteria can synthesize all their requirements from the simplest elements but others need a ready made supply of some of organic compounds necessary for their growth.

Most of the microbes require following major material

Water: major nutrient accounts for 80% to 90% of total weight of cells. In addition of H & O₂ which can be derived from water other elements required include C, N, P and S. these six account 95% of dry weight of cells.

Micronutrients or trace element required for the growth of bacteria includes Mg, Co, Cu, Nickel, Molybdenum and Zn.

Carbon: Bacteria derive energy from the oxidation of inorganic compounds. They use CO₂ as source of C

Autotrophs and heterotrophs should be discussed.

Nitrogen and Sulphur: these elements must be in reduced form for organic combinations eg. N from amino acids and S from sulphahydril compounds- discuss in detail

Oxygen: Microbes depends on aerobic respiration needs molecular O₂ for their growth.

Strict aerobes eg. Clostridium species

Facultative aerobes-grow either in presence or absence-ex E coli.

Obligate aerobes-grow best at partial pressure of oxygen less than 0.2 atm

Organic growth factors: essential organic compounds that an organism cannot synthesize itself from simple organic source but requires as a precursor or constituents of its organic cell material.

Falls into 3 categories- amino acids, purines & pyrimidines and vitamins

Energy : divided into four categories -Photoautotrophs- Photoheterotrophs- Chemoheterotrophs- Chemoautotrophs-description of these four

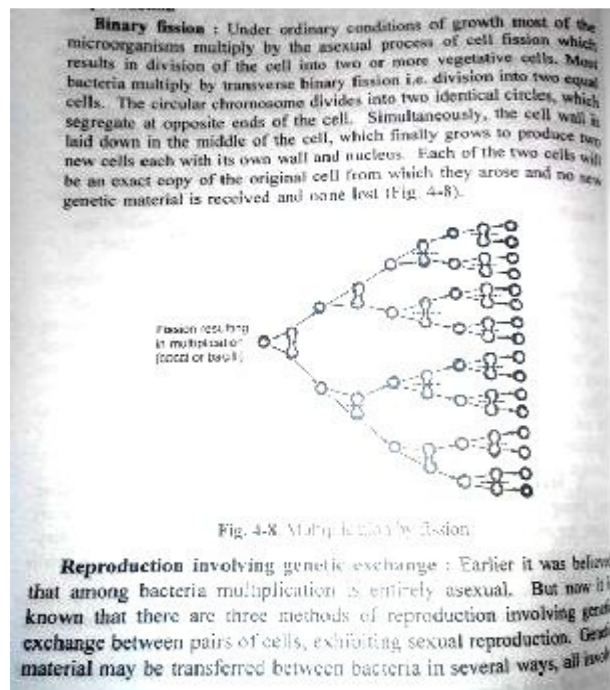
Temperature: favorable temperature for microbes is 37°C

Classified in 3 groups based on temperature- Psychophiles= cold loving; mesophiles=moderate temp. loving; thermophiles=hot loving

Group	Growth Temperature (°C)		
	Minimum	Maximum	Optimum
Psychrophiles			
Obligate	5 and below	15 to 22	15 to 18
Facultative	5 and below	30 to 35	25 to 30
Mesophiles	10 to 15	35 to 47	30 to 45
Thermophiles	40 to 45	60 to 85	55 to 75

Osmotic pressure : and pH:

Reproduction



Brief description of other routes of production i.e. **Transformation, Conjugation, Transduction**

Ans. 5 : Gaseous sterilization may be defined as the destruction of all living microbes with a chemical in gaseous or vapor state. Certain species of herb and smokes have been used since ancient times for preventing the disease. This method is important when some material cannot be sterilized by dry or moist heat sterilization. However these gases are harmful and difficult to remove from material.

-Their usefulness to be discussed

Commonly used agents are

- Ethylene oxide
 - o It is simplest cyclic ether mixed with gases generally with inert gas such as CO₂
 - o Materials can be sterilized using this and method to be discussed
- Ozone
 - o Materials can be sterilized using this and method to be discussed
- Formaldehyde
 - o Materials can be sterilized using this and method to be discussed
- B-Propiolactone (BPL)
 - o Materials can be sterilized using this and BPL characteristics

Test for sterility

The test for sterility are intended for detecting the presence of viable microbes in formulation. Test must be carried out in condition to avoid accidental contamination during test.

Culture media

Detailed composition of fluid thioglycolate media, alternative thioglycolate media and soybean casein digestive media should be discussed. Also, mention process pH and conditions for the media.

Test organism

Medium	Test Microorganisms (strains specified in LP)	Incubation	
		Temperature (°C)	Condition
Fluid Thioglycolate	1. <i>Bacillus subtilis</i>	30 to 35	Aerobic
	2. <i>Candida albicans</i>	30 to 35	Aerobic
	3. <i>Bacteroides vulgatus</i>	30 to 35	Aerobic
Alternative Thioglycolate	1. <i>Bacteroides vulgatus</i>	30 to 35	Anaerobic
Soybean-Casein Digest	1. <i>Bacillus subtilis</i>	20 to 25	Aerobic
	2. <i>Candida albicans</i>	20 to 25	Aerobic

Test procedure

Method a membrane filtration and Method B direct inoculation with details should be included.

Ans. 6: Quantitative chemical or physical methods can assay most of the substances, however limited no. of drugs of natural origin can not be assayed satisfactorily assayed. Biological assay refers to measurement of relative potency by measuring the anticipated effect. Microbial assay is a type of bioassay performed with microbes. E.g. yeast, bacteria, fungi. Many agents either inhibit the microbial growth or essential for their growth (Vitamins) can be standardized by microbial assay.

Microbial assay of antibiotics

The inhibition of microbes under standard conditions can be utilized for demonstrating the therapeutic efficacy of antibiotics.

Assay of antibiotics are based on either serial dilution or diffusion method.

Official assay method

Cylinder plate method or serial dilution method

-Details about the test organism and inoculum

-Composition of media

-apparatus, assay design and detailed method should be discussed

Antibiotic	Test organism	Assay method*	Medium**	Incubation temp. (°C)
Ampicillin	<i>Micrococcus luteus</i>	A	D	32-35
Chloramphenicol	<i>Escherichia coli</i>	B	C	35-37
Doxycycline	<i>Staphylococcus aureus</i>	B	C	35-37
Erythromycin	<i>Micrococcus luteus</i>	A	D	35-37
Oxytetracycline	<i>Bacillus cereus</i> Var. <i>Mycoides</i>	A	F	32-35
Rifampicin	<i>Bacillus subtilis</i>	A	B	32-35
Streptomycin sulphate	<i>Bacillus subtilis</i>	A	E	32-35
Tetracycline	<i>Bacillus cereus</i>	A	F	32-35
Tetracycline	<i>Staphylococcus aureus</i>	B	C	35-37

* Method A—Cylinder plate method
Method B—Turbidimetric method

Microbial assay of Vitamins

Official assay method

Microbial assay of Calcium pantothenate or niacin should be discussed as per following points

-Reagents

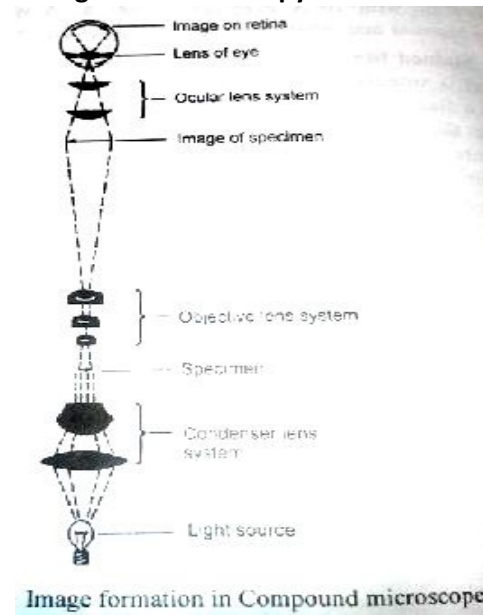
-Stock and standard solutions and Test solution

-Medium , stock culture of test organism and inoculum

Ans.7: a) Light microscopy: Basic microscope used in microbiology is compound microscope, it is a 2 lens system with the objective lens near the objective and ocular lens, near the eye. Usually have, 40X or 45 X and 100 X (oil immersion) lenses.

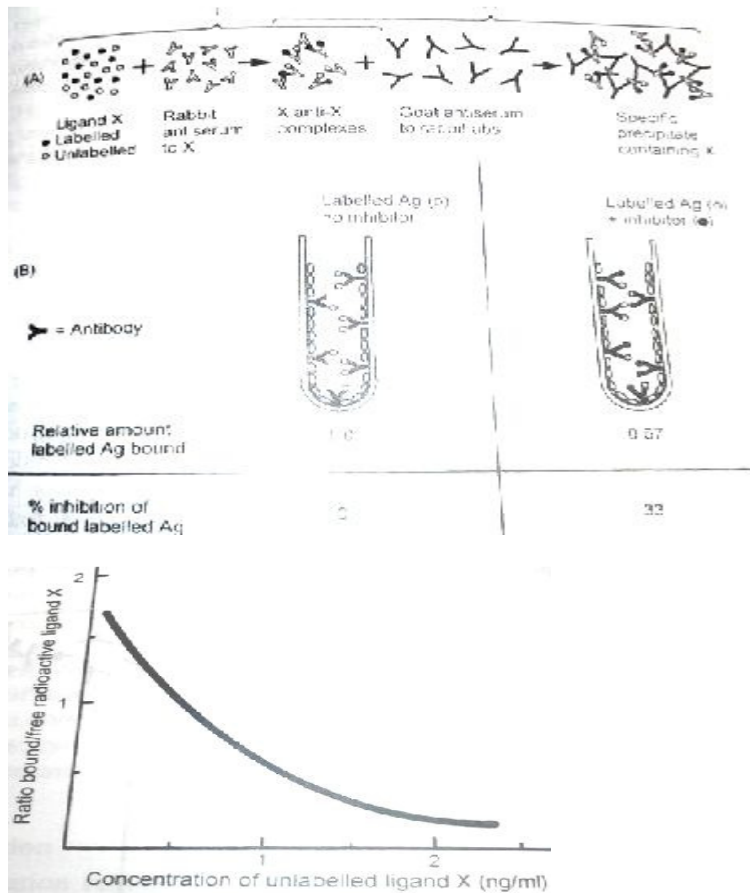
Also, brief description and method of operation of following microscopy should be discussed.

Dark ground microscopy and Phase contrast Microscopy



b) Immuno assays: Radioimmunoassays

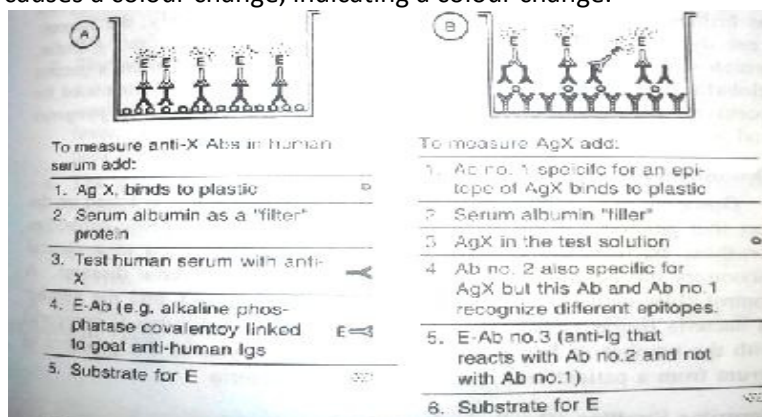
RIA assay is a sensitive, versatile technique using radiolabelled antigen or antibody. It is used to determine the small amounts of drugs, hormones, or antigens such as hepatitis B in donor blood.



Detail method and principles should be discussed.

Enzyme linked ImmunoSorbent assay

ELISA is a sensitive technique that use an enzyme-Ab-Ag combination absorbed onto the sides of a test well. If the patient has Ag or Ab for the diseases agents, the linkage is formed. Addition of substrate causes a colour change, indicating a colour change.



Detail method for the test should be discussed. Also applications of the ELISA should be included

c) Antigen Antibody reaction

The study of antigen-antibody reaction is called serology. Serological techniques are used in transfusion, blood banks, to diagnose diseases, to identify microbes, to detect allergy and in tissues transplantation. Major Ag-Ab reaction mentioned as follows- Discuss in detail

Agglutination

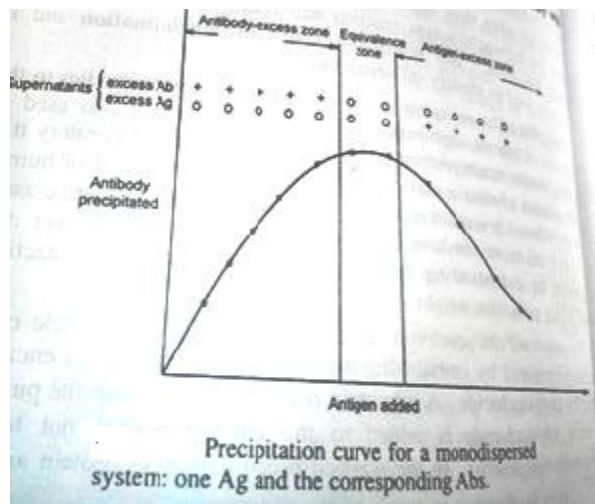
When suspension of bacteria or RBCs or mixed with their antisera they are usually clumped (agglutinated).

Discuss the method in detail of quantitative, qualitative and semi quantitative assay.

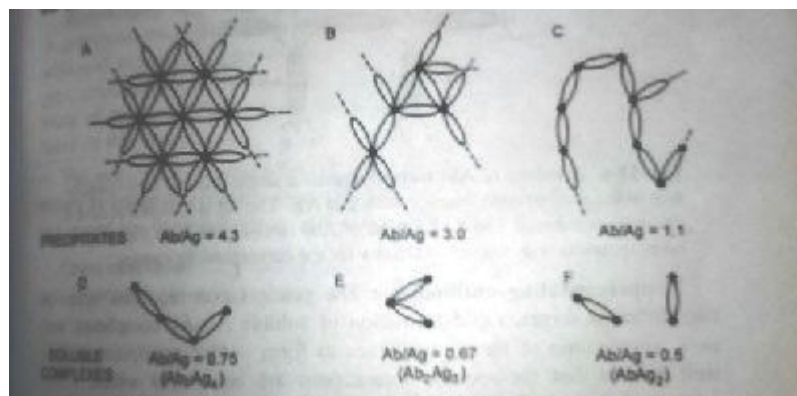
Also discuss the applications.

Precipitin

This test is useful in detecting Ab to the exotoxins of tetanus, diphtheria and scarlet fever. Also, used to identify various serum proteins in blood.



Discuss advantages and disadvantages as well as method in detail.



Lattice theory, reversibility of precipitation cross reactions discuss in detail.

Brief description of complement fixation and other reactions.