

BT102 - FINAL TERM SUBJECTIVE MEGA FILE

ALL DATA FROM PAST PAPERS

GROUP: BIOTECH BRAINY BUNCH

ADMINS: MEHWISH MUGHAL & BELLA

CONTACT: mehwishshabirhussain07@gmail.com

Current final term subjective – 2022

1-PCR steps

- 1- Denaturation step
- 2- Annealing step
- 3- Extension step

2-Western Blotting

Western blotting

- Bacterial Identification V
- Proteins from microbes are separated by electrophoresis
- Protein bands are blotted onto a nitrocellulose membrane.
- Proteins could be detected by electrophoresis and blotting.
- Blots can be detected by specific antibodies labelled with radioisotopes etc.

3-Immunity in detail

Definitions

✓ **Immunity:** Ability of the body to protect itself from harmful effects of diseases.

✓ **Susceptibility:** lack of immunity

Types of Immunity

A) **Innate:** Present at birth

b) **Adaptive:** Acquired by experience

Innate Immunity

✓ Present at birth

✓ Rapid to respond

✓ Nonspecific

✓ do not increase in response with repeated exposures to the same agent

✓ Have no memory

✓ Innate Immunity

✓ First line of defense: Physical barriers (skin, mucus membrane)

✓ Second line of defense: Phagocytic cells and their secretions

Adaptive Immunity

- ✓ Specific
- ✓ Have memory
- ✓ Increase with repeated exposure
- ✓ Slower to respond
- ✓ Humoral immune response
- ✓ Cellular immune response

4-Vaccine in detail

Concept of Vaccines

• People recovered from infections are immune to them. • Vaccine: Suspension of organism or fraction of organism that induces immunity

Principle of Vaccines

• It mimics the infection by the organism but without causing the disease

Types of Vaccines

• **Live attenuated:**

– Weakened organisms, – Louis Pasteur was the first to do that. • Pasteurella multocida

Benefits of Live Vac

• Better immunity: mimics actual infection, – Humoral, – Cellular • Virus replicates increasing the Ag dose • Long term immunity

• Inactivated Killed:

– Organism killed with formalin or phenol. • Cannot be weakened • Need frequent boosters. Inactivated Killed • Examples

– Rabies

– Polio

– Influenza

• Mostly induce humoral immunity

Other Vaccines

Toxoids: Toxins are inactivated and used as a vaccine. • Tetanus toxoid

Conjugated Vaccines:

• Poor antigens attached to proteins. • Haemophilus influenza type b

DNA vaccines:

• DNA injected into cells. • Proteins are expressed which stimulate immunity • West Nile Virus

Polysaccharides have poor success

Modern Vaccines

Subunit vaccines:

Portion of viral or bacterial proteins that can induce immunity. Also called recombinant vaccines

Modern Trends

Organisms in bulk are needed to make vaccines. Bacteria can be grown in flasks by providing medium of growth. Cell culture for viral vaccines. Chicken embryos can be used to grow viruses

Influenza vaccine

Animals can be used to grow viruses. Plants can be used in recombinant technology. Vaccines mimic infection but without harmful effects. Various types are available. Booster is required for better protection.

5-Drug sensitivity methods

Drug Sensitivity

Physicians use prescribe drugs that they know will work. If the organism has been identified, selection of drug is easy.

➤ **Disk Diffusion Method:**

- ❖ Standardized inoculum spread on agar plate
- ❖ Filter paper disks soaked with antibacterial
- ❖ Zone of inhibition is recorded
- ❖ Zone compared to a standard Table

❖ **Report:**

- Sensitive
- Intermediate
- Resistant

❖ **Minimum Inhibitory Concentration (MIC):**

- also called E test:
- the lowest conc. that inhibits visible growth

❖ **Broth Dilution Method:**

- ❖ Diffusion method has limitation
- ❖ does not tell you if the drug is bacteriostatic or bactericidal
- ❖ a broth dilution test is the answer.
- ❖ Various dilutions of the drug made and tested with test organism
- ❖ Tells both MIC and minimal bactericidal concentration (MBC)
- ❖ the wells that do not show growth are important.
- ❖ Culture the well that is one well higher in concentration than MIC.
- ❖ If growth occurs, the drug is bacteriostatic, otherwise cidal.

6-MHC I and MHC II

1) MHC I: Present on all nucleated cells

2) MHC II: Present only on APCs

7-Characteristics of yeast

Non-filamentous fungi, Unicellular, Reproduce by budding (Asexually) or sexually, some are dimorphic

– Yeast: at 37°C

– Molds: Room temp

Yeast are Larger than bacteria, No flagella or cilia, Facultative anaerobes, Larger than bacteria, No flagella or cilia, Facultative anaerobes, Gram Positive

• Yeasts are unicellular, some are dimorphic, both sexual and asexual reproduction, Bigger than bacteria.

8-Characteristics of Fungi

General Features

- Kingdom: Fungi
- Chemoheterotrophs
- Acquire nutrients by absorption
- Asexual and Sexual reproduction

Definitions

- Hyphae: Thread like filaments of a fungus
- Thallus: Collection of mycelia
- Mold: Filamentous fungi
- Yeast: unicellular fungus
- Mycology: Study of fungi

Mycelium: Collection of hyphae

Filamentous fungi

- Identified by morphology, not by biochemical testing
- Slide culture is made using Sabouraud's agar

- Spores are inoculated
- Slides stained with lactophenol cotton blue

9-Drugs which are used in cell wall inhibition

Penicillin, Chloramphenicol, Streptomycin, Tetracycline are used for the inhibition of cell wall.

10-Morbidity, Mortality, Pathogenicity

Morbidity: Incidence of a disease

Mortality: Deaths from a disease

Pathogenicity: The ability to cause disease

11-Virus, Virion, Viroid

Viruses: Acellular organisms only contain proteins and nucleic acids

Viroids: composed only of circular ssRNA (potato spindle tuber viroid)

Virusoids: Contain circular ssRNA, need helper viruses for replication and encapsidation also called satellite viruses

12-Primary and Secondary immune responses

Primary immune response

The first contact that an organism has with a particular antigen will result in the production of effector T and B cells which are activated cells that defend against the pathogen. The production of these effector cells as a result of the first-time exposure is called a primary immune response.

Secondary immune response

Secondary response: the immune response occurring on second and subsequent exposures to an antigen, with a stronger response to a lesser amount of antigen, and a shorter lag time compared to the primary immune response.

13-Spectrum of tetracycline

Wide: Targets many groups of microbes – Tetracycline.

14- Plaque Formation

Plaques formation:

– It is equivalent to bacterial colony, – Plaque is an area where the virus infection has lysed the cells.

15- NonComial infections

A nosocomial infection is an infection you get while you're in the hospital for another reason. It's also called a hospital-acquired infection or a health-care associated infection. Patients and healthcare professionals bring germs inside hospitals and pass them to each other.

16-Benefits of lichens

Lichen uses

- Dyes for clothes
- Usnic acid as antiseptic in China
- Erythrolitmin: for Litmus paper
- can cause allergic contact dermatitis

17- Hypersensitivity type 1

Type I hypersensitivity is also known as an immediate reaction and involves immunoglobulin E (IgE) mediated release of antibodies against the soluble antigen. This results in mast cell degranulation and release of histamine and other inflammatory mediators.

18-Procedure of capsule staining

Capsular Staining

- it is extracellular material (glycocalyx).
- When regular and thick, called capsule
- when loose and not organized, called slime
- Thickness of the capsule depends upon the culture conditions.
- mostly are water soluble.

- Capsular organisms use make the broth viscous and stringy
- Colonies look moist, glistening, and sticky.
- Involves in virulence
- those lacking capsule are rough strains.
- Prepare a thick smear in a loopful of congo red (1%) stain
- Fixation in acid alcohol for 15 sec
- Wash with dH₂O
- Cover it with acid fuchsin for 1 min.
- Wash with water
- Bacteria stains red
- Capsule colourless
- Dark blue background

Capsule can be seen with staining. Helps in identifying organisms.

19-Types of Algae

Phaeophyta (Kelp): Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll a and c, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.

Rhodophyta: Red Algae, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).

Chlorophyta: Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.

Bacillariophyta: Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels. Diatoms: Come in beautiful colours and shapes.

Dinoflagellata: These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll a and c, and carotene. These algae store starch in

them. Dinoflagellates in the genus *Alexandrium* produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of *Alexandrium* Gives Ocean a deep red color commonly known as red tide.

Oomycota (Water molds): They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores. See the cottony growth on the fish in the accompanying figure to the right for a water mold. Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s.

20-Portals of Entry of microbes

Mucus membranes: These include linings of digestive tract, respiratory tract, urogenital system, and conjunctiva. Most microbes enter through the digestive and respiratory tracts. Diseases that are commonly contracted via the respiratory tract include the common cold, pneumonia, tuberculosis, influenza, and measles. Microbes in the gastrointestinal tract can cause poliomyelitis, hepatitis A, typhoid fever, amebic dysentery, giardiasis, shigellosis (bacillary dysentery), and cholera. HIV infection, genital warts, chlamydia, herpes, syphilis, and gonorrhea are urogenital infections.

Skin: Surface area wise, skin is the largest organ of the body. Unbroken skin is impermeable to organisms. However, hair follicles and sweat gland ducts are some of the portals for microbial invasion.

Parenteral Route: Injury to the skin or mucous membrane leads to easy access for the organisms in the body. Any cut received on the skin for example becomes a portal of entry. Punctures, injections, bites, cuts, wounds, surgery, and splitting of the skin or mucous membrane due to swelling or drying can all establish parenteral routes. HIV, the hepatitis viruses, and bacteria that cause tetanus and gangrene can be transmitted parenterally. Also note please that most organisms prefer one route over the other. For example, *Salmonella* if swallowed will cause the disease; however, if rubbed on the skin will not cause typhoid other than a local inflammatory reaction.

21-What is natural, innate active and passive immunity

Innate (Natural):

Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific. Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body. Nonspecific: It means that it does not differentiate if it is an *E. coli* or a *Staph* or *Salmonella*. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize *E. coli*,

Staph or Salmonella at the same time (if these pathogens are present there). Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.

First line of defense: Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside.

Second line of defense: Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.

Adaptive Immunity: Adaptive (Acquired): It is the third line of defense. Acquired by experience, specific to an organism. Specific: It means that antibodies made against E. coli will destroy E. coli only, and will not do any harm to a Staph for example. Have memory: It means if antibodies have once been made against E. coli, this exposure will be remembered by the body, and if a second encounter is made with the same type of E. coli again, the body would mount a heightened immune response against E. coli because it has memory cells developed in the body which upon second stimulation proliferate quickly and mount a greater defensive response. Because of memory, body's immune response increase with every repeated exposure. Slower to respond: For the first time exposure, the body's response is slower and takes a long time compared with the innate immunity which is immediate.

Adaptive Immune Response can further be divided into:

Humoral immune response (antibody response by B cells or lymphocytes)

Cellular immune response (T cells are made which kills microbial harboring cells).

Naturally Acquired Active Immunity: This type of adaptive immunity develops in a person who gets infected with a microbe but then recovers from that illness making antibodies.

Naturally Acquired Passive Immunity: A typical example of this type of immunity is transfer of antibodies from colostrum or milk of a mother to the offspring.

Artificially Acquired Active Immunity: When a person receives a vaccine against a microbe and the body makes antibodies in response to that vaccine, it is called artificial acquired active immunity because body makes these antibodies actively by itself.

Artificially Acquired Passive Immunity: When premade antibodies are given to a person in order to protect a person from an illness or a snake bite, this constitutes an artificially acquired passive immunity type. The following figure illustrates these four types.

22-Features of Fungi

Some yeasts divide by fission which is even division compared with budding yeast which is a kind of uneven division. Yeast if grown on solid medium grows like bacteria. In other words,

colonies of yeast look like bacterial colonies but are bigger in size. Yeasts can grow as facultative anaerobes. Saccharomyces if given oxygen to grow, it converts carbohydrates aerobically and produces CO₂ and water; however, if oxygen is denied, carbohydrates get converted fermentative into ethanol and CO₂.

23-Features of lichens

Lichens are a combination of green alga (or a cyanobacterium) and a fungus. Green alga or cyanobacterium is also called a phycobiont and the fungus is called a mycobiont in this relationship. Both partners have a symbiotic relationship with each other. If separated apart, none can exist for long suggesting that both are benefited from each other. Lichens are the only microorganism that can grow where no other microbes can grow. This is the reason that you will see lichens growing on bare rocks, tree trunks and house roofs and bare soils. They typically grow extremely slowly (only 1 mm to 3 cm per year). Lichens are classified on the basis of the fungus. There are three types of lichens in general that are based on the physical appearance.

24- Prevalence and incineration

Prevalence: New + existing cases

Incineration: burning to ashes

25-Reverse transcript

They also have reverse transcriptase in their capsids which converts plus

strands into double stranded DNA molecules. These dsDNA are then are integrated into host genome as proviruses. While still remaining part of host chromosome, mRNAs are generated from the provirus which can make capsids and other proteases along with reverse transcriptase. New virions are assembled with two new copies of plus strands along with reverse transcriptase molecules in the capsid. The newly assembled virions are then released as enveloped viruses because they take part of the plasma membrane with them.

26-Characteristics of antibodies

Although antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese.

27-Mechanism of microbes damaging to the host cell

Microbes damage the host cells by four mechanisms:

By using the host nutrients: Bacteria can deplete nutrients needed by the host cells. For example, bacteria secrete proteins called siderophores which bind and concentrate iron. Iron is needed for bacterial growth. There are 3 proteins belonging to the host which normally carry iron. They include lactoferrin, transferrin, and ferritin. In other words, siderophores may bind all these proteins thus depleting the host cells of iron.

Direct Damage: Bacteria that grow in host cells rupture the cells eventually to release themselves and spread further. Rupturing obviously cause the damage to the tissues.

Production of Toxins: Toxins are poisonous substances released by microbes.

Exotoxins: These are released by bacteria when they are still alive. Antibodies made against them are called antitoxins which can bind with the toxins and can neutralize them. Toxins can be treated chemically to alter them so that they do not exhibit their toxicity, hence such toxins (called toxoids) could be used as vaccines. Exotoxins are disease specific. For example, tetanus toxin is released by *Clostridium tetani*. Exotoxins are also cell or tissue specific and they are sometimes named according to the cells or tissues they bind to. For example, neurotoxin binds to nerves, cardiotoxins attach heart muscles, hepatotoxins damage hepatocytes and leukotoxins attach leukocytes.

Types of Toxins: Based on the structure and function, exotoxins are divided into three types:

A-B Toxins: Such toxins consist of two parts designated as A and B, both of which are polypeptides. The A part is the active (enzyme) component, and the B part is the binding component. A model example of A-B toxin is diphtheria toxin. B part binds to the receptor on the cell and then A-B toxin is then internalized by the cell thus releasing the toxin in the cell which then shows its action.

Membrane Disrupting Toxins: These toxins disrupt plasma membrane and cause lysis of the cells. Some make channels in the plasma membrane while others disrupt phospholipid molecules. Membrane disrupting cells kills phagocytes thus contributing to their virulence. Membrane disrupting toxins that kill leukocytes are called leukocidins. They make membrane channels. Those that lyse RBCs are called hemolysins.

Superantigens: These antigens provoke very intense immune response. These are bacterial proteins that stimulate T cells nonspecifically resulting in widespread release of cytokines. The excessively high levels of cytokines released by T cells enter the bloodstream and give rise to a number of symptoms, including fever, nausea, vomiting, diarrhea, and sometimes shock and even death.

Endotoxins: They are part of the outer portion of cell wall of gram negative bacteria. LPS is an example. LPS is released when gram negative cells die and their cell wall disrupts. Endotoxins cause release of cytokines (IL-1 and TNF) from macrophages. LPS and cytokine result in high fever. Endotoxins cause chills, fever, weakness, generalized aches, and, in some cases, shock (endotoxins cause vasodilation and blood pooling) and even death. Presence of LPS in fluids can

be tested by limulus ameobocyte lysate assay which contains a clotting protein that clots when reacts with LPS.

28-Capsule function and capsulated organisms example

Capsule: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

- Plays important role in virulence (degree of pathogenicity)
- Can also be a part of the vaccine against the bacteria to which it belong
- Can be demonstrated by negative staining technique.
- Capsulated organism make glistening

Bacillus anthracis, streptococcus pneumoniae and Klebsiella are examples of capsulated organisms.

29-Symptoms of anaphylaxis

Symptoms of anaphylaxis

- Feeling lightheaded or faint.
- Breathing difficulties – such as fast, shallow breathing.
- Wheezing.
- A fast heartbeat.
- Clammy skin.
- Confusion and anxiety.
- Collapsing or losing consciousness.

30-Hypersensitivity type -2 diagnosis

Since this type of hypersensitivity targets body's own cells, these hypersensitivities can be diagnosed by looking into the number of cells such as RBCs, platelets etc. This hypersensitivity can be treated by avoid those drugs that cause it. Blood transfusion should be done with correct blood types.

31- Plasmogamy, karyogamy and meiosis

Plasmogamy: A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-). Both cells are haploid (one set of chromosomes)

Karyogamy: The (+) and (-) nuclei fuse to form a diploid zygote nucleus.

Meiosis: The diploid nucleus gives rise to haploid nuclei (sexual spores).

32-Factors affecting microbiota

Physical and chemical factors also influence the type of microbiota. Other factors include pH, O₂, and CO₂. Host defense, level of stress and age of an individual also matters for allowing microbiota of an individual.

33-Difference b/w capsules and slime

Capsule: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

Slime: If glycocalyx is loosely attached to the cell, it is called slime.

34-Types of symbiosis

Mutualism: both are benefited.

Parasitism: one is harmed.

Commensalism: One is benefited, other is unharmed.

35-When the thymus is removed why skin is not rejected

When the thymus gland was removed from the mice and skin grafts were inserted in those mice, grafts were accepted suggesting the involvement of thymus in this role. Later experiments recognized T cells to be involved in tissue rejection.

36-Inhibition of Cell wall synthesis

Inhibition of cell wall synthesis: Many antibiotics inhibit synthesis of peptidoglycan cell wall. Examples include Penicillins, cephalosporins, bacitracin, vancomycin. As the target is the peptidoglycan layer of the cell wall, the cell wall does not get synthesized, and cells swell up and are lysed as a result (cidal). So, Penicillin is a bactericidal drug or antibiotics. Target of such drugs is the growing cells that are actively involved in synthesizing of cell wall.

37-Inflammation

Damage to body tissues triggers a local defensive response which is called inflammation. It causes redness, pain, swelling, heat, and loss of function of that organ. During inflammation, various mediators of inflammation are released, some of which include:

- ✓ Histamine
- ✓ Kinins: Almost all cells.
- ✓ Prostaglandins by damaged cells
- ✓ Leukotrienes by mast cells

38-Transport system

Cardiovascular and lymphatics are the two transport systems of the body by which cells of the immune system patrol the whole body. Cardiovascular system consists of the heart and associated blood vessels (arteries and veins) in which blood circulates while lymphatics are channels that start from tissue spaces and collect tissue fluid (called lymph) from tissue spaces and bring them back to the heart. Along these lymphatic channels, there are strategically placed lymph nodes which filter lymph for microbes much like blood is filtered in the spleen. Lymph nodes contain aggregations of B and T cells along with macrophages and dendritic cells which interact with each other inducing adaptive immune responses.

39-NUCLEIC ACID BASED TESTS

There are many tests that involve nucleic acid sequence detection for the presence of microbes in a clinical sample. Please remember, as antibodies are specific to the organisms, DNA or RNA sequences must be uniquely specific for the microbe that we are going to detect. Such sequences that are specific to an organism are called signature sequences. In other words, it is these sequences that provide the specificity for the organisms. There are many such tests, however, we will only focus on polymerase chain reaction (PCR). PCR goes through 3 step cycles for about 30 to 35 times. Each cycle consists of a denaturing stage (about 95°C), primer annealing (50 to 56°C) and extending stage (72°C). A primer is a fragment of DNA which is specific to the signature sequence. Two primers span the signature sequence actually. At the end of 30 or so such cycles, enough DNA.

40-Location of algae in ocean on which things depends

Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O₂ on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such

algae include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located far from the surface and can use blue light from the sun as blue light is of shorter wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic.

41-Hypersensitivity type - 3

This hypersensitivity involves soluble antigen circulating in the serum and antibodies (IgG or sometimes IgM). Complement gets activated when these complexes get deposited in capillary beds as we see in the kidneys for example. A typical example will be the use of antivenom (antibodies) that was prepared in horses but given to a human to save the life. What happens is that horse antibodies will be recognized by human body as a foreign protein and human immune system will generate antibodies against this. These antibodies will bind with antivenom antibodies and make complexes. These complexes get deposited when kidney glomeruli filter plasma. Complement binds to these trapped complexes and cause damage to the kidneys. This type of hypersensitivity can be diagnosed by history and symptoms. Glomerulonephritis and cellulitis is very common. Deposition of immune complexes in the kidneys can be diagnosed by presence of IgG in kidney cells by using anti-IgG antibodies.

42-Antibiogram

Susceptibility of organisms is recorded over a period of time to see if there is a change in response. Drug sensitivity record that belongs to bacteria in a region is called an antibiogram. Such records are important for clinicians because they have to prescribe various antibacterials to patients for immediate treatment. Since sensitivity results become available only after 2-3 days, it is important for physicians to prescribe drugs that are known to work in an area.

43-Why T cells are called MHC restrictions

T cells are MHC restricted. This means that these cells recognize antigens only when these are associated with self MHC molecule. This also means that T cells do not bind or attach to free antigens. As B cells have B cell receptors, T cells have T cell receptor. B cell receptor binds antigenic epitope while T cells bind T cell specific antigenic epitopes.

44-Signature sequences

There are many tests that involve nucleic acid sequence detection for the presence of microbes in a clinical sample. Please remember, as antibodies are specific to the organisms, DNA or RNA

sequences must be uniquely specific for the microbe that we are going to detect. Such sequences that are specific to an organism are called signature sequences. In other words, it is these sequences that provide the specificity for the organisms.

45-Nucleic acid based microbial detection method

There are many such tests, however, we will only focus on polymerase chain reaction (PCR). PCR goes through 3 step cycles for about 30 to 35 times. Each cycle consists of a denaturing stage (about 95oC), primer annealing (50 to 56oC) and extending stage (72oC). A primer is a fragment of DNA which is specific to the signature sequence. Two primers span the signature sequence actually. At the end of 30 or so such cycles, enough DNA accumulates that one can visualize on an agarose gel easily.

46-Vaccine and its type

Definition:

A product that creates immunity against an infection is called a vaccine. It could be the whole organism or a part of the organism. The observation that people or animals once recover from an infection do not get sick easily to that organism again led to the discovery of vaccines and vaccination.

Principle of vaccination:

A vaccine mimics the infection by the organism but without causing the disease, so immune system gets stimulated in exactly the same way as is done by an infection. However, since vaccines do not harm the body, they provide a safe way for inducing immunity against real microbes that may be encountered in one's life.

Types:

Live Attenuated Vaccines: These are weakened organisms so that they do not harm the body, but divide or replicate in the same way as pathogenic version of the organism does in the body. So, when a live virus or bacteria multiplies in the body, it stimulates the immune system to create an adaptive immune response which is protective against the disease for which vaccine was given. The benefits of live vaccines are a better immunity because live microbes mimic actual infection (although they do not cause harm to the body). Live vaccines induce both humoral and cellular immune responses together, so live vaccines are a better choice if available. Because live microbes multiply in the body, this replication results in the increase of antigenic amount needed for stimulation of the immune system. Also, this antigen remains for a long time in the body, continuous presence of antigen continuously keeps the immune system active for longer time resulting in long term immunity.

Inactivated Killed Vaccines: Sometimes a non-pathogenic version of the microbe is not available, and to make a vaccine in this situation, the organism is killed with formalin or phenol and then used as a killed inactivated vaccine. Because organisms in such a vaccine do not replicate in the body, frequent boosters are required to achieve a good level of immunity (antibodies). Also, remember that this is a killed vaccine, so classical cellular immunity is not created with killed vaccines.

Toxoids: Some diseases are caused by bacterial toxins, and not by bacteria. In such cases, toxin is modified (inactivated) and used as a vaccine. Such a vaccine is known as a toxoid. Tetanus toxoid is a good example.

Conjugated Vaccines: Sometimes special parts of organisms are required for generating protective immunity. However, those substances are poor antigens if used alone. In order to enhance immunity, such poor antigens are attached to proteins, and such vaccines are called conjugated vaccines. Haemophilus influenza type b is a good example.

DNA vaccines: We now know that sometimes only certain proteins are required for generating a good immune response. In such cases, the genes responsible for those proteins can act as vaccines. DNA coding for such a protein is injected into cells, and that DNA gets transcribed by the cells of the body into the required protein and that protein induces the antibody production (because that protein is foreign to the body). West Nile Virus is a good example of DNA vaccine.

Subunit vaccines: Also called recombinant vaccines, such vaccines are composed of a portion of viral or bacterial proteins that can induce immunity. In other words, this is another version of DNA vaccines. In subunit vaccines, antigenic epitopes are included in the genome which is inserted into an expression vector that makes those subunit proteins. These subunit proteins are collected and used as vaccines to stimulate the immune system. Again, these act like a killed vaccine. There is another mechanism being explored in these days for recombinant vaccines. A subunit vaccine for Foot and Mouth disease virus is available. Such genes are being incorporated in plants now that are acting like an expression vector. Animals can eat those plants and subunit vaccines can induce immune response through M cells of the intestine. Success is limited though so far.

47-What is aerial and vegetative hyphae.

Vegetative Hyphae: Hyphae that are embedded in the medium and are used to obtain food are called vegetative hyphae.

Aerial or Reproductive Hyphae: The portion of hyphae that is concerned with reproduction. This portion is projected into the air. They bear reproductive spores. Hyphae look like a cottony growth on the medium while yeast growth looks like a powder.

48-Why hepten is considered poor antigen.

It is a molecule that by itself does not induce antibody production; however, can bind preformed antibodies. So, it needs a carrier molecule to induce antibody production. Penicillin is an example that acts like a hapten. So that's why it is considered as poor antigen.

49-What are symptoms of anaphylaxis?

Symptoms of anaphylaxis

- feeling lightheaded or faint.
- breathing difficulties – such as fast, shallow breathing.
- wheezing.
- a fast heartbeat.
- clammy skin.
- confusion and anxiety.
- collapsing or losing consciousness.

50-What is procedure of acid fast staining

Procedure for Acid-fast staining:

Carbol fuchsin: It is a primary stain heat is used to enhance penetration of carbol fuchsin.

Acid alcohol is a decolourizer.

Methylene blue is a counterstain.

Please note, Mycobacteria are gram positive, however, they look red in acid- fast staining.

51- Describe terms agglutination, opsonisation and Neutralization

Agglutination:

Agglutination is a process in which soluble antibodies interact with a particulate antigen. Antibodies can agglutinate antigens which are then cleared as a group by macrophages.

Opsonisation:

Antibodies can coat or cover antigens. Macrophages have receptors that can bind Fc portion of antibodies that have bound to antigens, thus engulfing the antigen and clearing it from the body.

Neutralization:

Toxins bind with antibodies and they become incapable of binding to their target receptors thus the toxins become harmless.

52- What are different ways of transmission of disease

Direct Transmission: person to person physical contact is a direct contact. Influenza spread is a typical example.

Indirect Transmission: A non-living object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

Droplet Transmission: Mucus droplets are created when you sneeze for example. These droplets carry organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

Vehicle Transmission: Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

53-ames antiviral drugs

Abacavir, Acyclovir, Umiferore.

54-Antibodies that inhibits protein synthesis

Inhibition of Protein Synthesis:

Many antibiotic target ribosomes and bind them so that the ribosomes are not able to make proteins. Such drugs include chloramphenicol, erythromycin, tetracycline and streptomycin. Selective cytotoxicity is ensured by the difference in the ribosomes of prokaryotes versus eukaryotes. Eukaryotes have 80S ribosome while prokaryotes have 70S ribosomes. There are many targets in the ribosomes to inhibit protein synthesis. For example, chloramphenicol binds to 50S ribosomes and inhibits peptide bonding, hence protein synthesis is stopped which arrests the growth of the organisms (see the figure for where this is happening). Another drug, streptomycin, binds to 30S part of ribosome and changes the shape of this part of the ribosome which in turn results in misreading of RNA codes by the ribosomes stopping the synthesis of proteins. Tetracyclines interfere with attachment of tRNA to mRNA ribosome complex thus inhibiting protein synthesis.

55-Factors that determine the composition and distribution of microbiota

Nutrients: Availability of various nutrients determines the type of organisms present in a system. Similarly, physical and chemical factors also influence the type of microbiota. Other factors include pH, O₂, and CO₂. Host defenses, level of stress and age of an individual also matters for allowing microbiota of an individual.

56-Name of antibiotics of nucleotide and nucleoside analogues

Nucleoside and nucleotide analogues:

Didanosine (ddI) – HIV. Zalcitabine (ddC) – HIV. Stavudine (d4T) – HIV. Abacavir (ABC) – HIV.

57-What is meant by spectrum of drugs

Narrow: Narrow spectrum antibiotics target only a group of organisms. For example, Penicillin kills G+ve bacteria only because Penicillin act on the cell wall which is more pronounced in Gram positive organisms. The advantage of using narrow spectrum is that these spare microflora of the body.

Wide: These antibiotics target many groups of microbes. For example, tetracycline is used against Gram negative and Gram positive organisms and many others. The main disadvantage of such antibiotics is that they also destroy the normal microflora because they target every single organism. You may have observed that when broad spectrum antibiotics are given especially to kids, they often develop candidiasis (caused by *Candida albicans*) commonly called thrush in their mouth cavity. It leaves white spots on the tongue and oral cavity.

58-Hypersensitivity and its types

It is a normal Immune response but in a damaging way. In other words, immune system is acting in a way for which it has made to. So when an antigen gets into the body, immune system recognizes it as foreign and start making antibodies against the antigen. However, the consequences are bad and damaging to the body. It may be called an abnormal antigenic response. Allergy is another name for hypersensitivity. Those antigens that act in a bad way are called allergens.

Types

Hypersensitivities can be grouped into 4 types based on the types of antibodies or cells involved.

Type I (Anaphylactic): Also called anaphylaxis or immediate hypersensitivity. This is IgE mediated.

Type II (Cytotoxic): IgG and IgM mediated as these antibodies attach to cells and destroy them by activating complement.

Type III (Immune complex mediated): Immune complexes are formed by IgM or IgG antibodies and these complexes lodge in the capillary beds and cause damage by complement activation.

Type IV (delayed type cell-mediated): CD4+ helper cells along with macrophages are involved.

59-What are two transport systems in body? Why they are necessary?

Cardiovascular and lymphatics are the two transport systems of the body by which cells of the immune system patrol the whole body. Cardiovascular system consists of the heart and associated blood vessels (arteries and veins) in which blood circulates while lymphatics are channels that start from tissue spaces and collect tissue fluid (called lymph) from tissue spaces and bring them back to the heart. Along these lymphatic channels, there are strategically placed lymph nodes which filter lymph for microbes much like blood is filtered in the spleen. Lymph nodes contain aggregations of B and T cells along with macrophages and dendritic cells which interact with each other inducing adaptive immune responses. Well, in unicellular organisms, nutrients can be absorbed directly from the environment in which a unicellular organism is living. However, a multicellular organism does not have this easy access to the nutrients. So, nutrients must surround the cells in a multicellular organism. These nutrients are provided by cardiovascular system which pumps blood to all organs. Remember that only fluid part of blood leaves blood vessels. RBCs never leave blood vessels. Sometimes proteins also leak from these blood vessels; however, once they leak from the blood vessels, they never can enter back into blood vessels because of their bigger size. If proteins remain in the tissue spaces, they exert osmotic pressure which attracts water into these space resulting in edema. However, nature has provided another mechanism in the form of lymphatic channels which start from tissue spaces and can drain tissue fluid and proteins back to the heart. This is the role of blood vessels and lymphatics that they ensure circulation of nutrients throughout the body without causing any edema or abnormality. B cells and T cells also keep circulating between these two channels (cardiovascular and lymphatics). Unfortunately, microbes can also use these two systems to spread themselves in the body. That is the reason, spleen and lymph nodes are placed in these channels by the nature to deal with these microbes that find their ways into blood and lymph.

Lymphatic System consists of the following:

- Bone marrow
- Lymph nodes
- Spleen
- Tonsils
- Thymus
- Mucosa associated
- Bursa of Fabricius in birds

60-What are algae? Describe its structure

These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemo heterotrophs. They are responsible for 80% atmospheric O₂ on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such algae include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located far from the surface and can use blue light from the sun as blue light is of shorter wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic.

Structure:

Body of multicellular alga such as seaweed is called a thallus which consists of branched holdfasts (anchor alga to rock) stem like hollow stipes and leaf like blades. There is no vascular tissue in these algae. Also, the stipe is not lignified or woody, so it does not provide support to the weed. Surrounding water provides the support for the thallus. Some algae have a gas filled body inside them which keeps them floating in the water. This gas filled structure is called pneumatocyst or float.

61-Why viruses grow on live cell

Viruses are obligate intracellular parasites. In other words, virus will only replicate inside a living cell. Hence, viruses can be grown in animals (mice, rabbits etc.), embryonated eggs (hen, duck), and cell cultures of animal and plant origin. Various cell lines are available commercially for this purpose.

62-Type II hypersensitivity diagnosis and how treated

This hypersensitivity involves antigenic cells and IgG or IgM antibodies that become reactive with these body cells. Whenever, IgM or IgG antibodies bind to their antigens, they always activate complement which then can punch holes in the cells lysing them altogether. Blood transfusion reaction is a good example of type II hypersensitivity. Sometimes, drugs bind to body cells and change them in such a way that cells of the immune system start reacting with body's own cells damaging them. See the figure for drug induced cytotoxicity. Since this type of hypersensitivity targets body's own cells, these hypersensitivities can be diagnosed by looking into the number of cells such as RBCs, platelets etc. This hypersensitivity can be treated by avoid those drugs that cause it. Blood transfusion should be done with correct blood types.

63-Types of T cells

Helper T – cell and Cytotoxic T – cells.

64-Peristalsis

Provides motility to the intestine propelling the digesta caudally and out of the rectum removing the organisms.

65-What are PAMPS

Pathogen associated molecular patterns (PAMPs): Formed by LPS, flagellin, peptidoglycan, DNA, RNA, these are repetitive patterns which the cells of the innate immune system recognize and get activated to release cytokines and reactive radicals that kill the organisms.

66-What is inflammation and consequences of inflammation

Inflammation is also part of the innate immunity. Damage to body tissues triggers a local defensive response which is called inflammation. It causes redness, pain, swelling, heat, and loss of function of that organ. During inflammation, various mediators of inflammation are released, some on which include:

- Histamine
- Kinins: Almost all cells.
- Prostaglandins by damaged cells
- Leukotrienes by mast cells

Cytokines are released (TNF-alpha), and they cause release of acute phase proteins from the liver.

67-Type IV hypersensitivity mechanism

It is called as delayed type because its bad effect becomes visible 2-3 days after exposure to the antigen. This is the only hypersensitivity that involves cells rather than antibodies. Robert Koch discovered this when he injected tuberculin in patients. Contact dermatitis is the most common example of this kind of hypersensitivity. This is basically an interaction between an antigen, T cells and macrophages. Transplant rejection is another good example of delayed type IV hypersensitivity. Reaction to hair dyes is another example. Poison Ivy is another example in which contact with this plant initiate type IV hypersensitivity response. Chemicals from poison ivy interact with skin proteins and change them into foreign-looking protein molecules. Body's

immune system mounts an immune response against these proteins resulting in a damaging type of intense inflammatory response.

68-Features of antigen

Antigen: A foreign substance that provokes immunity is called an antigen. More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably. Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens. Antigens are foreign to the body. Degradability: Inert molecules are not antigenic.

69-Name Antigen Presenting cells

Antigen Presenting Cells: These cells are able to present antigens to B cells for making antibodies by B cells. There are three cells that fall into this category: Dendritic cells, macrophages, and B cells.

70-What MRSA

MRSA (most commonly): Because of continuous use, resistance to original penicillin emerged after a few years of use. Scientists introduced synthetic penicillin called methicillin which was more effective against penicillin resistant Staph aureus. However, soon after the use of methicillin, Staph aureus also became resistant to the drug. So these Staphs were named as Methicillin Resistant Staphylococcus aureus (MRSA). Now the same term MRSA is applied to many organisms that have become resistant to a wide range of Penicillins and Cephalosporins. Originally, MRSA was confined to Staph aureus only which still is the most common MRSA pathogen, however, some other organisms have also been given MRSA status:

- Enterococcus faecium
- Klebsiella pneumoniae

71-Write brief note on lichen

Lichens are a combination of green alga (or a cyanobacterium) and a fungus. Green alga or cyanobacterium is also called a phycobiont and the fungus is called a mycobiont in this relationship. Both partners have a symbiotic relationship with each other. If separated apart, none can exist for long suggesting that both are benefited from each other. Lichens are the only microorganism that can grow where no other microbes can grow. This is the reason that you will see lichens growing on bare rocks, tree trunks and house roofs and bare soils. They typically grow extremely slowly (only 1 mm to 3 cm per year). Lichens are classified on the basis of the fungus. There are three types of lichens in general that are based on the physical appearance:

1. Crustose: These lichens form a thin crust on the surfaces of objects on which they grow

2. Foliose: These look like a leaf or they are leaf-like growths.

3. Fruticose: These are coral-like shrubby or bushy growths.

Structure:

Lichen thallus consists of 3 parts:

Medulla: It is mostly the middle portion. Microscopically, it consists of fungal hyphae and algal cells or cyanobacteria. In other words, fungal hyphae grow around algal cells in a tangled mass.

Cortex: This may divide into two layers: upper and lower layer

Rhizine: These are roots or holdfasts through which with fungal hyphae obtain nutrients and moisture.

Economic Benefits:

- o Various dyes for clothes are obtained from lichens.
- o Usnic acid is obtained from a lichen and used as antiseptic in China.
- o Erythrolitmin: for Litmus paper
- o Lichens can cause allergic contact dermatitis.
- o Lichens are the major food for tundra herbivores.
- o They can be used as indicator of environmental pollution.

72-Difference between antibiotics and antimicrobial

Antibiotics:

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections.[1][2] They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity.[3][4] Antibiotics are not effective against viruses such as the common cold or influenza;[5] drugs which inhibit viruses are termed antiviral drugs or antivirals rather than antibiotics.

Antimicrobials

An antimicrobial is an agent that kills microorganisms or stops their growth.[1] Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are microbicides, while those that merely inhibit their growth are called bacteriostatic agents. The use of antimicrobial

medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

73-Reproduction in fungi

Fungi can reproduce by both asexually and sexually.

74-Antibiotics prevent cell wall

Inhibition of cell wall synthesis: Many antibiotics inhibit synthesis of peptidoglycan cell wall. Examples include Penicillins, cephalosporins, bacitracin, vancomycin. As the target is the peptidoglycan layer of the cell wall, the cell wall does not get synthesized, and cells swell up and are lysed as a result (cidal). So, Penicillin is a bactericidal drug or antibiotics. Target of such drugs is the growing cells that are actively involved in synthesizing of cell wall.

75-Epidemiology and types

The study of where and when diseases occur and how they are transmitted in a population. There are three basic types of investigations relating to epidemiology:

Descriptive Epidemiology: (recording the data about disease). It includes location and time of cases of disease, gender, health, age of patients etc. (Basically, frequency and distribution of risk factors in population are recorded). Prevalence and incidence of disease are typical examples. Such studies can be retrospective (after they occur) or prospective (before they occur). So, in summary, in descriptive epidemiology, information about an already existing disease is recorded.

Analytical Epidemiology: It relates to determining the cause of the disease. Analysis of data, mode of transmission of diseases and means to prevent diseases also come in this discipline. Such studies can be done with case control methods in which factors that have preceded the disease are determined. A group of people who have the disease is compared with another group of people in the same location who are free of the disease. A Cohort method tracks two groups forward from exposure to outcome. Cohort study compares the experience of a group exposed to the factor with another group not exposed to the factor. An association between exposure and its outcome is determined which makes it easy to see the risk factor involved. In other words, cohort studies begin with a group of people (a cohort) free of disease. The people in the cohort are grouped by whether or not they are exposed to a potential cause of disease. The whole cohort is followed over time to see if the development of new cases of the disease (or other outcome) differs between the groups with and without exposure.

Experimental Epidemiology: A hypothesis relating to a disease is tested in this approach. For example, smoking cause cancer can be tested by designing an experiment in mice in which they

are exposed to certain levels of smoke over time and then the outcome is observed. Another example will be testing of a drug for prevention of a disease.

Case Reporting: Case reporting is essential part of epidemiology. It provides incidence and prevalence of a disease to epidemiologist. Health care workers report specified disease to local, state, and national offices for such purposes. Using these reports, epidemiologists begin various studies of the patients. If an epidemiological study shows that a large enough segment of the population is affected by a disease, an attempt is then made to isolate and identify its causative agent. Identification is accomplished by a number of different microbiological methods. Identifying the causative agent often provides valuable information regarding the reservoir for the disease. Once the chain of transmission is discovered, it is possible to apply control measures to stop the disease from spreading.

76-Consequences of viral infection

When virus invades body cells, it brings about microscopic and macroscopic changes called as cytopathic effects (CPE). Some viral infections result in lysis of cells (especially with the naked viruses). Such infections are called virulent infections. Other viruses do not replicate inside the cells and remain latent forever or for sometimes. These are called latent infections. Sometimes, latent viruses keep replicating inside the cells albeit at a slower rate that does not kill the cell. However, such individuals are carriers of the virus and may spread to other people. Some viruses are such that these transform host cells to cancerous cells.

77-Define virusoid and prions

Virusoid:

They are similar to viroids; however, they encode one or two gene products and need a helper virus to infect the host cells. The helper virus supplies gene products and other materials needed by the virusoid for completion of its replication cycle. Hepatitis D virusoid is a typical example which uses Hepatitis B virus as a helper.

Prions:

These are proteinaceous infectious particles that cause neurodegenerative diseases such as scrapie in sheep, bovine spongiform encephalopathy, and Kuru in humans. These are abnormal form of cellular proteins. How these proteins accumulate in the cell is not clear, although, some genetic components are known to be involved.

78-Define antigen and epitope

Antigen:

A foreign substance that provokes immunity is called an antigen. More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably. Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens. Antigens are foreign to the body. Degradability: Inert molecules are not antigenic.

Epitope:

Specific regions on an antigen. In other words, any antigen has multiple regions each one of those act as a small antigen. These are called epitopes. These epitopes are also called as antigenic determinants. Most antigens have MW >10,000 D. Molecules that are less than 10,000 D are generally poor antigens.

79-Mode of action of chloramphenicol, streptomycin, tetracycline.

Many antibiotic target ribosomes and bind them so that the ribosomes are not able to make proteins. Such drugs include chloramphenicol, erythromycin, tetracycline and streptomycin. Selective cytotoxicity is ensured by the difference in the ribosomes of prokaryotes versus eukaryotes. Eukaryotes have 80S ribosome while prokaryotes have 70S ribosomes. There are many targets in the ribosomes to inhibit protein synthesis. For example, chloramphenicol binds to 50S ribosomes and inhibits peptide bonding, hence protein synthesis is stopped which arrests the growth of the organisms (see the figure for where this is happening). Another drug, streptomycin, binds to 30S part of ribosome and changes the shape of this part of the ribosome which in turn results in misreading of RNA codes by the ribosomes stopping the synthesis of proteins. Tetracyclines interfere with attachment of tRNA to mRNA ribosome complex thus inhibiting protein synthesis.

80-Role of T- cell

T cell, also called T lymphocyte, type of leukocyte (white blood cell) that is an essential part of the immune system. T cells are one of two primary types of lymphocytes—B cells being the second type—that determine the specificity of immune response to antigens (foreign substances) in the body. T cells originate in the bone marrow and mature in the thymus. In the thymus, T cells multiply and differentiate into helper, regulatory, or cytotoxic T cells or become memory T cells. They are then sent to peripheral tissues or circulate in the blood or lymphatic system. Once stimulated by the appropriate antigen, helper T cells secrete chemical messengers called cytokines, which stimulate the differentiation of B cells into plasma cells (antibody-producing cells). Regulatory T cells act to control immune reactions, hence their name. Cytotoxic T cells, which are activated by various cytokines, bind to and kill infected cells and cancer cells.

81- From which sides antibiotics target in prokaryotes

Developing drugs against prokaryotes is relatively easy because prokaryotic cells differ from eukaryotic cells with respect to many structures that may be used as targets for antibiotics. However, developing drugs against viruses and fungi is difficult because viruses use eukaryotic cellular machinery for viral replication and fungi are eukaryotic cells. Any attempt to target any structure in the eukaryote basically amounts to killing the cell itself.

82-Bactericidal and bacteriostatic

Bactericidal: Those antibiotics that kill and lyse the cells are called bactericidal.

Bacteriostatic: Such antibiotics stop the growth of the organisms, they do not kill or lyse the cells. Cells of the immune system then clear them from the body.

83-Name the anti- fungal drugs

Amphotericin B, Ketoconazole.

84-Mode of action of Sulfanilamide and trimethoprim

Inhibition of Essential Metabolic Synthesis: Sulfanilamide and trimethoprim act as a substrate needed for making GABA. Sulfanilamide and para-aminobenzoic acid (PABA) look very much like each other. However, PABA is a substrate for an enzyme that is involved in folic acid synthesis. Folic acid is needed for purine and pyrimidine synthesis, hence when sulfa drugs compete with PABA, DNA and RNA are not made, stopping the growth of the organisms.

85-Explain term phaeophyta, Rhodophyta, chlorophyta, bacillariophyta and dinoflagellates

Phaeophyta (Kelp): Commonly known as brown algae, brownish in colour, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll a and c, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow up to 20 cm in one day.

Rhodophyta: Red Algae, these are reddish in colour and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).

Chlorophyta: Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.

Bacillariophyta: Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid

with which human can be intoxicated by eating contaminated mussels. Diatoms: Come in beautiful colours and shapes.

Dinoflagellata: These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll a and c, and carotene. These algae store starch in them. Dinoflagellates in the genus *Alexandrium* produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of *Alexandrium* Gives Ocean a deep red colour commonly known as red tide.

Past finals subjective - 2021

86-Define and type of algae.

Define:

The definition of algae is a single or multi-cellular organism that has no roots, stems or leaves and is often found in water. These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus) however, and they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O₂ on the face of the earth.

Types of algae:

- 1. Phaeophyta (Kelp):** Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll a and c, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.
- 2. Rhodophyta:** Red Algae, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).
- 3. Chlorophyta:** Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.
- 4. Bacillariophyta:** Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels. Diatoms: Come in beautiful colors and shapes.
- 5. Dinoflagellata:** These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the

membrane rigidity. They have chlorophyll a and c, and carotene. These algae store starch in them. Dinoflagellates in the genus *Alexandrium* produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of *Alexandrium* Gives Ocean a deep red color commonly known as red tide.

6. Oomycota (Water molds): They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores. Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s. *P. ramorum* causes sudden oak death.

87- HYPERSENSITIVITY AND ITS TYPES

It is a normal Immune response but in a damaging way. In other words, immune system is acting in a way for which it has made to. So when an antigen gets into the body, immune system recognizes it as foreign and start making antibodies against the antigen. However, the consequences are bad and damaging to the body. It may be called an abnormal antigenic response. Allergy is another name for hypersensitivity. Those antigens that act in a bad way are called allergens

Types of hypersensitivity:

Hypersensitivities can be grouped into 4 types based on the types of antibodies or cells involved.

- 1. Type I (Anaphylactic):** Also called anaphylaxis or immediate hypersensitivity. This is IgE mediated.
- 2. Type II (Cytotoxic):** IgG and IgM mediated as these antibodies attach to cells and destroy them by activating complement.
- 3. Type III (Immune complex mediated):** Immune complexes are formed by IgM or IgG antibodies and these complexes lodge in the capillary beds and cause damage by complement activation.
- 4. Type IV (delayed type cell-mediated):** CD4+ helper cells along with macrophages are involved.

88-Define antigen and epitope

1. Antigen: A foreign substance that provokes immunity is called an antigen.

The definition of antigen is a harmful substance which enters the body which causes the body to make antibodies as a response to fight off disease. More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably. Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid

alone are poor antigens. Antigens are foreign to the body. Degradability: Inert molecules are not antigenic.

2. Epitopes: Specific regions on an antigen. In other words, any antigen has multiple regions each one of those act as a small antigen. These are called epitopes. These epitopes are also called as antigenic determinants. Most antigens have MW >10,000 D. Molecules that are less than 10,000 D are generally poor antigen. A small molecular region of an antigen that binds to a particular antibody or antigen receptor on a T cell; an antigenic determinant. A single antigen can have multiple epitopes.

89-Describe two transported system in the blood and why it is necessary.

Cardiovascular and lymphatics are the two transport systems of the body by which cells of the immune system patrol the whole body. Cardiovascular system consists of the heart and associated blood vessels (arteries and veins) in which blood circulates lymphatics are channels that start from tissue spaces and collect tissue fluid (called lymph) from tissue spaces and bring them back to the heart. Along these lymphatic channels, there are strategically placed lymph nodes which filter lymph for microbes much like blood is filtered in the spleen. Lymph nodes contain aggregations of B and T cells along with macrophages and dendritic cells which interact with each other inducing adaptive immune responses.

Why Cardiovascular and lymphatics are needed?

Well, in unicellular organisms, nutrients can be absorbed directly from the environment in which a unicellular organism is living. However, a multicellular organism does not have this easy access to the nutrients. So, nutrients must surround the cells in a multicellular organism. These nutrients are provided by cardiovascular system which pumps blood to all organs. Remember that only fluid part of blood leaves blood vessels. RBCs never leave blood vessels. Sometimes proteins also leak from these blood vessels; however, once they leak from the blood vessels, they never can enter back into blood vessels because of their bigger size. If proteins remain in the tissue spaces, they exert osmotic pressure which attracts water into these space resulting in edema. However, nature has provided another mechanism in the form of lymphatic channels which start from tissue spaces and can drain tissue fluid and proteins back to the heart. This is the role of blood vessels and lymphatics that they ensure circulation of nutrients throughout the body without causing any edema or abnormality. B cells and T cells also keep circulating between these two channels (cardiovascular and lymphatics). Unfortunately, microbes can also use these two systems to spread themselves in the body. That is the reason, spleen and lymph nodes are placed in these channels by the nature to deal with these microbes that find their ways into blood and lymph. Lymphatic System consists of the following: Bone marrow, Lymph nodes, Spleen, Tonsils, Thymus, and Mucosa associated, Bursa of Fabricius in birds.

90-Disk diffusion method

A standardized inoculum of the organisms is spread on agar plate. Filter paper disks soaked with anti bacterials are then placed on the lawn of the organism. Plates are incubated at 37oC and results are recorded. Drugs diffuse from the paper disks radially and if the organisms are sensitive to the drug, a clear area with no growth around the disks develops. The size of the zone directly relates to the degree of sensitivity of the drug. This zone is compared to a standard table for recording the results of sensitivity test. Results are reported as sensitive or resistant or intermediate (between the two extremes).

91-Define immunity and difference between innate immunity and adaptive immunity.

Immunity: Ability of the body to protect itself from harmful effects of diseases.

Susceptibility: lack of immunity which leads to easy establishment of disease.

Types of immunity

Innate (Natural):

Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific. Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body. Nonspecific: It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there). Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.

First line of defense: Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside.

Second line of defense: Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.

Adaptive Immunity: This will be studied in detail in a separate lecture soon.

Adaptive (Acquired): It is the third line of defense. Acquired by experience, specific to an organism.

Specific: It means that antibodies made against E. coli will destroy E. coli only, and will not do any harm to a Staph for example. Have memory: It means if antibodies have once been made against E. coli, this exposure will be remembered by the body, and if a second encounter is made with the same type of E. coli again, the body would mount a heightened immune response against E. coli because it has memory cells developed in the body which upon second stimulation proliferate quickly and mount a greater defensive response. Because of memory, body's immune

response increase with every repeated exposure. It has a limit also! Slower to respond: For the first time exposure, the body's response is slower and takes a long time compared with the innate immunity which is immediate.

Adaptive Immune Response can further be divided into:

Humoral immune response (antibody response by B cells or lymphocytes)

Cellular immune response (T cells are made which kills microbial harboring cells).

92- Agglutination and Neutralization

Agglutination: Antibodies can agglutinate antigens which are then cleared as a group by macrophages

Neutralization: Toxins bind with antibodies and they become incapable of binding to their target receptors thus the toxins become harmless.

What is plaque

Plaques are equivalent to bacterial colonies. Basically a plaque is an area where the virus infection has lysed the cells. These plaques are formed on cultured cells in a culture dish. A monolayer of the cells is infected with the virus to be enumerated and then the culture is overlaid with the semisolid agar so that infected and lysed cells do not move away from the site in the dish. Plaques can be counted easily much like bacterial colonies

93-Difference between capsule and slime

Capsule: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

- Plays important role in virulence (degree of pathogenicity)
- Can also be a part of the vaccine against the bacteria to which it belongs.
- Can be demonstrated by negative staining technique.

Slime: If glycocalyx is loosely attached to the cell, it is called slime.

94-PCR method

PCR goes through 3 step cycles for about 30 to 35 times. Each cycle consists of a denaturing stage (about 95oC), primer annealing (50 to 56oC) and extending stage (72oC). A primer is a fragment of DNA which is specific to the signature sequence. Two primers span the signature sequence actually. At the end of 30 or so such cycles, enough DNA accumulates that one can visualize on an agarose gel easily.

95-Type of T cell

T helper cells

TH1 (CD4+)

TH2 (CD4+)

T cytotoxic cells (CD8+)

96-Inhibits growth cell wall drug name

Penicillins and cephalosporins are the major antibiotics that inhibit bacterial cell wall synthesis. They are called beta-lactams because of the unusual 4-member ring that is common to all their members.

97-Microbiota factor and distribution composition

Nutrients: Availability of various nutrients determines the type of organisms present in a system. Similarly, physical and chemical factors also influence the type of microbiota. Other factors include pH, O₂, and CO₂. Host defenses, level of stress and age of an individual also matters for allowing microbiota of an individual.

98-Capsule function

The capsule impairs phagocytosis and help the organisms evade the immune system; however, antibodies can help cells of the immune system in phagocytosis of such encapsulated organisms. Only a few bacteria produce capsule which include Strep pneumonia, Klbesiella pneumonia.

99-Viral replication stages

Viral multiplication goes through various stages in the cell. These include viral attachment, entry into the cell by pinocytosis or fusion with the plasma membrane, uncoating of the virus, biosynthesis of capsids and nucleic acids, maturation (assembly of the virus) and final release of the virus from the cell.

100-Economic benefits of fungi

Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentatively). However, if Saccharomyces are incubated aerobically (for bread

making), they metabolize glucose to produce CO₂ (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise. This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from *Aspergillus niger*; cellulase from *Trichoderma*, antibiotics from many fungi and anticancer Taxol from *Taxomyces* are some other examples of useful fungal products. Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.

101-Types of symbiosis

Interaction of microbiota with the host leads to a relationship called symbiosis. There are a few outcomes of this symbiosis which we must know. The two participants of this relationship are microbiota and the host. At least one of these participants is benefited and sometimes both can be benefited from this mutual relationship. Different names or terms have been given to these mutual relationships as under:

Mutualism: both are benefited

Parasitism: one is harmed.

Commensalism: One is benefited, other is unharmed.

102-Inflammation and write its consequence

Damage to body tissues triggers a local defensive response which is called inflammation. It causes redness, pain, swelling, heat, and loss of function of that organ during inflammation, various mediators of inflammation are released, some of which include:

- Histamine
- Kinins: Almost all cells.
- Prostaglandins by damaged cells
- Leukotrienes by mast cells
- Cytokines are released (TNF-alpha), and they cause release of acute phase proteins from the liver

103- Cause and diagnose of anaphylaxis

The patient also experiences difficulty in breathing. It is an emergency as it may kill the person. Anaphylaxis may be local or systemic. Shock from drug reactions, venoms, and common allergens causing asthma are some samples of type I hypersensitivity. Hypersensitivity can be diagnosed by demonstration of IgE by intradermal injections of allergens one by one. If specific IgE are present in the serum, a reaction will be visible on the skin locally in the form of a wheel

of inflammation. Moreover, IgE levels can be measured by ELISA. Measurement of IgE is more practical as nature of allergens is not known most of the time. Rx: Epinephrine & antihistaminic drugs provide a means of treatment.

104-Two name of bacteria which is capsulated

Bacillus anthracis, streptococcus pneumoniae and Klebsiella are examples of capsulated organisms.

105-What is Epidemiology and its types

The study of where and when diseases occur and how they are transmitted in a population. There are three basic types of investigations relating to epidemiology

1. Descriptive Epidemiology
2. Analytical Epidemiology
3. Experimental Epidemiology:

106- Capsomere

Capsid is made up of protein subunits called capsomeres that are arranged in 3 possible ways to give a helical, or icosahedral or complex symmetry to the viruses. Capsomeres: Subunit called protomers aggregate to form capsomeres.

107-Character of antigen

Antigen: A foreign substance that provokes immunity is called an antigen. More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably. Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens. Antigens are foreign to the body. Degradability: Inert molecules are not antigenic.

108- Algae structure

Body of multicellular alga such as seaweed is called a thallus which consists of branched holdfasts (anchor alga to rock) stemlike hollow stipes and leaflike blades. There is no vascular tissue in these algae. Also, the stipe is not lignified or woody, so it does not provide support to the weed. Surrounding water provides the support for the thallus. Some algae have a gas filled

body inside them which keeps them floating in the water. This gas filled structure is called pneumatocyst or float.

109-Acid fast staining

Some bacteria such as Mycobacteria have a waxy material in their cell wall. Specific name for this waxy substance is mycolic acid Mycobacterium tuberculosis: Causative agent for tuberculosis M. leprae: Causative agent for leprosy

• **Principle of Acid-fast staining:** Mycobacteria are lipophilic, not easy to stain But once stained, they are resistant to acidic alcohol decolorization process.

• **Overall Procedure for Acid-fast staining:**

Carbol fuchsin: It is a primary stain Heat is used to enhance penetration of carbol fuchsin.

Acid alcohol is a decolorizer.

Methylene blue is a counterstain.

Please note, Mycobacteria are gram positive, however, they look red in acidfast staining.

110-Capsule staining

Capsule: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

- Plays important role in virulence (degree of pathogenicity)
- Can also be a part of the vaccine against the bacteria to which it belongs.
- Can be demonstrated by negative staining technique.
- Capsulated organism make glistening colonies as seen in the accompanying diagram below:

Bacillus anthracis, streptococcus pneumoniae and Klebsiella are examples of capsulated organisms. Thickness of the capsule depends upon the culture conditions. Capsules are mostly water soluble. Also remember that capsular organisms usually make the broth viscous and stringy

Capsule staining procedure

1. Prepare a thick smear in a loopful of congo red (1%) stain
2. Fixation in acid alcohol for 15 sec
3. Wash with dH₂O
4. Cover it with acid fuchsin for 1 min.

5. Wash with water
6. A bacterium stains red, capsule remains colorless and is seen in the dark blue background

111-Define vaccine and its types

A product that creates immunity against an infection is called a vaccine. It could be the whole organism or a part of the organism. The observation that people or animals once recover from an infection do not get sick easily to that organism again led to the discovery of vaccines and vaccination.

Types of vaccine:

Live Attenuated Vaccines: These are weakened organisms so that they do not harm the body, but divide or replicate in the same way as pathogenic version of the organism does in the body. So, when a live virus or bacteria multiplies in the body, it stimulates the immune system to create an adaptive immune response which is protective against the disease for which vaccine was given. The benefits of live vaccines are a better immunity because live microbes mimic actual infection (although they do not cause harm to the body). Live vaccines induce both humoral and cellular immune responses together, so live vaccines are a better choice if available. Because live microbes multiply in the body, this replication results in the increase of antigenic amount needed for stimulation of the immune system. Also, this antigen remains for a long time in the body, continuous presence of antigen continuously keeps the immune system active for longer time resulting in long term immunity.

Inactivated Killed Vaccines: Sometimes a non-pathogenic version of the microbe is not available, and to make a vaccine in this situation, the organism is killed with formalin or phenol and then used as a killed inactivated vaccine. Because organisms in such a vaccine do not replicate in the body, frequent boosters are required to achieve a good level of immunity (antibodies). Also, remember that this is a killed vaccine, so classical cellular immunity is not created with killed vaccines.

Toxoids: Some diseases are caused by bacterial toxins, and not by bacteria. In such cases, toxin is modified (inactivated) and used as a vaccine. Such a vaccine is known as a toxoid. Tetanus toxoid is a good example.

Conjugated Vaccines: Sometimes special parts of organisms are required for generating protective immunity. However, those substances are poor antigens if used alone. In order to enhance immunity, such poor antigens are attached to proteins, and such vaccines are called conjugated vaccines. Haemophilus influenzae type b is a good example.

DNA vaccines: We now know that sometimes only certain proteins are required for generating a good immune response. In such cases, the genes responsible for those proteins can act as vaccines. DNA coding for such a protein is injected into cells, and that DNA gets transcribed by

the cells of the body into the required protein and that protein induces the antibody production (because that protein is foreign to the body). West Nile Virus is a good example of DNA vaccine

Subunit vaccines: Also called recombinant vaccines, such vaccines are composed of a portion of viral or bacterial proteins that can induce immunity. In other words, this is another version of DNA vaccines. In subunit vaccines, antigenic epitopes are included in the genome which is inserted into an expression vector that makes those subunit proteins. These subunit proteins are collected and used as vaccines to stimulate the immune system. Again, these act like a killed vaccine. There is another mechanism being explored in these days for recombinant vaccines. A subunit vaccine for Foot and Mouth disease virus is available. Such genes are being incorporated in plants now that are acting like an expression vector. Animals can eat those plants and subunit vaccines can induce immune response through M cells of the intestine. Success is limited though so far.

112-Describe features of fungi

These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O₂ on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such algae include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located r from the surface and can use blue light from the sun as blue light is of shorter wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic.

113- Treatment of hypersensitivity ii

This type of hypersensitivity can be diagnosed by history and symptoms. Glomerulonephritis and cellulitis is very common. Deposition of immune complexes in the kidneys can be diagnosed by presence of IgG in kidney cells by using anti-IgG antibodies.

114-What are antibiotics

Antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese

115-Define virion and viroid

Virion: A virion is an infectious particle composed of nucleic acid that is surrounded by a protein coat (capsid). Viruses are classified based on their nucleic acid and protein coat. Viruses have an extracellular and an intracellular state. Outside of a cell, in the extracellular state, a virus is called a virion.

VIROIDS: They are composed only of circular single stranded (ss) RNA.

116-Write entry point of microorganism

Mucus membranes: These include linings of digestive tract, respiratory tract, urogenital system, and conjunctiva
Skin: Surface area wise, skin is the largest organ of the body. Unbroken skin is impermeable to organisms
Parenteral Route: Injury to the skin or mucous membrane leads to easy access for the organisms in the body. Any cut received on the skin for example becomes a portal of entry.

117-Role of thin clonal selection

According to this theory, B cells are generated in the bone marrow randomly without seeing the antigen. When such a B cell encounters its specific antigen in the body, it starts proliferating into a clone which ultimately becomes plasma cells and start secreting antibodies. A beautiful example to understand clonal selection theory is like going to a shoe store and select a shoe size that fits you very well. Note, that the shoe that you selected was made randomly without any regard to your foot size.

118-Antigen presenting cell

These cells are able to present antigens to B cells for making antibodies by B cells. There are three cells that fall into this category: Dendritic cells, macrophages, and B cells

119-Reverse transcription

A reverse transcriptase is an enzyme used to generate complementary DNA from an RNA template, a process termed reverse transcription

120-Bactericidal and bacteriostatic drug

Bactericidal: Those antibiotics that kill and lyse the cells are called bactericidal.

Bacteriostatic: Such antibiotics stop the growth of the organisms, they do not kill or lyse the cells. Cells of the immune system then clear them from the body

121-Define plasmogamy karyogamy and meiosis

1.Plasmogamy: A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-). Both cells are haploid (one set of chromosomes)

2. Karyogamy The (+) and (-) nuclei fuse to form a diploid zygote nucleus.

3. Meiosis: The diploid nucleus gives rise to haploid nuclei (sexual spores).

122-Mechanism of hypersensitivity iv

It is called as delayed type because its bad effect becomes visible 2-3 days after exposure to the antigen. This is the only hypersensitivity that involves cells rather than antibodies. Robert Koch discovered this when he injected tuberculin in patients. Contact dermatitis is the most common example of this kind of hypersensitivity. This is basically an interaction between an antigen, T cells and macrophages. Transplant rejection is another good example of delayed type IV hypersensitivity. Reaction to hair dyes is another example. Poison Ivy is another example in which contact with this plant initiate type IV hypersensitivity response. Chemicals from poison ivy interact with skin proteins and change them into foreign-looking protein molecules. Body's immune system mounts an immune response against these proteins resulting in a damaging type of intense inflammatory response

123-Cytokines

Cytokines are released as a result of defense cell stimulation by PAMPs and TLRs interactions. Cytokines recruits more immune cells and also stimulate adaptive immune response

124-Note on infectious disease transmission with vector vehicle and contacts

Direct Transmission: person to person physical contact is a direct contact. Influenza spread is a typical example.

Indirect Transmission: A non-living object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

Droplet Transmission: Mucus droplets are created when you sneeze for example. These droplets carry organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

Vehicle Transmission: Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

125-Define magnification

Magnification is the enlargement of an object its formula is Magnification of objective lens x magnification of ocular lens

126-Define Buffer

A buffer is the substance that maintains the desired pH in the solution or any medium.

127-What is taxonomic hierarchy

Microbes are placed in groups based on similarities that they share with into a series of subdivisions that make up the taxonomic hierarchy.

128-Give two advantages of solid media in bacterial culturing.

- Pure culture is easy to obtain by streaking the organisms
- Colony characters can be studied

129- Physical Requirements of Media

Physical: Requirements • Temperature • pH • Osmotic Pressure

130-Passive movement

The movement of molecules from high concentration to new concentration without use of ATP (energy) is called passive transport. Example, Simple Diffusion, ciliated diffusion and Osmosis

131-Flagellum and its function

Long filamentous structure that propels bacteria, the primary role of the flagellum is locomotion, but it also often has function as a sensory organelle being sensitive to chemicals and temperatures outside the cell. Example Chemotaxis —Phototaxis

132-Glycocalyx, its composition and function

It is the outermost layer, viscous and gelatinous in nature that surrounds the cells. It is composed of polysaccharide and polypeptide. Capsule: If glycol calyx is organized and firmly attached to the cell, it is called a capsule. They Plays important role in virulence (degree of pathogenicity). Can also be a part of the vaccine against the bacteria to which it belong. Can be demonstrated by negative staining technique

133-Characteristics of microaerophiles

A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (i.e. <21% O₂; typically 2-10% O₂). Many microaerophiles are also capnophiles, requiring an elevated concentration of carbon dioxide (e.g. 10% CO₂ in the case of Campylobacter species).

134- Write composition of teichoic Acid

Teichoic Acid Composition: - (glycerol or ribitol + Phosphate)

135- D/f b/w chemotaxis and phototaxis

Phototaxis: - The movement of organism or its part towards the light whiles the movement of an organism towards specific stimulus or chemical substance called chemotaxis.

136-What is western blotting

We can use this technique for the detection of antigen in the serum. Microbial proteins can be separated on SDS-PAGE by electrophoresis and the presence of these proteins can be detected by enzyme-tagged antibodies specific to those proteins

137-Define virulence

The ability of an agent of infection to produce disease, the virulence of a microorganism is a measure of the severity of the disease it causes.

138-Two teichoic acid present in bacterial cell wall.

1) Lipoteichoic Acid 2) Wallteichoic acid

139-Define antiseptics

An antiseptic is a substance that stops or slows down the growth of microorganisms. Like ethanol, hydrogen peroxide etc.

140-What is high frequency combinations?

If F factor gets integrated into the chromosome of the recipient cells, the bacterium becomes high frequency of recombination cell, it is a kind of conjugation

141-Differentiate between clone and strain

Clone: Population of cells derived from a single cell that is genetically identical.

Strain: A genetic variant of a clone is called a strain

142-Difference between positive sense and negative sense RNA

The main difference between positive and negative sense RNA virus is that positive sense RNA virus consists of viral mRNA that can be directly translated into proteins whereas negative sense RNA virus consists of viral RNA that is complementary to the viral mRNA.

143-Write characteristics of Microaerophiles

1) Aerobes but oxygen is required in low amounts. 2) Produce O₂ toxic compounds if exposed to more oxygen.

144-Name two basic dyes

Crystal violet • Methylene blue • Malachite green • Safranin

145-Two methods for viable counting

1) Spread plate 2) Pour plate

146-What is microbiology?

Studying microbes is microbiology

147-Characteristics of Integral proteins

Imbedded in the membrane —Insoluble in water —Amphipathic in nature

148-Formula of magnification

Magnification of objective lens x magnification of ocular lens

149-Substrate level phosphorylation

The Direct transfer of P to ADP

150- MacConkey agar

Nutrient agar + Bile salt + CV • Selective & differential, Used for gut bacteria.

151-What is virulence?

Virulence: The ability of an agent of infection to produce disease. The virulence of a microorganism is a measure of the severity of the disease it cause

152-Advantages of solid medium.

Pure culture is easy to obtain by streaking the organisms • Colony characters can be studied.

153-Pasturization method

It is a method in which the liquid usually milk is pasteurized to Reduces spoilage organisms and pathogens.

154-Two types of fermentation

Lactic acid fermentation and alcoholic fermentation.

155-Define L-form bacteria.

L-form organisms are not found without the cell wall. In other words, normally, they have cell wall but under certain conditions, they may lack a cell wall.

156-Industrial microbiology

Use of microbe r their enzymes for large scale production of biomolecules.

157-What are the types of a media?

• General purpose media • Enriched media • Selective media • Differential media

158-Describe transduction, conjugation transformation

Transduction:-

Transferring of a gene from a bacterium to another bacterium via a virus is called transduction.

Conjugation:-

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell to cell contact or by a bridge-like connection between two cells.

Transformation:-

Transformation is the process by which a DNA molecule is taken u from a external environment and incorporated into the genome of the recipient cell or uptake of n robes.

159-Economical use of fungi

- Saccharomyces are used for alcoholic beverages under anaerobic conditions.
- Saccharomyces are incubated aerobically (for bread making), they metabolise to produce CO₂ which is used in bread.
- Yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from Aspergillus Niger.
- Antibiotics from many fungi and anticancer are some other examples of useful fungal products
- Mushrooms can be cooked and eaten as source of proteins.
- Entomophaga (kills gypsy moth), hence is used as pest controller.

160-Difference between vertical and horizontal

• Vertical Gene Transfer: -

This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

- **Horizontal Gene transfer:** -

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms.

161-What are interferons? Give their functions

These are antiviral proteins, produced by macrophages and lymphocytes in response to viral infections. Fibroblasts can also secrete it. These proteins interfere with viral replication.

Types of Interferon:

- Alpha: It is released by all infected cells.
- Beta: Also produced by infected cells.
- Gamma: This one is released by lymphocytes only. Lymphocytes belong to adaptive immune system (just remember that).

162-Function of cell wall in bacteria

- 1) Shape 2) Protection from osmotic lysis 3) Pathogenicity

163-Define Virus, virusoid, viroids

- **VIRUSES:** They are acellular structures that consist of a protein capsid enclosing either DNA or RNA (but never both) and sometimes may contain an extra membrane called envelope
- **VIROIDS:** They are composed only of circular single stranded (ss) RNA
- **VIRUSOIDS:** They contain circular ssRNA that need helper viruses for replication and encapsidation. They are also called as satellite virus.
- **PRIONS:** These are basically infectious proteins formed inside the body. They behave like organisms as they can be transmitted from one individual to another. Extremely resistant to heat.

164-DNA lateral transfer

Lateral gene transfer or Horizontal gene transfer: -

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms.

165-Types of Spiral Bacteria

Vibrio: curved rods. **Spirillum**: Helical but rigid **Spirochete**: Helical but flexible.

166-Characteristic of bacterial colony.

- Forms of bacterial colonies like Punctiform, circular, filamentous etc.
- Elevation of bacterial colonies like Flat, raised, convex etc.
- Margins of Bacterial colonies like entire look circular, undulate, curled etc.

167-Define buffers.

Buffers are used to maintain desired pH. Peptones and amino acids are used as buffers. Phosphates are also used for buffers.

168-What is sporulation?

Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

169-Zoonosis

Primarily an animal infection, but can be transmitted to humans. It can assume epidemic proportions in humans and is difficult to control or eradicate.

170-What is flagellum? Write its functions.

A flagellum consists of the following 3 parts as shown in the accompanying diagram below:

- **Filament**: It is made of a protein called flagellin, which makes H antigen that can be used for serovar identification in gram negative bacteria.
- **Hook**: A different protein
- **Basal Body**: The most complex.

171-Describe classification system proposed by Robert H Whittaker.

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

- Plantae: plants
- Animalia: Animals
- Fungi: Yeasts, molds and mushrooms
- Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

172-What is taxonomic hierarchy?

Microbes are placed in groups based on similarities that they share with each other. All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. A bacterial species represents —a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminative phenotypic property (definition taken from the internet). A genus consists of various species; however, these species differ from each other in certain ways, although these are related by descent with each other. Related genera make up a family. A group of similar families constitutes an order, and a group of similar orders makes up a class. Related classes, in turn, make up a phylum. All phyla that are related to each other make up a kingdom, and related kingdoms are grouped into a domain.

173-Enlist types of passive transport.

Passive Movement is divided into two groups:

1. Simple Diffusion

- Area of high concentration to low concentration
- This continues until ions or molecules are evenly distributed. When this happens (equal distribution throughout), it is called a point of equilibrium.
- O₂ and CO₂ are examples of simple diffusion.

2. Facilitated Diffusion

i. Integral proteins act as channels or carriers in facilitated diffusion. Integral proteins are called transporters or permeases

- ii. No energy is required during transportation of substances through these integral proteins.
- iii. Two kinds of transporters are known:

Nonspecific transporters

- Ions
- specific transporters
 1. Change in shape
 2. Sugars, vitamins
- Examples of facilitated diffusion include:
 - i. Large molecules degraded by extracellular enzymes
 - ii. Smaller molecules then bind transporters
 - iii. Water molecules can pass through lipid bilayer by simple diffusion or through water channels, aquaporin.

174-What is function of lysozymes

Lysozyme breaks the sugar-derived backbone of cell wall in bacteria.

175-What are fimbriae? Give location and function.

Hair-like small appendages on G-negative cells. Composed of pilin, a protein. Can be at one pole or around the entire cell. Used for attachment to surfaces or epithelial cells

- Fimbria: Singular
- Fimbriae help bacteria attach to surfaces before they can secrete biofilm.
- Fimbriae also help attach to epithelial cells
- Neisseria gonorrhoeae
- E.coli O157: Diarrhea

176-TYPES OF MICROSCOPES

Dark field microscope:

This microscope makes use of a special condenser that provides illumination from the sides. This special arrangement of the condenser throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night. The beauty

of this microscope is that one does not have to stain the organisms to see them under the microscope.

Phase contrast microscope:

This instrument is built on the concept of phases of light waves. The light ray that passes through an organism gets retarded about $\frac{1}{4}$ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the centre than the ends. This arrangement causes the retarded light to further slow down about $\frac{1}{4}$ of its wavelength, which when recombines with the un-retarded light creates a contrast which makes the cells/organisms visible. This method for visualizing organisms also does not involve staining.

Florescent microscopy:

This microscope is equipped with UV light that provides the source of light for illumination. However, this light source does not pass through the slide, it instead falls over the slide (having specimen) and creates fluorescence. Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum). But how is this fluorescence created? Well, the fluorochromes are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies. See the accompanying diagrams for details. Look for UV lamp (Mercury Lamp).

Transmission Electron microscope:

Electron microscope (EM) makes use of electron beam as an alternative to light rays. Since electron beam has much shorter wavelength, resolution of EM is 0.1nm compared with light microscope which is 200nm. Second difference of EM with light microscope is that focusing of electron beam is done not by glass lenses but by electromagnets that acts like lenses. The third difference in EM is that the image of the specimen is formed on a fluorescent screen like a TV screen.

Scanning Electron microscope:

This is a modified version of electron microscope. Electron beam is allowed to fall on to the specimen and the image is formed from the reflected electrons that are collected and assembled into an image by the detector. It gives a three dimensional picture as shown for Staphylococcus aureus.

178-What do you know about types of teichoic acid

G-positive cell wall contains teichoic acid which may be divided into wall teichoic (remains within the cell wall) acid and lipoteichoic acid (is inserted into the cell membrane).

Teichoic acid is antigenic in nature and helps the cell wall by providing rigidity.

179-Write characteristics of integral proteins.

Integral proteins are inserted in the membrane or embedded in the membrane

- Insoluble in water
- Amphipathic in nature

Cell membrane has been referred to as a mosaic fluid model. This means that integral proteins are not static in position in the membrane. They can diffuse laterally and change positions in the membrane from time to time. So, plasma membrane is like water pond and integral proteins are like plastic bags people throw in the pond. These plastic bags swim and move by air currents. Membranes are much like that.

180-Define refractive index

Refractive Index: Ability of a medium to bend the light. Light rays move in a straight line through a single medium. Light rays bend when they pass from one medium to another. We use cedar wood oil to eliminate this bending of light in microscopy. Cedar wood oil has the same refractive index as that of the glass (1.5), so that the light rays do not bend when they pass through the specimen into the objective lens of the microscope. This provides a brighter and better image.

181-Define medical microbiology.

Medical microbiology, also known as clinical microbiology, is a sub discipline of microbiology dealing with the study of microorganisms (parasites, fungi, bacteria, viruses, and prions) capable of infecting and causing diseases in humans.

182-What is resolution

Resolution is the ability of the lenses to distinguish between two closely lying objects as separate. See the accompanying diagram for visual concept of resolution. Light microscope resolving power is $0.2 \mu\text{m}$.

183-Write three differences between prokaryotes and eukaryotes.

Prokaryotes have the following features:

- DNA is not enclosed in a nuclear membrane.
- Chromosome: mostly single, circular

- DNA not associated with histones
- No membrane enclosed organelles
- Cell wall has peptidoglycan, a complex carbohydrate.
- Divide by binary fission.

Eukaryotes have the following features:

- DNA is enclosed in a membrane- bound nucleus.
- DNA is found in multiple chromosomes.
- Chromosomes are linear, thread- like structures.
- DNA is associated with histones.

184-Define virulence.

Virulence: The extent of pathogenicity

185-Write steps of germination of spores.

Spore germinates when it finds a conducive environment for its growth.

Germination has 3 phases or stages:

Activation: It prepares the spore for germination. Heat can activate the spore when appropriate moisture and nutrients are present in the environment.

Germination: Spore starts swelling and losing its coats etc. It becomes metabolically active.

Outgrowth: New components are made.

186-Write parts of lipopolysaccharides

Lipopolysaccharide molecule consists of 3 parts:

1. Lipid A: endotoxin – Fever, vasodilation and shock
2. Core polysaccharide, Structural support
3. O side chain- Antigenic much like teichoic acid in gram positive bacteria

187-Define culture, media and inoculum.

Inoculum

An inoculum can be defined as the population of microorganisms or cells that is introduced in the fermentation medium or any other suitable medium.

Media

Culture media, also known as growth media, are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds).

Culture:

The propagation of microorganisms in a growth medium. Any body tissue or fluid can be evaluated in the laboratory by using culture techniques to detect and identify infectious processes. Culture techniques can be used to determine sensitivity to antibiotics.

188-Define antiseptics

Antiseptic agents in dermatologic surgery commonly include chlorhexidine, povidone-iodine, chloroxylenol, isopropyl alcohol, hexachlorophene, benzalkonium chloride, and hydrogen peroxide. They should be used for most, if not all, procedures that enter the dermis of the skin or deeper.

189-Define phosphorylation.

The addition of an inorganic phosphate group to a chemical compound is called phosphorylation. So, a phosphate is added to ADP to form ATP molecules in cells. In other words, energy derived from hydrogen containing organic molecules such as carbohydrates and fats is captured and concentrated in the form of ATP molecules.

190-Explain phases of growth curve.

There are four distinct phases of this curve.

1. The Lag Phase: cells prepares for growth in this phase. No growth is observed during this period or phase, however. Cells are metabolically very active during this phase.

2. The Log Phase: During this phase, organisms multiply exponentially or logarithmically. Generation time becomes constant during this phase and that is the reason, the log graph will show a straight line. Cells are in the most active stage during this phase of growth curve. For commercial applications such as vaccine production, cells have to remain in this phase in order to reproduce most efficiently resulting in increased cell mass or number. Another application of this phase of growth is to determine the generation time.

3. The Stationary Phase: This is also called a period of equilibrium as microbial deaths equal production of new cells. In other words, organisms start dying during this phase, however, the number of dead organisms is replaced by new organisms because there is still replication of cells going on. So, overall number of organisms does not change. This is the reason, it is called a stationary phase.

4. The Death Phase: The number of deaths exceeds the number of new cells formed during this phase. In other words, overall number decreases. It is also called logarithmic decline phase. Why there is a decline phase or death phase. The reason is simple: Nutrients are depleted and waste products which are toxic to the cells accumulate suppressing the growth and killing the cells.

191-What is high frequency recombination?

High frequency of recombination. A strain of bacteria that has incorporated an F factor into its chromosome and can then transfer the chromosome during conjugation. In Escherichia coli, a cell having its fertility factor integrated into the bacterial chromosome; a donor (male) cell.

192-Differentiate between clone and strain?

The key difference between clone and strain is that clone is a genetically identical organism to its progenitor while strain is a genetic variant or subtype of an organism. Clone and strain are two concepts used when defining organisms.

193-Differentiate between positive sense and negative sense RNA

The key difference between the negative and positive sense RNA virus is that the negative sense RNA virus comprises viral RNA, which is complementary to the viral mRNA, while positive sense RNA virus comprises viral mRNA, which can be translated into proteins directly.

194-Explain methods of microbial control in detail.

Physical methods:

Heat: It is the cheapest sources of all and easily available to control microbial growth. There are a few heat related concepts that we must appreciate. Heat denatures enzymes.

Thermal Death Point: It is the lowest temperature at which all cells in a culture are killed in 10 min. It will be a specific temperature for a specific species of organism.

Thermal Death Time: Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures.

Methods that use heat can further be classified into dry and moist heat methods.

- Heat kills by oxidation
- Dry Heat
- Incineration Dry oven
- Moist Heat
- Autoclaving
- Boiling Water
- (Disinfection)

Form of dry heat include: 1. Dry heat (hot-air oven), 2. Flaming (Platinum loop sterilization in the flame of Bunsen burner), 3. Incineration (burning to ashes). Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at 100°C, and

Tyndallization (100°C for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than 100°C).

Pasteurization: This technique typically employs low heat for killing pathogenic and food spoiling bacteria in milk. However, it does not kill thermophilic organisms. Thermophilic organisms are not pathogenic though. Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. Dairy industry tests phosphatase to determine if pasteurization of dairy product has occurred effectively because phosphatase present in raw milk gets inactivated with effective pasteurization temperatures. The following three equivalent heat treatments can be given to achieve pasteurization of milk:

- o 63°C for 30 min
- o High-temperature short-time: 72°C for 15 sec
- o Ultra-high-temperature: 140°C for 4 sec

Other Physical Methods

Low temperature inhibits microbial growth by slowing down chemical reactions.

Refrigeration: Materials stored at 2-8°C can last for a day or two.

Deep-freezing: Long term storage of materials is possible at minus temperatures in the deep freezers.

Lyophilisation: Freeze drying is another method for long term storage of food and other materials.

High pressure reshapes and denatures proteins: This technique is used for preserving juices and wines.

Desiccation prevents metabolism: This method is very old and still in use today. Food stored in high concentrations of salt or sugar solution can keep for long.

Osmotic pressure causes plasmolysis. High salt concentrations for example can take the water content of the cells out leaving them starved of water.

Filtration: It is also a physical method. It can be used to filter air (using high efficiency particulate air filter) or liquid medium using membrane filters.

Radiation:

- o Ionizing and non-ionizing radiation
- o Ionizing Radiation: High energy waves that destroys microbes.

Ionization radiations include gamma rays, X rays, or high-energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.

Non-ionizing Radiation: wavelengths longer than 1nm fall into non-ionizing radiation. The best example is UV light. UV light causes mutations in the DNA by dimerization of thymidine bases. These thymine dimers inhibit correct replication of the DNA during reproduction of the cell. The most useful wavelength is 260 nm. This wavelength is specifically absorbed by DNA. UV radiation is used to control organisms in the air mostly. However, surfaces are also sterilized by UV radiation. UV light cannot penetrate deep into the fluids, so only surface bacteria are killed. See the range of UV light that is useful for microbial killing.

ETHYLENE OXIDE FOR MICROBIAL CONTROL

Ethylene Oxide: Many heat-sensitive items such as disposable plastic, petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized by ethylene oxide. Ethylene oxide is a strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity. It rapidly penetrates packing materials, even plastic wraps. EtO is explosive, supplied in a 10% to 20% concentration mixed with either CO₂ or dichlorodifluoromethane. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L.

CHEMICALS AS ANTIMICROBIAL AGENTS I

Chemical methods

Chemicals are used to control microbial growth. Factors that influence the efficacy of a chemical disinfectant include the concentration of the disinfectant, presence of organic matter in the environment (matrix) where the disinfectant is being used, pH of the environment (disinfectants are more effective at acidic pH), and time of exposure to the disinfectant.

- It should be effective at low concentration.

- It should be nontoxic to body tissues.
- It should be effective in organic matter.
- It should have a broad spectrum of activity.
- There is no ideal disinfectant available that provides all of the above features.

Phenol and Phenolics

Lister was the first to use phenol (carbolic acid) as a disinfectant to control microbes in surgery. Derivatives of phenols are called phenolics. Phenols disrupt plasma membranes and are effective in the presence of organic matter. They are mostly used microbes in Pus, saliva, feces. The structure of phenol is shown to the right of this page. Phenol is irritating to the skin. Derivatives of phenols are less irritant.

Cresol is an example of such a derivative and o-phenylphenol is an example of a cresol.

Bisphenols: These are also derivative of phenol. Hexachlorophene is an example of a bisphenol. This disinfectant is very commonly used in surgeries in hospitals.

Trichlosan is another example of bisphenol. Trichlosan inhibits synthesis of lipid that are needed in the plasma membrane of microbes.

Biguanide: Made by condensation of two guanidine molecules. These compounds disrupt plasma membrane. Examples include chlorhexidine and alexidine.

Halogens: Iodine and chlorine are very good antimicrobial agents. Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

Tincture of Iodine: A solution of iodine in alcohol is called a tincture.

Iodophor: A combination of iodine with an organic molecule from which iodine is released slowly. An example is Betadine.

Choline: when chlorine gas is mixed with water, it forms hypochlorous acid which has germicidal activity. It is a very strong oxidizing agent which inactivates cellular enzymes.

Calcium Hypochlorite: Commonly used as disinfectant for dairy equipment.

Sodium Hypochlorite: Bleach consists of this compound. It is also is very good disinfectant for inanimate objects.

Chloramine: It is a combination of chlorine and ammonia. Used for dairy equipment mostly.

195-Explain methods of microbial identifications.

Microbes especially bacteria (disease causing) can be identified by three methods:

o Classical or Conventional Method

- o Serological Methods
- o Nucleic Acid based Methods

196-How fungi can be identified by biochemical testing

Filamentous fungi are mainly identified by morphology, and not by biochemical testing.

197-Write functions of cell membrane of bacteria.

- Selective permeable barrier
- Passive and Active Transport
- Respiration in microbes (bacteria)
- Photosynthesis in microbes
- Lipid synthesis
- Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
- PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

198-Write names of 5 types of bacteria with respect to temperature

Organisms are basically classified into three groups based on the temp requirements:

1. Psychrophiles: These are further divided into strict psychrophiles and psychrotrophs:
Psychrotrophs: Cold loving: 15 0C. Psychrotrophs: Optimum temp is 20-30 0C. Food spoilage bacteria that can spoil food during refrigeration.

2. Mesophiles: 25 – 40 0C: These are the ones that cause diseases in animals and humans.
Moderate temp loving organisms. Optimum: 37 0C as this is the body temperature of humans and animals.

3. Thermophiles: 50 – 60 0C. Heat loving. Important in organic compost piles. These are further divided into hyperthermophiles that grow optimally at 80 0C. They live in hot springs.

Final term subjective – 2020

199-Write characteristics of metachromatic granules.

Metachromatic Granules

- Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different colour as methylene blue gives blue colour but the colour on these granules is red).
- Large inclusions
- These inclusions contain inorganic phosphates. Inorganic phosphates are used up in ATP synthesis.
- Characteristics of *Corynebacterium diphtheria*: This bacterium can be identified by the presence of these granules in it.

200-Explain five kingdom classification systems.

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

- Plantae: plants
- Animalia: Animals
- Fungi: Yeasts, molds and mushrooms
- Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

201-Explain layers of endospore.

Endospore and its various parts/structures

- **Exosporium:** A thin delicate outermost covering of the spore
- **Spore coat:** 2nd layer underneath the exosporium. It is thick and composed of several protein layers. Resistant to chemicals It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favourable for their growth.
- **Cortex:** It is the 3rd layer from outside in. It has peptidoglycan in it.
- **Spore cell wall or core wall:** Surrounds the protoplast or spore core
- **Spore core:** Contains nucleoid and ribosomes

202-Enlist types of bacteria on the basis of oxygen.

Based on the use and concentration of Oxygen, microbes can be classified into five groups:

Obligate Aerobes: Oxygen must be present for their growth. These organisms do not grow if O₂ is not present in the medium. If you test these organisms in a tube containing nutrient agar, the growth will remain confined to the surface only (see the diagram for the concept).

Mycobacterium tuberculosis is an example of such an organism.

Facultative Anaerobes: These organisms prefer aerobic respiration; however, if oxygen is not available, they can use anaerobic respiration or fermentative modes for generating ATPs. These organisms will be present throughout the tube, however, more growth will be seen on the top (close to the surface) because these organisms prefer using oxygen if it is available.

Obligate Anaerobes: Although, these organisms do not use oxygen, they cannot grow in the presence of oxygen. The reason is that oxygen creates toxic compounds which can kill these cells. Normally, oxygen toxic compounds are eliminated by the cells, however, strict anaerobe do not have a system to get rid of these toxic compounds when they are made in the cells. So using or not using oxygen is one thing and growing in the presence of oxygen (and not utilizing it) is another. The growth will remain confined to the bottom which has little oxygen.

Aerotolerant Aerobes: These organisms do not use oxygen and also are not bothered by the presence of oxygen. In other words, they have a better system to dispose of toxic oxygen compounds. They will grow evenly throughout the tube of medium.

203-Explain ELISA.

There are some interactions (Antigens and Antibodies) that are not directly evident. Interactions may have taken place, but there is no visible direct clue if the interaction has taken place or not. For such interactions, we use indirect methods. Say, there is big body of smoke visible to you from a kilometre ahead of you. What is your guess? Yes, you guessed it right. There is a big fire there. Did you see the fire? Probably not. You guessed on the basis of smoke that there is a fire there. This is called indirect way of interpretations. A beautiful example of such a test is ELISA where we do not know if the interaction has taken place or not. However, we can see it indirectly. We will see that in the following paragraph which discusses ELISA.

Enzyme-Linked Immunosorbent Assay: This test is done by coating the ELISA plastic plates either with the antigen or the antibodies. Suppose we have *Brucella* antigen coated ELISA plates that can detect antibodies from the serum of a *Brucella* infected animal. You take the serum and put into the wells of ELISA plate. You allow time for antigen and antibody to interact and then wash these wells with some buffer to remove unbound antibodies. These bound antibodies are not visible at this step. So, how do we see this interaction? Well, we use another antibody that has been made against the antibody bound to the antigen. This special antibody has been tagged with an enzyme. After allowing time for interaction of this 2nd antibody with the 1st antibody, 2nd antibody is also given a washing to remove unbound antibody molecules. Again, we cannot

say if interaction has taken place or not. Here comes an indirect way of detecting this interaction. Remember that the 2nd antibody has a molecule of an enzyme attached to it. Now, if we provide substrate to the enzyme in the wells, enzyme will convert the substrate into a colour compound visible to the eyes. The color change can be measured by a spectrophotometer as well which gives a quantitative data. See a photo below for ELISA plate.

204-Explain types of conidiophores.

Conidiospores: This type of spore is not enclosed in a sac. It is produced in a chain at the end of a conidiophore (hypha that bears conidiospores). Aspergillus produces such spores.

205-Name types of spiral bacteria.

These are curved shaped bacteria. They are further divided into 3 more subgroups.

Vibrio: curved rods

Spirillum: Helical but rigid

Spirochete: Helical but flexible

206-What is function of lysozymes

A lysosome is a membrane-bound organelle found in nearly all animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. Lysosomes are organelles that contain digestive enzymes. They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria.

207-What are benefits of solid medium

Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other. A well isolated colony is assumed to be the progeny of a single cell. Secondly, the solid media are used to study the colony characteristics.

208-How to make smear?

- Staining starts with making a smear using a glass slide.
- Using a platinum loop, a colony of a drop of broth culture can be smeared into a thin film on a glass slide for making a smear.
- The specimen is spread into a thin film (smear).
- Smear is air-dried.
- Smear is fixed (attached) to the

slide before staining. • Heating the slide is one way of fixing the smear. • Methyl alcohol can be used.

209-Structure and function of pili

These are hair-like structure composed of pilin, usually one to ten in number. • Longer than fimbriae • Used for attachment to: • Host cells • Bacteria • Used for DNA transfer from one bacterium to another: Conjugation (Sex pili) • Also function in twitching Motility • Gliding Motility is also the function of the pili.

210-Respiration of glucose takes place in three general steps

- **Glycolysis:** Oxidation of glucose to pyruvic acid
- **Krebs cycle:** Oxidation of acetyl CoA to CO₂
- **Electron Transport Chain:** Coenzymes that carry electrons from Krebs cycle or glycolysis are oxidized to create ATP.

211-Epidemiology and methods

The study of where and when diseases occur and how they are transmitted in a population ♣
Descriptive Epidemiology: (recording the data about disease). It includes location and time of cases of disease, gender, health, age of patients etc. (Basically, frequency and distribution of risk factors in population are recorded). Prevalence and incidence of disease are typical examples. Such studies can be retrospective (after they occur) or prospective (before they occur). So, in summary, in descriptive epidemiology, information about an already existing disease is recorded. ♣ Analytical Epidemiology: It relates to determining the cause of the disease. Analysis of data, mode of transmission of diseases and means to prevent diseases also come in this discipline. Such studies can be done with case control methods in which factors that have preceded the disease are determined. A group of people who have the disease is compared with another group of people in the same location who are free of the disease. A Cohort method tracks two groups forward from exposure to outcome. Cohort study compares the experience of a group exposed to the factor with another group not exposed to the factor. An association between exposure and its outcome is determined which makes it easy to see the risk factor involved. In other words, cohort studies begin with a group of people (a cohort) free of disease. The people in the cohort are grouped by whether or not they are exposed to a potential cause of disease. The whole cohort is followed over time to see if the development of new cases of the disease (or other outcome) differs between the groups with and without exposure. ♣ Experimental Epidemiology: A hypothesis relating to a disease is tested in this approach. For example, smoking cause cancer can be tested by designing an experiment in mice in which they are exposed to certain levels of smoke over time and then the outcome is observed. Another example will be testing of a drug for

prevention of a disease. O Case Reporting: Case reporting is essential part of epidemiology. It provides incidence and prevalence of a disease to epidemiologist. Health care workers report specified disease to local, state, and national offices for such purposes. Using these reports, epidemiologists begin various studies of the patients. If an epidemiological study shows that a large enough segment of the population is affected by a disease, an attempt is then made to isolate and identify its causative agent. Identification is accomplished by a number of different microbiological methods. Identifying the causative agent often provides valuable information regarding the reservoir for the disease. Once the chain of transmission is discovered, it is possible to apply control measures to stop the disease from spreading.

212-Advantage of Dry Heat relatively

Advantages of dry heat sterilization include: it is inexpensive, it does not pollute or cause toxic fumes, and it does not corrode or rust metal objects.

Disadvantages include: it is relatively slow and many objects cannot withstand the high temperatures.

213-How Microorganism cause damaged the body

Infection with a pathogen does not necessarily lead to disease. Infection occurs when viruses, bacteria, or other microbes enter your body and begin to multiply. ... Pathogenic microbes challenge the immune system in many ways. Viruses make us sick by killing cells or disrupting cell function.

214-Microbial Identification

Microbial identification can be defined as “microbial characterization by a limited spectrum of tests pre-chosen and appropriate to the problem being studied”

Methods: Classical or Conventional Method, Serological testing, Nucleic acid based testing.

215-Write two method? Viral Replication and Its mechanism? Stages of Viral Replication?

Viral replication involves six steps:

- 1) Attachment,
- 2) Penetration,
- 3) uncoating,
- 4) Replication,

5) Assembly

6) Release.

During uncoating, replication, and assembly, the viral DNA or RNA incorporates itself into the host cell's genetic.

216-Gram Staining Procedure

Crystal violet is added. – Primary stain • Crystal violet is washed off. • Iodine is added to enhance binding. – Mordant. • The slide is washed off with alcohol. – Decolorizing agent • Gram positive bacteria retain crystal violet • Gram negative bacteria appear colorless. • Crystal violet and iodine make a complex in the cytoplasm • Peptidoglycan layer is thicker in Gram positive bacteria and CV-I is retained. • Alcohol is rinsed off and stained with safranin. – Counterstain • The smear is washed again. • Blotted dry and examined microscopically.

217-Difference Simple and Differential Media

Selective and differential media are used to isolate or identify particular organisms. Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. ... Differential media are used to differentiate closely related organisms or groups of organisms. material and induces it to replicate the viral genome.

218-Difference LB Broth and LB Agar

LB Broth: broth (LB) is a nutritionally rich medium primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended LB to stand for lysogeny broth, but LB has also come to be commonly referred to as Luria broth, Lennox broth, or Luria-Bertani medium.

LB Agar:

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. The addition of agar to LB results in the formation of a gel that bacteria can grow on, as they are unable to digest the agar but can gather nutrition from the LB within.

219-Chemical used in Gram staining

Both gram-positive and gram-negative cells have peptidoglycan in their cell walls, so initially, all bacteria stain violet. Gram's iodine (iodine and potassium iodide) is applied as a mordant or fixative. Gram-positive cells form a crystal violet-iodine complex. Alcohol or acetone is used to decolorize the cells.

220-UV uses in lab

- Trans illuminators. Used for the visualization of molecular samples, trans illuminators are indispensable tools for researchers at life-sciences laboratories. ...
- UV Cross linkers.
- Fluorescence Analysis Viewing Cabinets and Work Stations
- Ultraviolet Lamps.
- Digital Radiometers/Photometers

221-Streaking Plates method

Streak plate technique is used for the isolation into pure culture of the organisms (mostly bacteria), from mixed population. The inoculum is streaked over the agar surface in such a way that it “thins out” the bacteria. Some individual bacterial cells are separated and well-spaced from each other.

222-Process of bacterial identification

The identification of bacteria is a careful and systematic process that uses many different techniques to narrow down the types of bacteria that are present in an unknown bacterial culture. It produces benefits for many aspects of the research of microorganisms and helps physicians correctly treat patients.

223-Physical Method of Sterilization

Heat Method of Sterilization

This is the most common method of sterilization. The heat used kills the microbes in the substance. In heat sterilization process, the longer the exposure to heat the better is the sterilization at a given temperature.

224-Disadvantage of Chlorine Sterilization

Disadvantages as well; First of all high concentration of this agent corrodes metals and also damages cloths. Secondly the strength of this solution decreases with time so whenever you want to use it always prepare its fresh solution.

225-Advantage of dry heat Sterilization

Advantages of dry heat sterilization include: it is inexpensive, it does not pollute or cause toxic fumes, and it does not corrode or rust metal objects. Disadvantages include: it is relatively slow and many objects cannot withstand the high temperatures.

226-Pili and its functions

These are hair-like structure composed of pilin, usually one to ten in number. Longer than fimbriae Used for attachment to:

Host cells

Bacteria

Used for DNA transfer from one bacterium to another during Conjugation (Sex pili). Also function in twitching Motility. Gliding Motility is also the function of the pili.

227-Physical nature of media

Solid Media

Liquid Media

228-Write two method? Viral Replication and Its mechanism?Stage of Viral Replication?

Viral replication involves six steps: attachment, penetration, uncoating, replication, assembly, and release. During uncoating, replication, and assembly, the viral DNA or RNA incorporates itself into the host cell's genetic material and induces it to replicate the viral genome.

229-Difference Simple and Differential Media?

Selective and differential media are used to isolate or identify particular organisms. Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. Differential media are used to differentiate closely related organisms or groups of organisms.

230-Disadvantage of Chlorine Sterilization

Disadvantages as well; First of all high concentration of this agent corrodes metals and also damages cloths. Secondly the strength of this solution decreases with time so whenever you want to use it always prepare its fresh solution.

231-Spectrum of drugs

- **Narrow:** targets only a group of organisms – Penicillin for G+ve. well targeted – Spare microflora
- **Wide:** Targets many groups of microbes – Tetracycline. Targets every organism – Destroy normal microflora as well

232-Consequence of viral infection

Lysis of cells

- Microscopic and Macroscopic Degenerative changes (Cytopathic Effects).
- Virulent infection: lysis of cells
- Latent infection: Virus does not replicate
- Persistent infection: Virus replicates but slowly
- Transformation: Tumor cells

233- what is Endemic, Epidemic and pandemic.

Endemic: consistently present, but with low incidence. Infected ones are reservoir of infection

Epidemic: within an area, unusually high incidence of a disease

Pandemic: Worldwide epidemic

234-Define Cytokines

Cytokines are released as a result of defense cell stimulation by PAMPs and TLRs interactions. Cytokines recruits more immune cells and also stimulate adaptive immune response.

235-Name two antifungal drugs

- Amphotericin B
- Ketoconazole

236-Name the drugs that inhibit the cell wall of fungi

Caspofungin, micafungin, and anidulafungin.

237-Use of antibodies in body defence the body

Ag-Ab Binding

Agglutination

- Opsonization
- Complement Activation
- Ab-dependent cell mediated cytotoxicity
- Neutralization

238-Define Clonal selection

B cells are selected by the antigen to secrete antibodies.

239-Distribution of MHC I and MHC II.

- MHC I: Present on all nucleated cells
- MHC II: Present only on APCs (Antigen Presenting Cells)

240-Define Symbiosis

Symbiosis can be defined as living together is called symbiosis

241-How adaptive immunity was discovered

Observations: Individuals once recovered from smallpox, measles, or chickenpox become immune to it. Experiments led to antibodies discovery in serum and vaccination.

242-Write mode of action of chloramphenicol, streptomycin and tetracycline.

Target: Protein Synthesis Inhibition:

Chloramphenicol:

– Binds to 50S

- Inhibits peptide bonding
- Bacteriostatic

Streptomycin:

- Change's shape of 30S
- Misreading of RNA codes
- Bacteriostatic

Tetracyclines:

- Interfere with attachment of tRNA to mRNA ribosome complex

243-Difference between epitope and antigen

Antigen: A foreign substance that provokes immunity

- More specifically called as Immunogen
- Protein in nature
- Could be large polysaccharide

Epitopes: Specific regions on an antigen

- Also called as antigenic determinants
- Most antigens have MW >10,000 D Bacteriostatic

244-Algae and its structure.

Mostly aquatic (water is necessary for physical support, reproduction and diffusion of nutrients). Cell covering thallus can carry photosynthesis some algae float by gas-filled bladders

Structure

Body of multicellular alga is called a thallus which consists of branched holdfasts (anchor alga to rock) stem like hollow stipes and leaf like blades.

245-Protein inhibiting antibiotics names

- Chloramphenicol
- Erythromycin
- Tetracyclines

– Streptomycin

246-Factors affecting Location of Algae in the ocean

Their location in oceans depends on the availability of nutrients, wavelength of light and the surface on which to grow.

Separate Whatsapp Groups for Girls and Boys
Special Internship Group with Senior Guideline
Zero Percentage Irrelevant Chatting in study groups

Follow this Link for Joining Study Group:

<https://chat.whatsapp.com/D2yLdl6LFFpJuS2EKWYmbE>

Remember Us in Your Prayers