I Internal Examination (2019-20)

B.Sc. Biotechnology III Year

Medical Biotechnology

SET-A

Time: 1:30 Hours **Max. Marks-30**

Q1. Answer the following questions in very short: **1×5=5**

1). Define Pandemic Disease?

Ans: A pandemic is an epidemic disease that has spread across a large region; for instance multiple continents or even worldwide.

2). What do you mean by Active Immunization?

Ans: Active immunization stimulates the immune system to produce antibodies against a particular infectious disease.

3). Define Fungemia?

Ans: Fungemia is the presence of fungus or yeast in the blood.

4). Which component play important role in detection of immune component in ELISA?

Ans: labelled Antibody

5). Write the antigen associated with blood group 'A'?

Ans: Antigen a

Q2. Explain the following terms with examples: **4×2=8**

i). Disease Cycle

Ans: The chain of events that leads to the development of a disease is called the disease cycle – which may be different to the pathogen's life cycle. The incidence and severity of the majority of plant diseases vary on a distinct cyclic basis. Each cycle includes two alternating phases; the parasitic phase and the survival or over summering phase. The seasonal nature of the production of annual crops and the seasonal nature of climate are the main factors contributing to the cyclical nature of plant diseases.

Survival over summer is dependent on environmental conditions. Rhizoctonia and take-all survive well during dry summers when there is little break down of plant residues or

competition from other organisms in the soil. Rusts survive during a wet summer because conditions suit the growth of host plants.

The first requirement for the establishment of a disease is for the pathogen to come into contact with the host. Disease inoculum is generated by previous infections and liberated into the environment; it may come from the same location or have travelled over great distances. Inoculum may be primary (resulting from infections in the previous season), or secondary (arising from infections in the same season).

Wind is the most important way in which fungal spores, (eg. rust spores), are disseminated over long distances. Water is important for some fungal pathogens (eg. Septoria) to spread, especially over short distances. For other diseases (eg. Rhizoctonia, take-all) the inoculum comes from infected plant debris remaining in the soil.

The size, distribution and genetic diversity of host populations are of great importance in determining the degree and rate of epidemic development. For example, the risk of major losses in our wheat crops would be high if all the cultivars relied on the same stripe or stem rust resistance genes.

A key component of disease control is accurate diagnosis and knowledge of the disease cycle for the particular pathogen

ii). Numerical Chromosomal Aberration

Ans: Numerical aberrations (whole chromosomal aneuploidy) represent a significant proportion of chromosomal changes found in humans. These aberrations can occur as a consequence of chromosome segregation defects during cell division. Segregation errors that arise during reductive cell division, or meiosis, are upon fertilisation and subsequent embryo development constitutively present in all cells, resulting in whole organismal aneuploidy. Missegregation in mitosis leads to a mosaic distribution of aneuploidy in somatic cells. Aneuploidy is associated with pathological states in most organisms. Numerical aberrations represent a significant cause of pregnancy loss as well as abnormalities found in live births. Moreover, numerical aberrations are frequently found in ageing tissues or in tumour cells. Although the association of aneuploidy and cancer is known for almost a century, the dispute whether this is a cause or a consequence of cell transformation is still ongoing. Recently, new evidence is emerging that numerical aberrations significantly alter the physiology of eukaryotic cells and might indeed directly contribute to tumourigenesis.

Key Concepts

- Numerical chromosomal aberrations result from errors in chromosome segregations.

- Trisomy, monosomy and polyploidy are among the major causes of spontaneous human abortions.
- Trisomies compatible with survival often result in multiple defects.
- Numerical chromosomal aberrations significantly alter physiology of eukaryotic cells.
- Numerical chromosomal aberrations are frequently found in cancer cells and can contribute to tumourigenesis.

Q3. Write short notes on: **4×2=8**

i). Exotoxins

Ans: Endotoxins are cell-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. In the context of this article, endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) located in the outer membrane of Gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics. Endotoxins generally act in the vicinity of bacterial growth or presence.

Exotoxins are usually secreted by bacteria and act at a site removed from bacterial growth. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides, that act enzymatically or through direct action with host cells and stimulate a variety of host responses. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However, some bacterial exotoxins act at the site of pathogen colonization and may play a role in invasion.

BACTERIAL PROTEIN TOXINS

Exotoxins are usually secreted by living bacteria during exponential growth. The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only Clostridium tetani produces tetanus toxin; only Corynebacterium diphtheriae produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria). At one time, it was thought that exotoxin production was limited mainly to Gram-positive bacteria, but clearly both Gram-positive and Gram-negative bacteria produce soluble protein toxins.

Bacterial protein toxins are the most powerful human poisons known and retain high activity at very high dilutions. The lethality of the most potent bacterial exotoxins is compared to the lethality of strychnine, snake venom, and endotoxin in Table 1 below.

TABLE 1. LETHALITY OF BACTERIAL PROTEIN TOXINS

Toxin	Toxic Dose (mg)	Host	Lethal toxicity compared with:		
			Strychnine	Endotoxin (LPS)	Snake Venom
Botulinum toxin	0.8×10^{-8}	Mouse	3×10^{6}	3×10^{7}	3×10^{5}
Tetanus toxin	4×10^{-8}	Mouse	1×10^{6}	1×10^{7}	1×10^{5}
Shiga toxin	2.3×10^{-6}	Rabbit	1×10^{6}	1×10^{7}	1×10^{5}
Diphtheria toxin	6×10^{-5}	Guinea pig	2×10^{3}	2×10^{4}	2×10^{2}

Usually the site of damage caused by an exotoxin indicates the location for activity of that toxin. Terms such as enterotoxin, neurotoxin, leukocidin or hemolysin are descriptive terms that indicate the target site of some well-defined protein toxins. A few bacterial toxins that obviously bring about the death of an animal are known simply as lethal toxins, and even though the tissues affected and the target site or substrate may be known, the precise mechanism by which death occurs is not clear (e.g. anthrax LF).

Some bacterial toxins are utilized as invasins because they act locally to promote bacterial invasion. Examples are extracellular enzymes that degrade tissue matrices or fibrin, allowing the bacteria to spread. This includes collagenase, hyaluronidase and streptokinase. Other toxins, also considered invasins, degrade membrane components, such as phospholipases and lecithinases.

Some protein toxins have very specific cytotoxic activity (i.e., they attack specific types of cells). For example, tetanus and botulinum toxins attack only neurons. But some toxins (as

produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Toxins that are phospholipases act in this way. This is also true of pore-forming hemolysins and leukocidins.

Bacterial protein toxins are strongly antigenic. In vivo, specific antibody neutralizes the toxicity of these bacterial exotoxins (antitoxin). However, in vitro, specific antitoxin may not fully inhibit their activity. This suggests that the antigenic determinant of the toxin may be distinct from the active portion of the protein molecule. The degree of neutralization of the active site may depend on the distance from the antigenic site on the molecule. However, since the toxin is fully neutralized in vivo, this suggests that other host factors must play a role in toxin neutralization in nature.

Protein exotoxins are inherently unstable. In time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich who coined the term "toxoid" for this product. Toxoids are detoxified toxins which retain their antigenicity and their immunizing capacity. The formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37 degrees at pH range 6 to 9 for several weeks. The resulting toxoids can be used for artificial immunization against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin production. Toxoids are effective immunizing agents against diphtheria and tetanus that are part of the DPT (DTP) vaccine.

Toxins with Enzymatic Activity

As proteins, many bacterial toxins resemble enzymes in a number of ways. Like enzymes, they are denatured by heat, acid and proteolytic enzymes, they act catalytically, and they exhibit specificity of action. The substrate (in the host) may be a component of tissue cells, organs or body fluid.

A plus B Subunit Arrangement

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components: one component (subunit A) is responsible for the enzymatic activity of the toxin; the other component (subunit B) is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits

are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are a variety of ways that toxin subunits may be synthesized and arranged: A + B indicates that the toxin is synthesized and secreted as two separate protein subunits that interact at the target cell surface; A-B or A-5B indicates that the A and B subunits are synthesized separately, but associated by noncovalent bonds during secretion and binding to their target; 5B indicates that the binding domain of the protein is composed of 5 identical subunits. A/B denotes a toxin synthesized as a single polypeptide, divided into A and B domains that may be separated by proteolytic cleavage.

Attachment and Entry of Toxins

There are at least two mechanisms of toxin entry into target cells:

In one mechanism called direct entry, the B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

In an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of receptor-mediated endocytosis (RME). The toxin is internalized in the cell in a membrane-enclosed vesicle called an endosome. H+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases above, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is reflected in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways. If the B subunit contains a hydrophobic region (of amino acids) that insert into the membrane (as in the case of the diphtheria toxin), it may be referred to as the T (translocation) domain of the toxin.

A few bacterial toxins (e.g. diphtheria) are known to utilize both direct entry and RME to enter into host cells, which is not surprising since both mechanisms are variations on a theme. Bacterial toxins with similar enzymatic mechanisms may enter their target cells by different

mechanisms. Thus, the diphtheria toxin and Pseudomonas exotoxin A, which have identical mechanisms of enzymatic activity, enter their host cells in slightly different ways. The adenylate cyclase toxin of Bordetella pertussis (pertussis AC) and anthrax EF produced by Bacillus anthracis, act similarly to catalyze the production of cAMP from host cell intracellular ATP reserves. However, the anthrax toxin enters cells by receptor mediated endocytosis, whereas the pertussis adenylate cyclase traverses the cell membrane directly.

The specific receptors for the B subunit of toxins on target cells or tissues are usually sialogangliosides (glycoproteins) called G-proteins on the cell membrane. For example, the cholera toxin utilizes the ganglioside GM1, and tetanus toxin utilizes ganglioside GT1 and/or GD1b as receptors on host cells.

Diphtheria Toxin

The best known and studied bacterial toxin is the diphtheria toxin, produced by Corynebacterium diphtheriae. Diphtheria toxin is a bacterial exotoxin of the A/B prototype. It is produced as single polypeptide chain with a molecular weight of 60,000 daltons. The function of the protein is distinguishable into two parts: subunit A, with a m.w. of 21,000 daltons, contains the enzymatic activity for inhibition of elongation factor-2 involved in host protein synthesis; subunit B, with a m.w. of 39,000 daltons, is responsible for binding to the membrane of a susceptible host cell. The B subunit possesses a region T (translocation) domain which inserts into the endosome membrane thus securing the release of the enzymatic component into the cytoplasm.

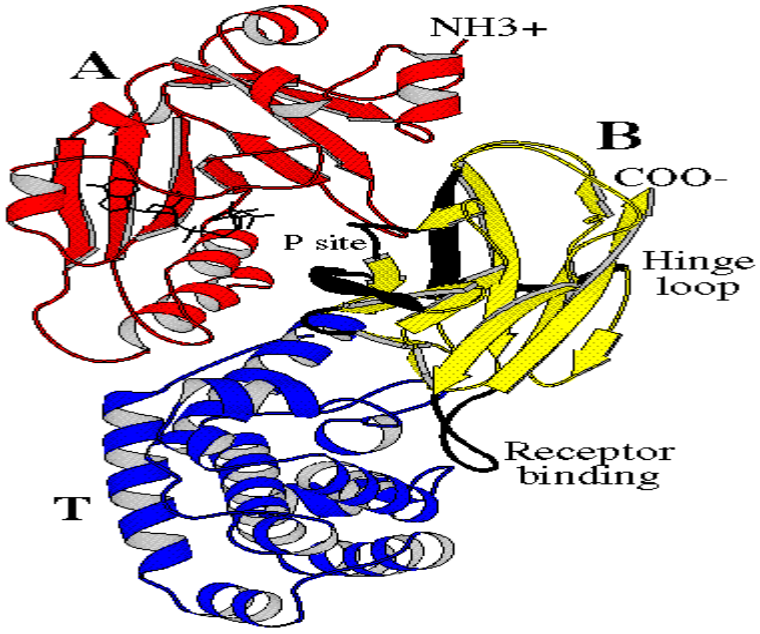


Figure 1. Diphtheria Toxin (Dtx). A (red) is the catalytic domain; B (yellow) is the binding domain which displays the receptor for cell attachment; T (blue) is the hydrophobic domain responsible for insertion into the endosome membrane to secure the release of A. The protein is illustrated in its "closed" configuration.

In vitro, the native toxin is produced in an inactive form which can be activated by the proteolytic enzyme trypsin in the presence of thiol (reducing agent). The enzymatic activity of Fragment A is masked in the intact toxin. Fragment B is required to enable Fragment A to reach the cytoplasm of susceptible cells. The C terminal end of Fragment B is hydrophilic and contains determinants that interact with specific membrane receptors on sensitive cell membranes and the N-terminal end of Fragment B (called the T domain) is strongly hydrophobic. The specific membrane receptor for the B fragment has been shown to be a transmembranous heparin-binding protein on the susceptible cell's surface.

The diphtheria toxin enters its target cells by either direct entry or receptor mediated endocytosis. The first step is the irreversible binding of the C-terminal hydrophilic portion of Fragment B (AA 432-535) to the receptor. During RME, the whole toxin is then taken up in an endocytic vesicle. In the vesicle, the pH drops to about 5 which allows unfolding of the A and B chains. This exposes hydrophobic regions of both the A and B chains that can insert into the vesicle membrane. The result is exposure of the A chain to the cytoplasmic side of the membrane. There, reduction and proteolytic cleavage releases the A chain in the

cytoplasm. The A fragment is released as an extended chain but regains its active (enzymatic) globular conformation in the cytoplasm. The A chain catalyzes the ADP ribosylation of elongation factor-2 (EF-2) as shown in Figure 2.

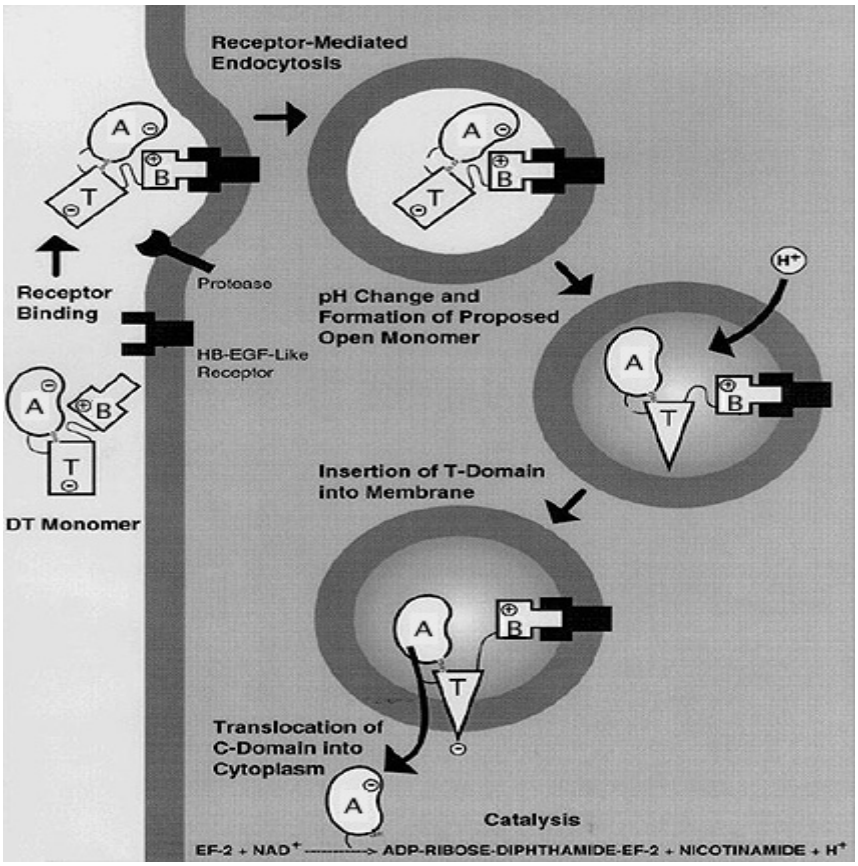


Figure 2. Entry and activity of diphtheria toxin (Dtx) in susceptible cells.

The B domain of the toxin binds to a cognate receptor on a susceptible cell. The toxin is taken up in an endosome by receptor mediated encocytosis. Acidification of the endocytic vesicle allows unfolding of the A and B chains exposing the hydrophobic T domain of the toxin. The T domain inserts into the endosome membrane translocating the A fragment into the cytoplasm where it regains its enzymatic configuration. The enzymatic A component utilizes NAD as a substrate. It catalyzes the attachment of the ADP-ribose portion of NAD to elongation factor (EF-2) which inactivates its function in protein synthesis.

Table 2 describes several bacterial toxins with known enzymatic activity and the biological effects of the toxins in humans.

TABLE 2. BIOLOGICAL EFFECTS OF SOME BACTERIAL EXOTOXINS WITH ENZYMATIC ACTIVITY

TOXIN (subunit arr)*	**ENZYMATIC ACTIVITY**	**BIOLOGICAL EFFECTS**
Cholera toxin (A-5B)	ADP ribosylates eucaryotic adenylate cyclase Gs regulatory protein	Activates adenylate cyclase; increased level of intracellular cAMP promote secretion of fluid and electrolytes in intestinal epithelium leading to diarrhea
Diphtheria toxin (A/B)	ADP ribosylates elongation factor 2	Inhibits protein synthesis in animal cells resulting in death of the cells
Pertussis toxin (A-5B)	ADP ribosylates adenylate cyclase Gi regulatory protein	Blocks inhibition of adenylate cyclase; increased levels of cAMP affect hormone activity and reduce phagocytic activity
E. coli heat-labile toxin LT (A-5B)	ADP ribosylates adenylate cyclase Gs regulatory protein	Similar or identical to cholera toxin
Shiga toxin (A/5B	Glycosidase cleavage of ribosomal RNA (cleaves a single	Inactivates the mammalian 60S ribosomal subunit and

	Adenine base from the 28S rRNA)	leads to inhibition of protein synthesis and death of the susceptible cells; pathology is diarrhea, hemorrhagic colitis (HC) and/or hemolytic uremic syndrome (HUS)
Pseudomonas Exotoxin A (A/B)	ADP ribosylates elongation factor-2 analogous to diphtheria toxin	Inhibits protein synthesis in susceptible cells, resulting in death of the cells
Botulinum toxin (A/B)	Zn^{++} dependent protease acts on synaptobrevin at motor neuron ganglioside	Inhibits presynaptic acetylycholine release from peripheral cholinergic neurons resulting in flaccid paralysis
Tetanus toxin (A/B)	Zn^{++} dependent protease acts on synaptobrevin in central nervous system	Inhibits neurotransmitter release from inhibitory neurons in the CNS resulting in spastic paralysis
Anthrax toxin LF (A2+B)	Metallo protease that cleaves MAPKK (mitogen-	Combined with the B subunit (PA), LF

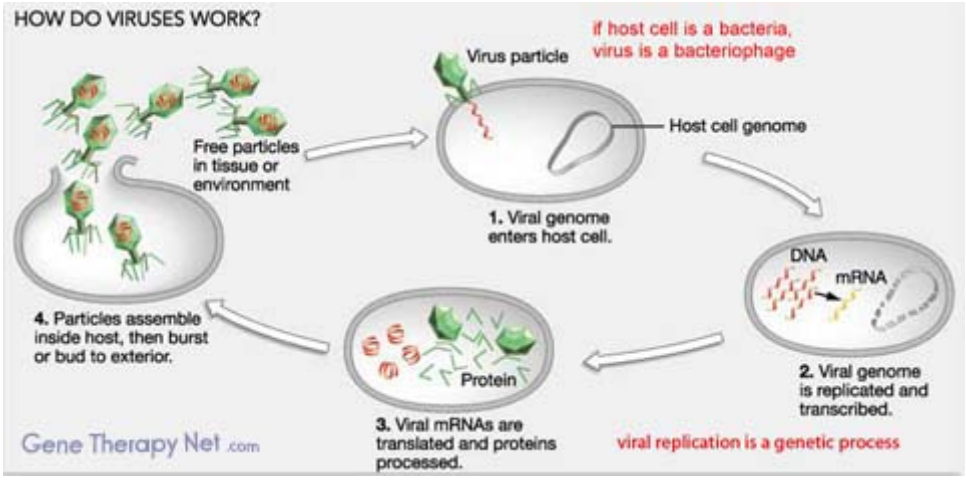
	activated protein kinase kinase) enzymes	induces cytokine release and death of target cells or experimental animals
Bordetella pertussis AC toxin (A/B) and Bacillus anthracis EF (A1+B)	Calmodulin-regulated adenylate cyclases that catalyze the formation of cyclic AMP from ATP in susceptible cells, as well as the formation of ion-permeable pores in cell membranes	Increases cAMP in phagocytes leading to inhibition of phagocytosis by neutrophils and macrophages; also causes hemolysis and leukolysis
Staphylococcus aureus Exfoliatin B	Cleaves desmoglein 1, a cadherin found in desmosomes in the epidermis (also a superantigen)	Separation of the stratum granulosum of the epidermis, between the living layers and the superficial dead layers.

* toxin subunit arrangements: A-B or A-5B indicates subunits synthesized separately and associated by noncovalent bonds; A/B denotes subunit domains of a single protein that may be separated by proteolytic cleavage; A+B indicates subunits synthesized and secreted as separate protein subunits that interact at the target cell surface; 5B indicates that the binding domain is composed of 5 identical subunits.

ii). Biological Vector associated with gene therapy

Ans: All viruses attack their hosts and introduce their genetic material into the host cell as part of their replication cycle. This genetic material contains basic 'instructions' of how to produce more copies of these viruses, hijacking the body's normal production machinery to serve the needs of the virus (see figure). The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells becoming infected. Some types of viruses actually physically insert their genes into the host's genome. This incorporates the genes of that virus among the genes of the host cell for the life span of that cell.

Viruses like this could be used as vehicles to carry 'good' genes into a human cell. First, a scientist would remove the genes in the virus that cause disease. Then they would replace those genes with genes encoding the desired effect (for instance, insulin production in the case of diabetics). This procedure must be done in such a way that the genes which allow the virus to insert its genome into its host's genome are left intact.



Many gene therapy clinical trials rely on retroviruses or adenoviruses to deliver the desired gene. Other viruses used as vectors include adeno-associated viruses, lentiviruses, pox viruses, alphaviruses, and herpes viruses. These viruses differ in how well they transfer genes to the cells they recognize and are able to infect, and whether they alter the cell's DNA permanently or temporarily (see figure).

		Adenovirus	Adeno-associated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Particle characteristics	Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
	Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
	Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
	Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
	Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
	Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb
							
	Family	*Adenoviridae*	*Parvoviridae*	*Togaviridae*	*Herpesviridae*	*Retroviridae*	*Poxviridae*
Gene Therapy Properties	Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
	Host genome interaction	Non-integrating	Non-integrating*	Non-integrating	Non-integrating	Integrating	Non-integrating
	Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
	Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

Q4. Write a detailed note on chickenpox epidemiology, pathogenesis & its prevention? 9

Ans: Varicella (chickenpox) is an acute infectious disease. It is caused by varicella-zoster virus (VZV), which is a DNA virus that is a member of the herpesvirus group. After the primary infection, VZV stays in the body (in the sensory nerve ganglia) as a latent infection. Primary infection with VZV causes varicella. Reactivation of latent infection causes herpes zoster (shingles).

Incubation Period:

The average incubation period for varicella is 14 to 16 days after exposure to a varicella or a herpes zoster rash, with a range of 10 to 21 days. A mild prodrome of fever and malaise may occur 1 to 2 days before rash onset, particularly in adults. In children, the rash is often the first sign of disease.

Transmission: Varicella is highly contagious. The virus can be spread from person to person by direct contact, inhalation of aerosols from vesicular fluid of skin lesions of acute varicella or zoster, and possibly through infected respiratory secretions that also may be aerosolized. A person with varicella is contagious beginning 1 to 2 days before rash onset until all the chickenpox lesions have crusted. Vaccinated people may develop lesions that do not crust. These people are considered contagious until no new lesions have appeared for 24 hours.

It takes from 10 to 21 days after exposure to the virus for someone to develop varicella. Based on studies of transmission among household members, about 90% of susceptible close

contacts will get varicella after exposure to a person with disease. Although limited data are available to assess the risk of VZV transmission from zoster, one household study found that the risk for VZV transmission from herpes zoster was approximately 20% of the risk for transmission from varicella.

People with breakthrough varicella are also contagious. One study of varicella transmission in household settings found that people with mild breakthrough varicella (<50 lesions) who were vaccinated with one dose of varicella vaccine were one-third as contagious as unvaccinated people with varicella. However, people with breakthrough varicella with 50 or more lesions were just as contagious as unvaccinated people with the disease.

Varicella is less contagious than measles, but more contagious than mumps and rubella.

Complications

The most common complications from varicella are:

- In children: Bacterial infections of the skin and soft tissues
- In adults: Pneumonia

Severe complications caused by varicella include cerebellar ataxia, encephalitis, viral pneumonia, and hemorrhagic conditions. Other severe complications are due to bacterial infections and include:

- Septicemia
- Toxic shock syndrome
- Necrotizing fasciitis
- Osteomyelitis
- Bacterial pneumonia
- Septic arthritis

I Internal Examination (2019-20)

B.Sc. Biotechnology III Year

Medical Biotechnology

SET-B

Time: 1:30 Hours **Max. Marks-30**

Q1. Answer the following questions in very short: **1×5=5**

1). Define Epidemic Disease?

Ans: An epidemic is the rapid spread of infectious disease to number large number of people in a given population within a short period of time, usually two weeks or less.

2). Write one example of Passive immunization?

Ans; Hepatitis A (gamma globulin)

3). Define meningitis?

Ans: A serious disease in which there is inflammation of the meninges, caused by viral or bacterial infection, and marked by intense headache and fever, sensitivity to lights and muscular rigidity.

4). Name any two viral Diseases?

Ans: Chickenpox and Mumps

5). Write the antigen associated with blood group 'O'?

Ans: No Antigen

Q2. Explain the following terms with examples: **4×2=8**

i). Numerical Chromosomal Aberration

Ans: Numerical aberrations (whole chromosomal aneuploidy) represent a significant proportion of chromosomal changes found in humans. These aberrations can occur as a consequence of chromosome segregation defects during cell division. Segregation errors that arise during reductive cell division, or meiosis, are upon fertilisation and subsequent embryo development constitutively present in all cells, resulting in whole organismal aneuploidy. Missegregation in mitosis leads to a mosaic distribution of aneuploidy in somatic cells. Aneuploidy is associated with pathological states in most organisms. Numerical aberrations represent a significant cause of pregnancy loss as well as abnormalities found in live births.

Moreover, numerical aberrations are frequently found in ageing tissues or in tumour cells. Although the association of aneuploidy and cancer is known for almost a century, the dispute whether this is a cause or a consequence of cell transformation is still ongoing. Recently, new evidence is emerging that numerical aberrations significantly alter the physiology of eukaryotic cells and might indeed directly contribute to tumourigenesis.

Key Concepts

- Numerical chromosomal aberrations result from errors in chromosome segregations.
- Trisomy, monosomy and polyploidy are among the major causes of spontaneous human abortions.
- Trisomies compatible with survival often result in multiple defects.
- Numerical chromosomal aberrations significantly alter physiology of eukaryotic cells.
- Numerical chromosomal aberrations are frequently found in cancer cells and can contribute to tumourigenesis.

ii). Synthetic vectors for gene therapy

Ans: Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses.

1. Naked DNA:

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection. In addition to trials with plasmids, there have been trials with naked PCR product, which have had similar or greater success. This success, however, does not compare to that of the other methods, leading to research into more efficient methods for delivery of the naked DNA such as electroporation and the use of a "gene gun", which shoots DNA coated gold particles into the cell using high pressure gas.

2. Oligonucleotide:

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique

sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA, and therefore expression of the gene.

A further strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression.

3. Lipoplexes and Polyplexes:

To improve the delivery of the new DNA into the cell, the DNA must be protected from damage and its entry into the cell must be facilitated. To this end new molecules, lipoplexes and polyplexes, have been created that have the ability to protect the DNA from undesirable degradation during the transfection process.

Plasmid DNA can be covered with lipids in an organized structure like a micelle or a liposome. When the organized structure is complexed with DNA it is called a lipoplex. There are three types of lipids, anionic (negatively charged), neutral, or cationic (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors. However, in spite of the facts that there is little toxicity associated with them, that they are compatible with body fluids and that there was a possibility of adapting them to be tissue specific; they are complicated and time consuming to produce so attention was turned to the cationic versions.

Cationic lipids, due to their positive charge, naturally complex with the negatively charged DNA. Also as a result of their charge they interact with the cell membrane, endocytosis of the lipoplex occurs and the DNA is released into the cytoplasm. The cationic lipids also protect against degradation of the DNA by the cell.

The most common use of lipoplexes has been in gene transfer into cancer cells, where the supplied genes have activated tumor suppressor control genes in the cell and decrease the activity of oncogenes. Recent studies have shown lipoplexes to be useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.

Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions. One large difference between the methods of action of polyplexes and lipoplexes is that polyplexes cannot release their DNA load into the cytoplasm, so to this end, co-transfection with endosome-lytic agents (to lyse the endosome that is made during endocytosis, the process by which the polyplex enters the cell) such as inactivated adenovirus must occur. However this isn't always the case,

polymers such as polyethylenimine have their own method of endosome disruption as does chitosan and trimethylchitosan.

Q3. Write short notes on: **4×2=8**

i). Mumps

Ans: Mumps is a viral infection that primarily affects saliva-producing (salivary) glands that are located near your ears. Mumps can cause swelling in one or both of these glands.

Mumps was common in the United States until mumps vaccination became routine. Since then, the number of cases has dropped dramatically.

However, mumps outbreaks still occur in the United States, and the number of cases has crept up in recent years. These outbreaks generally affect people who aren't vaccinated, and occur in close-contact settings such as schools or college campuses.

Complications of mumps, such as hearing loss, are potentially serious but rare. There's no specific treatment for mumps.

Symptoms: Some people infected with the mumps virus have either no signs or symptoms or very mild ones. When signs and symptoms do develop, they usually appear about two to three weeks after exposure to the virus.

The primary sign of mumps is swollen salivary glands that cause the cheeks to puff out. Other signs and symptoms may include:

- Pain in the swollen salivary glands on one or both sides of your face
- Pain while chewing or swallowing
- Fever
- Headache
- Muscle aches
- Weakness and fatigue
- Loss of appetite

Causes

Mumps is caused by a virus that spreads easily from person to person through infected saliva. If you're not immune, you can contract mumps by breathing in saliva droplets from an infected person who has just sneezed or coughed. You can also contract mumps from sharing utensils or cups with someone who has mumps.

Complications

Complications of mumps are rare, but some are potentially serious.

Most mumps complications involve inflammation and swelling in some part of the body, such as:

- **Testicles.** This condition, known as orchitis, causes one or both testicles to swell in males who've reached puberty. Orchitis is painful, but it rarely leads to the inability to father a child (sterility).
- **Brain.** Viral infections such as mumps can lead to inflammation of the brain (encephalitis). Encephalitis can cause neurological problems and become life-threatening.
- **Membranes and fluid around the brain and spinal cord.** This condition, known as meningitis, can occur if the mumps virus spreads through your bloodstream to infect your central nervous system.
- **Pancreas.** The signs and symptoms of this condition, known as pancreatitis, include pain in the upper abdomen, nausea and vomiting.

Other complications of mumps include:

- **Hearing loss.** Hearing loss can occur in one or both ears. Although rare, the hearing loss is sometimes permanent.
- **Heart problems.** Rarely, mumps has been associated with abnormal heartbeat and diseases of the heart muscle.
- **Miscarriage.** Contracting mumps while you're pregnant, especially early in your pregnancy, may lead to miscarriage.

Prevention

The best way to prevent mumps is to be vaccinated against the disease. Most people have immunity to mumps once they're fully vaccinated.

The mumps vaccine is usually given as a combined measles-mumps-rubella (MMR) inoculation, which contains the safest and most effective form of each vaccine. Two doses of the MMR vaccine are recommended before a child enters school. Those vaccines should be given when the child is:

- Between the ages of 12 and 15 months
- Between the ages of 4 and 6 years

College students, international travelers and health care workers in particular are encouraged to make sure they've had two doses of the MMR vaccine. A single dose is not completely effective at preventing mumps.

A third dose of vaccine isn't routinely recommended. But your doctor might recommend a third dose if you are in an area that is experiencing an outbreak. A study of a recent mumps

outbreak on a college campus showed that students who received a third dose of MMR vaccine had a much lower risk of contracting the disease.

ii). Transmission of Pathogen

Ans: To survive as a species, pathogens must infect new people or animals. To do this, they must leave the body of the host, find their way to a new susceptible person or animal, and enter the body of that person or animal. As the exit, transmission, and entry of the pathogens are closely associated, we will cover them together. Water and environmental sanitation interventions that aim to improve the health of a population usually try to reduce the risk of transmission of infection. To do this appropriately, the WES specialist needs to be familiar with the pathogens' transmission route(s). It is this understanding that enables the specialist to determine which control measures will be most effective in a particular situation. As many infections are linked to WES, it is useful to categorise the different diseases.

For a water and sanitation specialist the most useful categorisation is based on the transmission cycles of the infections. Generally speaking, diseases with similar transmission cycles can be controlled by similar preventive measures, and will occur in similar environments. The infections are categorised and their transmission routes described at the same time. Some terms relating to the transmission or classification of infections are defined here:

Food-borne infections: infections which can be transmitted through eating food containing the pathogen.

Vector-borne infections: infections transmitted through vectors. We use vector borne infections only for infections with a biological vector, that is a vector in which the pathogen goes through a development before further transmission is possible (e.g. mosquitoes, tsetse fly, body louse). We do not classify as vector borne those infections which are transmitted by mechanical vectors, that is the animal is only a vehicle for transporting the pathogen (e.g. domestic flies, cockroaches).

Water-borne infections: infections which can be transmitted through drinking water which contains the pathogen.

Water-washed infections: infections caused by pathogens whose transmission can be prevented by improving personal hygiene. Infections can have either direct or indirect transmission routes.

Q4. Write a detailed note on HIV epidemiology, pathogenesis & its Diagnosis? 9

Ans: Human immunodeficiency virus (HIV) is a virus that attacks immune cells called CD4 cells, which are a type of T cell.

These are white blood cells that move around the body, detecting faults and anomalies in cells as well as infections. When HIV targets and infiltrates these cells, it reduces the body's ability to combat other diseases.

This increases the risk and impact of opportunistic infections and cancers. However, a person can carry HIV without experiencing symptoms for a long time.

HIV is a lifelong infection. However, receiving treatment and managing the disease effectively can prevent HIV from reaching a severe level and reduce the risk of a person passing on the virus.

AIDS is the most advanced stage of HIV infection. Once HIV infection develops into AIDS, infections and cancer pose a greater risk.

Without treatment, HIV infection is likely to develop into AIDS as the immune system gradually wears down. However, advances in ART mean than an ever-decreasing number of people progress to this stage.

By the close of 2015, around 1,122,900 people were HIV-positive. To compare, figures from 2016 show that medical professionals diagnosed AIDS in an estimated 18,160 people.

Causes

People transmit HIV in bodily fluids, including:

- blood
- semen
- vaginal secretions
- anal fluids
- breast milk

In the United States, the main causes of this transfer of fluids are:

- anal or vaginal intercourse with a person who has HIV while not using a condom or PrEP, a preventive HIV medication for people at high risk of infection
- sharing equipment for injectable illicit drugs, hormones, and steroids with a person who has HIV

A woman living with HIV who is pregnant or has recently given birth might transfer the disease to her child during pregnancy, childbirth, or breastfeeding.

The risk of HIV transmitting through blood transfusions is extremely low in countries that have effective screening procedures in place for blood donations.

Undetectable = untransmittable

To transmit HIV, these fluids must contain enough of the virus. If a person has 'undetectable' HIV, they will not transmit HIV to another person, even if after a transfer of fluids.

Undetectable HIV is when the amount of HIV in the body is so low that a blood test cannot detect it. People may be able to achieve undetectable levels of HIV by closely following the prescribed course of treatment.

Confirming and regularly monitoring undetectable status using a blood test is important, as this does not mean that the person no longer has HIV. Undetectable HIV relies on the person adhering to their treatment, as well as the effectiveness of the treatment itself.

Progression to AIDS

The risk of HIV progressing to AIDS varies widely between individuals and depends on many factors, including:

- the age of the individual
- the body's ability to defend against HIV
- access to high-quality, sanitary healthcare
- the presence of other infections
- the individual's genetic inheritance resistance to certain strains of HIV
- drug-resistant strains of HIV

Symptoms

For the most part, infections by other bacteria, viruses, fungi, or parasites cause the more severe symptoms of HIV.

These conditions tend to progress further in people who live with HIV than in individuals with healthy immune systems. A correctly functioning immune system would protect the body against the more advanced effects of infections, and HIV disrupts this process.

Early symptoms of HIV infection

Sweats are an early sign of HIV, but many people do not know they have the disease for years.

Some people with HIV do not show symptoms until months or even years after contracting the virus.

However, around 80 percent of people may develop a set of flu-like symptoms known as acute retroviral syndrome around 2–6 weeks after the virus enters the body.

The early symptoms of HIV infection may include:

- fever
- chills
- joint pain

- muscle aches
- sore throat
- sweats. particularly at night
- enlarged glands
- a red rash
- tiredness
- weakness
- unintentional weight loss
- thrush

These symptoms might also result from the immune system fighting off many types of viruses.

However, people who experience several of these symptoms and know of any reason they might have been at risk of contracting HIV over the last 6 weeks should take a test.

Asymptomatic HIV

In many cases, after the symptoms of acute retroviral syndrome, symptoms might not occur for many years.

During this time, the virus continues to develop and cause immune system and organ damage. Without medication that prevents the replication of the virus, this slow process can continue for an average of around 10 years.

A person living with HIV often experiences no symptoms, feels well, and appears healthy.

Complying rigidly to a course of ART can disrupt this phase and suppress the virus completely. Taking effective antiretroviral medications for life can halt on-going damage to the immune system.

Late-stage HIV infection

Without medication, HIV weakens the ability to fight infection. The person becomes vulnerable to serious illnesses. This stage is known as AIDS or stage 3 HIV.

Symptoms of late-stage HIV infection may include:

- blurred vision
- diarrhea, which is usually persistent or chronic
- dry cough
- a fever of over 100 °F (37 °C) lasting for weeks
- night sweats
- permanent tiredness
- shortness of breath, or dyspnea

- swollen glands lasting for weeks
- unintentional weight loss
- white spots on the tongue or mouth

During late-stage HIV infection, the risk of developing a life-threatening illness increases greatly. A person with late-stage HIV can control, prevent and treat serious conditions by taking other medications alongside HIV treatment.

Opportunistic infections

Toxoplasmosis, found in cat and animal feces, is a dangerous opportunistic infection for people who have AIDS.

HIV treatment is nowadays often effective enough to keep many infections at bay.

In reducing the activity of the immune system, late-stage HIV reduces the ability of the body to combat a range of infections, diseases, and cancers. Infections that caused minimal or no health problems before the development of AIDS might pose a serious health risk once the condition has weakened the immune system.

Medical professionals refer to these as opportunistic infections (OIs). Once any of these infections occur, a doctor will diagnose AIDS.

These include:

Candidiasis of the bronchi, trachea, esophagus, and lungs: As a fungal infection that normally occurs in the skin and nails, this frequently causes serious problems in the esophagus and lower respiratory tract for people with AIDS.

Invasive cervical cancer: This type of cancer begins in the cervix and spreads to other areas in the body. Regular checks with a cancer care team can help prevent the cancer or limit the spread.

Coccidioidomycosis: People sometimes refer to the self-limited version of this disease in healthy individuals as valley fever. Inhalation of the fungus *Coccidioides immitis* causes this infection.

Cryptococcosis: *Cryptococcus neoformans* is a fungus that can infect any part of the body, but most often enters the lungs to trigger pneumonia or the brain to cause swelling.

Cryptosporidiosis: The protozoan parasite *Cryptosporidium* causes this infection that leads to severe abdominal cramps and watery diarrhea.

Cytomegalovirus disease (CMV): CMV can cause a range of diseases in the body, including pneumonia, gastroenteritis, and encephalitis, a brain infection. However, CMV retinitis is of particular concern in people with late-stage HIV, and it can infect the retina at the back of the eye, permanently removing sight. CMV retinitis is a medical emergency.

HIV-related encephalopathy: An acute or chronic HIV infection can trigger this brain disorder. While doctors do not fully understand the cause, they consider it to be linked to post-infection inflammation in the brain.

Herpes simplex (HSV): This virus, usually sexually transmitted or passed on in childbirth, is extremely common and rarely causes health issues or causes self-limiting recurrences in people with healthy immune systems. However, it can reactivate in people with HIV, causing painful cold sores around the mouth and ulcers on the genitals and anus that do not resolve. The sores, rather than a herpes diagnosis, are an indicator of AIDS. HSV can also infect the breathing tube, lungs, or esophagus of people with AIDS.

Histoplasmosis: The fungus *Histoplasma capsulatum* causes extremely severe, pneumonia-like symptoms in people with advanced HIV. This condition can become progressive disseminated histoplasmosis and can impact on organs outside of the respiratory system.

Chronic intestinal isosporiasis: The parasite *Isospora belli* can infect the body through contaminated food and water, causing diarrhea, fever, vomiting, weight loss, headaches, and abdominal pain.

Kaposi's sarcoma (KS): *Kaposi's sarcoma herpesvirus* (KSHV), also known as *human herpesvirus 8* (HHV-8), causes a cancer that leads to the growth of abnormal blood vessels anywhere in the body. If KS reaches organs, such as the intestines or lymph nodes, it can be extremely dangerous. KS appears as solid purple or pink spots on the surface of the skin. They might be flat or raised.

Lymphoma: People refer to cancer of the lymph nodes and lymphoid tissues as lymphoma, and many different types might occur. However, Hodgkin and non-Hodgkin lymphoma have strong links to HIV infection.

Tuberculosis (TB): The bacteria *Mycobacterium tuberculosis* causes this disease and can transfer in droplets if a person with an active form of the bacteria sneezes, coughs, or speaks. TB causes a severe lung infection as well as weight loss, fever, and tiredness, and can also infect the brain, lymph nodes, bones, or kidneys.

Mycobacteria, including *Mycobacterium avium* and *Mycobacterium kansasii*: These bacteria occur naturally in the environment and pose few problems for people with fully-functioning immune systems. However, they can spread throughout the body and cause life-threatening health issues for people with HIV, especially in its later stages.

***Pneumocystis jirovecii* pneumonia (PJP):** A fungus called *Pneumocystis jirovecii* causes breathlessness, dry cough, and high fever in people with suppressed immune systems, including those with HIV.

Recurrent pneumonia: Many different infections can cause pneumonia, but a bacteria called Streptococcus pneumoniae is one of its most dangerous causes in people with HIV. Vaccines are available for this bacteria, and every person who has HIV should receive vaccination for *Streptococcus pneumoniae*.

Progressive multifocal encephalopathy (PML): The John Cunningham (JC) virus occurs in a vast number of people, usually lying dormant in the kidneys. However, in people with compromised immune systems, either due to HIV or medications, such as those for multiple sclerosis (MS), the JC virus attacks the brain, leading to a dangerous conditon called progressive multifocal leukoencephalopathy (PML). PML can be life-threatening, causing paralysis and cognitive difficulties.

Recurrent *Salmonella* septicemia: This type of bacteria often enters the body in contaminated food and water, circulates the entire body, and overpowers the immune system, causing nausea, diarrhea, and vomiting.

Toxoplasmosis (toxo): *Toxoplasma gondii* is a parasite that inhabits warm-blooded animals, including cats and rodents, and leaves the body in their feces. Humans contract the diseases by inhaling contaminated dust or eating contaminated food, but it can also occur in commercial meats. *T. gondii* causes severe infection in the lungs, retina, heart, liver, pancreas, brain, testes, and colon. Take care to wear protective gloves while changing cat litter and thoroughly wash the hands afterward.

Wasting syndrome: This occurs when a person involuntarily loses 10 percent of their muscle mass through diarrhea, weakness, or fever. Part of the weight loss may also consist of fat loss.

Prevention

Preventing OIs is key to extending life expectancy with late-stage HIV. Aside from managing HIV viral load with medications, a person who lives with the disease must take precautions, including the following steps:

- Wear condoms to prevent other STIs.
- Receive vaccinations for potential OIs. Discuss these with your primary care physician.
- Understand the germs in your surrounding environment that could lead to an OI. A pet cat, for example, could be a source of toxoplasmosis. Limit exposure and take precautions, such as wearing protective gloves while changing litter
- Avoid foods that are at risk of contamination, such as undercooked eggs, unpasteurized dairy and fruit juice, or raw seed sprouts.

- Do not drink water straight from a lake or river or tap water in certain foreign countries. Drink bottled water or use water filters.
- Ask your doctor about work, home, and vacation activities to limit exposure to potential OIs. Antibiotic, antifungal, or antiparasitic drugs can help treat an OI.