

**INSTRUCTIONS: Attempt ALL the questions**

**TIME: 3 HOURS**

**1. What do you understand by the following words? (20 points)**

- 1) Metabolome - The metabolome refers to the complete set of small-molecule chemicals found within a biological sample. The biological sample can be a cell, a cellular organelle, an organ, a tissue, a tissue extract, a biofluid or an entire organism.
- 2) Non-targeted metabolomics - Nontargeted metabolomics aims to profile the entire metabolome present in cells, biofluids, or tissues. Nontargeted metabolomics measures as many metabolites as possible to compare biological samples and is useful for hypothesis generation
- 3) Transcriptomics. - Transcriptomics is the study of the transcriptome—the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell—using high-throughput methods, such as microarray analysis. Comparison of transcriptomes allows the identification of genes that are differentially expressed in distinct cell populations, or in response to different treatments.
- 4) Toxicogenomics. - Toxicogenomics is a field of science that deals with the collection, interpretation, and storage of information about gene and protein activity within particular cell or tissue of an organism in response to toxic substances. Toxicogenomics combines toxicology with genomics or other high throughput molecular profiling technologies such as transcriptomics, proteomics and metabolomics. Toxicogenomics endeavours to elucidate molecular mechanisms evolved in the expression of toxicity, and to derive molecular expression patterns (i.e., molecular biomarkers) that predict toxicity or the genetic susceptibility to it.
- 5) Interactomics - In molecular biology, an interactome is the whole set of molecular interactions in a particular cell. The term specifically refers to

physical interactions among molecules (such as those among proteins, also known as protein-protein interactions) but can also describe sets of indirect interactions among genes (genetic interactions). Mathematically, interactomes are generally displayed as graphs.

- 6) Antibody - An antibody (Ab), also known as an immunoglobulin (Ig), is a large, Y-shaped protein used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an antigen. Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can tag a microbe or an infected cell for attack by other parts of the immune system, or can neutralize it directly (for example, by blocking a part of a virus that is essential for its invasion).
- 7) DNA microarray - (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome
- 8) Proteome - this is the entire set of proteins that can be expressed by a genome, cell, tissue, or organism at a certain time. It is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions.

**2. Name Six applications of transcriptomics (10 points)**

- a) Sequencing and Next-Generation Sequencing
- b) Genotyping
- c) Copy number variation (CNV)
- d) Methylation Analysis
- e) Gene Expression
- f) miRNA Analysis
- g) ChIP-on-chip (Chromatin Immunoprecipitation)

h) Metagenomics: Bacteria and Fungus Identification.

3. How does an antibody tests work? **(20 points)**

Antibody tests detect whether the body has produced detectable quantities of antibodies to a certain molecule, and can therefore reveal whether someone has been infected by a specific virus or bacteria in the past. Usually, these tests are detecting IgM or IgG. For instance, SARS-CoV-2 (COVID 19) antibody tests typically detect either part or all of the coronavirus' spike protein and can reveal whether someone has had COVID-19 in the past. Because the body takes time to ramp up its production of antibodies, people usually only test positive about two weeks after they were first exposed to the pathogen. There are two common types of antibody tests — lateral flow assays and enzyme-linked immunosorbent assay (ELISA) tests. Both involve fixing an antigen to a surface and then detecting whether an antibody binds to that antigen. Usually, a chemical reaction, such as fluorescence or a colour-change, is triggered when the antibody binds to the antigen. Lateral flow assays are similar to pee-on-a-stick pregnancy tests; rather than pee, for antibody tests, blood or serum is washed over the flat surface, which is usually paper. A good antibody test is one that produces few false positives and few false negatives.

4. Discuss factors affecting the protein binding of drugs. (Three drug related and three protein related) **(25 points)**

a. DRUG RELATED FACTORS

- i. Physiochemical characteristics of the drug – Protein binding is directly related to the lipophilicity of drug. An increase in lipophilicity increases the extent of binding. Acidity/ anionic drugs bind to HSA; basic/ cationic drugs to AAG; neutral/ unionized drugs to lipoproteins.
- ii. Concentration of the drug in the body – Alteration in the concentration of drug substance as well as the protein molecules or surfaces subsequently brings alteration in the protein binding process. At low concentrations,

most drugs may be bound to proteins. At high concentrations, more free drugs may be present owing to saturation of binding sites on proteins.

- iii. Drug's affinity towards protein/ tissue. – This factor entirely depends upon the degree of attraction or affinity the protein molecule or tissues have towards drug moieties.

b. PROTEIN RELATED FACTORS

- i. Physiochemical characteristics of the protein or binding agent – Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core. The physiologic pH determines the presence of active anionic and cationic groups on the albumin molecules to bind a variety of drugs.
- ii. Concentration of protein/ binding component – Among the plasma protein, binding predominantly occurs with albumin, as it is present in high concentration in comparison to other plasma protein. The amount of several proteins and tissue components available for binding, change during disease state.
- iii. Number of binding sites on the protein – Albumin has a large number of binding sites as compared to other proteins. Indomethacin binds to 3 sites on albumin. AAG is a protein with limited binding capacity due to low concentration and molecular size. Lidocaine binds to 2 sites on AAG in presence of HSA.

5. State and explain SIX significances of protein and tissue binding of drugs. (25 Points)

- I. Absorption - As we know the conventional dosage form follow first order kinetics. So, when there is more protein binding then it disturbs the absorption equilibrium.
- II. Distribution - A protein bound drug in particular does not cross the BBB, the placental barrier, the glomerulus, thus, protein binding decreases the distribution of drugs.

- III. Metabolism - Protein binding decreases the metabolism of drugs & enhances the biological half-life.
- Only unbound fraction gets metabolized e.g. Phenylbutazone & Sulfonamide.
- IV. Elimination - Only the unbound drug is capable of being eliminated. Protein binding prevents the entry of drug to the metabolizing organ (liver) & to glomerular filtration e.g., Tetracycline is eliminated mainly by glomerular filtration.
- V. Systemic solubility of drug - Lipoprotein acts as vehicle for hydrophobic drugs like steroids, heparin, etc.
- VI. Drug action - Protein binding inactivates the drugs because sufficient concentration of drug cannot be built up in the receptor site for action e.g., Naphthoquinone
- VII. Sustain release - The complex of drug protein in the blood acts as a reservoir & continuously supplies the free drug e.g., Suramin sodium-protein binding for antitrypanosomal action.
- VIII. Diagnosis - The chlorine atom of chloroquine replaced with radiolabeled I-131 can be used to visualize melanomas of eye & disorders of thyroid gland.