

**ANIMAL CELL CULTURE & ANIMAL BIOTECHNOLOGY  
(BIOT 4135)**

**Time Allotted : 2½ hrs**

**Full Marks : 60**

*Figures out of the right margin indicate full marks.*

***Candidates are required to answer Group A and any 4 (four) from Group B to E, taking one from each group.***

***Candidates are required to give answer in their own words as far as practicable.***

**Group – A**

1. Answer any twelve:

**12 × 1 = 12**

*Choose the correct alternative for the following*

- (i) For making transgenic animals in fertilized egg the best place to insert trans-gene is in?  
 (a) female pronuclei (b) cytoplasm  
 (c) male pronuclei (d) cleavage cell.
- (ii) Monoclonal antibodies can be modified for better research and therapeutic applications. Several such approaches are mentioned below in Group-I and Group-II.

<b>Group-I</b>	<b>Group-II</b>
(P) Binding site of the original mouse mAb are placed onto the Fc regions of human antibodies	(1) Abzymes
(Q) Antibodies are modified by conjugation to toxins designed to kill cells to which the antibody will bind.	(2) Reduce side effect of xenogeneic antibodies in immunotherapy.
(R) Generation of mAb that specifically bind and stabilize the transition state of a chemical reaction.	(3) Immunotoxin

Which one of the following options represents correct match of group-I and Group-II?

- (a) P – 1; Q - 2; R – 3 (b) P – 2; Q - 1; R – 3  
 (c) P – 3; Q - 2; R – 1 (d) P – 2; Q - 3; R – 1
- (iii) Laminin is used as a substrate coating because it interacts with \_\_\_\_\_ on the animal cell surface  
 (a) Ornithine (b) Poly-D-lysine  
 (c) Integrin (d) Concanavalin
- (iv) In the case of monoclonal antibody production by hybridoma technology, myeloma cells used lack the enzyme hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) such that fused cells can only survive when selected on hypoxanthine-aminopterin-thymidine (HAT) What is the role of aminopterin in this medium?  
 (a) To be used as cell cycle inhibitor of myeloma cells.  
 (b) To block the pathway of nucleotide synthesis.  
 (c) To facilitate fusion of myeloid B cells and antibody producing B cells.  
 (d) To facilitate production antibody producing B cells.

- (v) Which of the following analytical techniques does not involve an optical measurement?  
 (a) ELISA (b) Microarray  
 (c) Flow cytometry (d) Differential Scanning calorimetry.
- (vi) For culturing, plasma from the adult chicken is preferred to mammalian plasma because  
 (a) it forms a clear and solid coagulum even after dilution  
 (b) it is too opaque  
 (c) it doesn't produce solid clots,  
 (d) it forms a semi solid coagulum.

(vii) Match different strategy of gene therapy in group-I with their meaning in group-II

Group-I	Group-II
(P) Gene augmentation	(1) To revert specific mutation in the gene of interest
(Q) Gene correction	(2) to eliminate the activity of a gene that encourages the growth of disease-related cells
(R) Killing of specific cells	(3) to insert DNA into a diseased cell that causes that cell to die
(S) Gene inhibition therapy	(4) To add DNA containing a functional version of the lost gene back into the cell to eliminate the activity of a gene that encourages the growth of disease-related cells

Which one of the following options represents correct match of group-I and Group-II

- (a) P - 2; Q - 4; R - 1; S - 3, (b) P - 1; Q - 2; R - 3; S - 4,  
 (c) P - 3; Q - 4; R - 2; S - 1, (d) P - 4; Q - 1; R - 3; S--2

(viii) Biopharmaceuticals are classified into groups.

Group-I	Group-II
(P) Protein therapeutics with enzymatic or regulatory activity	(1) Hepatitis B surface antigen
(Q) Protein therapeutics with special targeting activity	(2) Insulin aspart
(R) Protein vaccines	(3) Secretin
(S) Protein diagnostics	(4) Transtuzmab

Which one of the following options represents correct match of group-I and Group-II?

- (a) P - 2; Q - 4; R - 1; S - 3, (b) P - 1; Q - 2; R - 3; S - 4,  
 (c) P - 3; Q - 4; R - 2; S - 1, (d) P - 4; Q - 1; R - 3; S - 2,

- (ix) Accumulation of lactate leads to  
 (a) increase in pH,  
 (b) no change in pH  
 (c) reduction in the pH of culture hence loss of cell viability  
 (d) no loss of cell viability.

- (x) Which of the following cannot be used to produce corona virus vaccine?  
 (a) Animal cell-based vaccine (b) Egg-based vaccine,  
 (c) Recombinant vaccine, (d) Genetically mutated.

*Fill in the blanks with the correct word*

- (xi) TALEN is \_\_\_\_\_ tool.
- (xii) Two most popular mammalian cell used for recombinant protein expressions are \_\_\_\_\_ and \_\_\_\_\_.
- (xiii) Monod model in is a \_\_\_\_\_ model.

- (xiv) DMSO used in animal cell culture as \_\_\_\_\_.
- (xv) We know  $\mu = [\mu_{\max} S / \{K_s + S\}]$  Monod Model If  $\mu \rightarrow \mu_{\max}$   $K_s \rightarrow$  \_\_\_\_\_.

### Group - B

2. (a) What are the different types of methods used for disaggregation of animal tissue or organ fragment? What are the different enzymes used for disaggregation of animal tissues? [[CO2](Remember/LOCQ)]
- (b) Explain the procedure involved in warm and cold trypsinization for the preparation of primary culture from animal tissue fragment. [[CO2](Explain/HOCQ)]
- (c) What are the optimum conditions required in the ATC lab for the growth of animal cells? Write the name of the instruments which will provide all those conditions. [[CO1](Remember/IOCQ)]
- (2 + 2) + 5 + 3 = 12**
3. (a) Draw and write names three type of animal culture vessels for the following types of animal cell cultivation systems: (i) Static cultivation system, (ii) Semi-dynamic cultivation system, (iii) Dynamic cultivation system. [[CO1](Remember/LOCQ)]
- (b) (i) Explain the principle of flowcytometry and different parts of flowcytometer with a diagram. (ii) Explain the application of flowcytometry to analyse human blood cells based on their size, with standard experimental result. [[CO2](Remember/LOCQ)]
- (c) What are the names of three types of Mammalian cells based on their morphology? Describe the special features of each of the types of mammalian cells with example. [[CO2](Apply/IOCQ)]
- 3 + (3 + 3) + (1 + 2) = 12**

### Group - C

4. (a) Define structured model. [[CO4](Understand/IOCQ)]
- (b) A special type animal cell culture growth data obtained when chemostat Operation were carried out.  
 Doubling time = 2.5 hrs.,  $K_s = 1.2 \text{ kg/m}^3$ ,  $Y_{x/s} = 0.42$ ,  $S_0 = 35 \text{ kg/m}^3$   
 Calculate the following :-----  
 (i) Cell concentration at  $D = \frac{1}{2} D_{\max}$   
 (ii) Substrate concentration at  $D = 0.75 D_{\max}$   
 (iii) Maximum dilution rate for maximum output of cells. [[CO4] (Evaluate/IOCQ)]
- 3 + 9 = 12**
5. (a) With the help of a clean diagram write the mass balance equation for non-ideal CSTR model. [[CO4](Remember/IOCQ)]
- (b) Blood plasma is used for animal cell culture Flowing growth data were obtained from bioreactors
- |                      |      |      |      |      |      |     |      |      |     |
|----------------------|------|------|------|------|------|-----|------|------|-----|
| t, hr.               | 0    | 2    | 4    | 8    | 10   | 12  | 14   | 16   | 18  |
| X, kg/m <sup>3</sup> | 0.2  | 0.2  | 0.3  | 0.98 | 1.77 | 3.2 | 5.6  | 6.15 | 6.2 |
| S, kg/m <sup>3</sup> | 9.23 | 9.21 | 9.07 | 8.03 | 6.8  | 4.6 | 0.92 | 0.77 | 0.0 |
- (i) Find maximum growth rate, (ii) Saturation constant (iii) Mass doubling time, (iv) Yield coefficient. [[CO2](Apply/IOCQ)]
- 4 + (2 × 4) = 12**

## Group - D

6. (a) Write the names different physical, chemical and biological based gene transfer methods into animal cell. Describe the principle and steps of gene transfer into animal by calcium phosphate precipitation method. [[CO3](Remember/LOCQ)]
- (b) Explain the principle of stable and transient transfection in animal cell with labelled diagram. [[CO3](Explain/IOCQ)]
- (c) Explain the steps of production of multimeric proteins in mammalian cell system, based on (i) Two-gene expression vector, with labelled diagram, (ii) bicistronic vector system. [[CO3](Explain/IOCQ)]
- (2 + 2) + (1.5 × 2) + (2.5 + 2.5) = 12**
7. (a) Explain and write the steps of creation of KO mouse using ES cells and through classical HR method with diagram. [[CO5](Understand/LOCQ)]
- (b) Explain in details how ES cell carrying KO gene was selected by positive and negative selection. [[CO5](Analyse/HOCQ)]
- (c) Explain and write the steps of creation of KO mouse using cre-loxP method with diagram. [[CO5](Understand/IOCQ)]
- 5 + 3 + 4 = 12**

## Group - E

8. (a) Why Tissue Engineering (TE) is required? [[CO6](Explain/IOCQ)]
- (b) What is TE triad? Describe different scaffold materials in TE. [[CO6](Remember/LOCQ)]
- (c) What is regenerative medicine? How stem cells can be used as a source of neurons for transplantation in Parkinsonism's disease? [[CO6](Explain/IOCQ)]
- 2 + (2 + 3) + (1 + 4) = 12**
9. (a) A genetic disease causes due to defect in one gene-X. Now, explain and write the steps for curing from this disease to remove the defective gene, by gene therapy using CRISPR-Cas9 with a labelled diagram. [[CO6](Explain/IOCQ)]
- (b) Write three differences between 3-D and 2-D cell culture. [[CO6](Remember/LOCQ)]
- (c) Explain and write all the steps of animal cell culture-based vaccine (inactivated whole virus) production for an animal virus using labelled diagram. [[CO3](Explain/IOCQ)]
- 5 + 3 + 4 = 12**

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	31.25	60.42	8.33

### Course Outcome (CO):

After the completion of the course students will be able to

CO1. Understand the fundamental scientific principles animal cell culture; describe the condition, media, special instruments and laboratory design required for animal cell culture.

CO2. Acquire knowledge for isolation, maintenance, counting, preservation and growth of animal cell; develop proficiency in establishing and maintaining of cell lines.

CO3. Acquire knowledge in animal cloning and its applications.

CO4. Understand and analyze growth kinetics and scale up of animal cell culture. Do analysis and solve problems related to animal cell culture.

CO5. Understand and explain the basics of animal biotechnology and the creation of transgenic animal with the help of modern gene targeting and editing technology.

CO6. Understand and demonstrate the application of animal cell culture and animal biotechnology in production of monoclonal antibody, organ transplantation, production of human and animal viral vaccines and pharmaceutical proteins, gene therapy, stem cell technology.

\*LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question.