

**ANIMAL CELL CULTURE & ANIMAL BIOTECHNOLOGY  
(BIOT 4111)**

**Time Allotted : 3 hrs**

**Full Marks : 70**

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

*Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.*

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

**Group - A  
(Multiple Choice Type Questions)**

1. Choose the correct alternative for the following: **10 × 1 = 10**

- (i) Stem cells are widely used for their regenerative property and capacity to differentiate into different lineages. A person with a damaged liver approaches a stem cell therapist. Which of the following therapeutic strategies would be safest?
- (a) Transplanting adult liver cells from healthy donor and grafting them into patient.
- (b) Transforming skin cells from patient into iPSC and using them for further differentiation and grafting in liver.
- (c) Injecting embryonic stem cell into the damaged liver.
- (d) Injecting cord blood cells into the liver directly.
- (ii) Match different strategy of gene therapy in group-I with their meaning in group-II

<b>Group-I</b>	<b>Group-II</b>
(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.

- (iii) What are the main differences between a cloned animal by SCNT method and an animal which is produced by normal sexual reproduction?  
(a) The SCNT animal has genetic material primarily from two animal instead of one  
(b) There is no significant difference  
(c) The SCNT animal has genetic material primarily from one animal instead of two  
(d) The animal produced by sexual reproduction has genetic material primarily from one animal instead of two
- (iv) Identify the cell culture medium below that normally contains phenol red as an additive to detect pH changes  
(a) LB medium  
(b) Cell culture medium for sf9 cells  
(c) YPD yeast cell culture medium  
(d) Cell culture medium used for mammalian cells.
- (v) Which of the following is not a cryopreservation technique for animal cells or organs?  
(a) Slow programmable freezing  
(b) Vitrification  
(c) Persulfflation (PSF; gaseous oxygen perfusion)  
(d) Thawing
- (vi) The growth medium for mammalian cells contains serum. One of the major functions of serum is to stimulate the cell growth and attachment. However, it must be filter sterilized to  
(a) remove collagen only  
(b) remove mycoplasma and microorganisms  
(c) remove foaming agents  
(d) large lipids and proteins
- (vii) A animal cell bioreactor is operated at a dilution rate of  $0.5 \text{ hr}^{-1}$ . At steady state, the biomass concentration in the exit stream was found to be 20 g/L. Evaluate the biomass productivity in g/L/hr after 3h of steady state operation.  
(a) 18                      (b) 10                      (c) 12                      (d) 20
- (viii) At small substrate concentration Monod model behaves as a reaction of the type  
(a) first order    (b) zero order  
(c) second order    (d) pseudo first order
- (ix) Non-ideality of reactors is determined by  
(a) Reynold's number    (b) Mixing time  
(c) dispersion number    (d) Residence time distribution
- (x) A batch bioreactor is to be scaled up from 10 to 15,000 L. The diameter of the large bioreactor is 15 times that of the small bioreactor. The agitator speed in the small bioreactor is 750 rpm. Evaluate the agitator speed (rpm) of the large bioreactor with the same impeller tip speed as that of the small bioreactor and scale up ratio.  
(a) 50 and 15              (b) 45 and 10              (c) 40 and 15              (d) 45 and 15.

**Group-B**

2. (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 application of flowcytometry to know the stages of mammalian cell cycle like G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub> and M with standard experimental results.  
(ii) Explain the application of flowcytometry to analyse human blood cells based on their size, with standard experimental result. [(CO2) (Understand/LOCQ)]
- (c) What are the names of three types of Mammalian cells based on their morphology? Analyze the special features of each of the types of mammalian cells with example. [(CO2) (Analyze/IOCQ)]
- (1 × 3) + (2.5 + 2.5) + (1 + 3) = 12**
3. (a) Analyze with reasons a special required laboratory animal cell culture? [(CO1) (Analyze/IOCQ)]
- (b) Explain the basic differences in the working principle between horizontal and vertical Laminar Air Flow Hood, with diagram. [(CO1) (Understand/LOCQ)]
- (c) Analyze the design and principle for the storage animal cells are stored in the laboratory and write steps of storage for animal cells. [(CO2) (Explain/IOCQ)]
- 2 + (3 + 3) + 4 = 12**

**Group - C**

4. (a) Why do you assume steady state situation to derive Monod chemostat model? [(CO4) (Analyze / IOCQ)]
- (b) The following set of growth data is available for a new recombinant animal cell line.

t, (hr)	0.0	0.5	1.0	1.5	2.0
X, dry cell mass (gm/lit.)	0.1	0.15	0.23	0.34	0.51

The above set of data is to be fitted to the model as given below

$$(dx/dt) = K X (1 - \beta X)$$

Where,  $\beta = (1/ X_s)$  and  $X_s$  is the cell mass concentration in the stationery phase. Evaluate the constant K and  $\beta$ . [(CO4) (Evaluate/HOCQ)]

**2 + 10 = 12**

5. (a) How do you avoid washout condition ? [(CO4) (Understand/LOCQ)]
- (b) The specific growth rate for an inhibited growth in a chemostat is given by the following equation  $\mu_g = [(\mu_{max} S) / \{Ks + S + (I K_s / K_I)\}]$ . [(CO4) (Evaluate/HOCQ)]

- i) Determine X & S as a function of "D" when I = 0
- ii) With inhibitor added to a chemostat, determine the effluent substrate concentration and "X" as a function of "D".  
Determine the cell productivity DX as a function of dilution rate.

**2 + 10 = 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-vector expression system and (ii) Two-gene expression vector, with labelled diagram. [(CO3) (Explain/IOCQ)]

**(2 + 3) + (1.5 + 1.5) + (2 + 2) = 12**

7. (a) Write the names of three types of strategy used for animal cloning. Explain the strategy for animal cloning which was used for creation of 'Noori' with diagram. [(CO3) (Remember/LOCQ)]
- (b) Write the names three chemical reagent techniques for gene transfer into animal cells. Explain any one of the techniques for gene transfer into animal cells, from those you mentioned, with rational and diagram. [(CO3) (Explain/IOCQ)]
- (c) Explain the transfection of gene into animal cells based on retroviral vector with diagram. [(CO3) (Explain/IOCQ)]

**(1 + 3) + (1 + 3) + 4 = 12**

### **Group - E**

8. (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) (Understand/LOCQ)]
- (b) Write three differences between 3-D and 2-D cell culture. [(CO6) (Differentiate/IOCQ)]
- (c) Briefly analyze all the steps of animal cell culture-based vaccine (inactivated whole virus) production for an animal virus using a labelled diagram. [(CO3) (Analyze/IOCQ)]

**5 + 3 + 4 = 12**

9. (a) Explain generation of human induced pluripotent stem cells for use in cell therapy. [(CO6) (Explain/IOCQ)]

- (b) Analyze the application of stem cells for the treatment of hemophilia and diabetes mellitus. [(CO6) (Explain/LOCQ)]
- (c) Describe the process of cell fusion by virus mediated and by electrofusion method in the production of monoclonal antibody along with a labelled diagram. [(CO6) (Explain/IOCQ)]
- 4 + 4 + (3 + 1) = 12**

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	31%	48%	21%

**Course Outcome (CO):**

After the completion of the course students will be able to:

1. Understand the fundamental scientific principles animal cell culture; describe the condition, media, special instruments and laboratory design required for animal cell culture.
2. Acquire knowledge for isolation, maintenance, counting, preservation and growth of animal cell; develop proficiency in establishing and maintaining of cell lines.
3. Acquire knowledge in animal cloning and its applications.
4. Understand and analyze growth kinetics and scale up of animal cell culture. Do analysis and solve problems related to animal cell culture.
5. Understand and explain the basics of animal biotechnology and the creation of transgenic animal with the help of modern gene targeting and editing technology.
6. 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

Department & Section	Submission Link
BT	<a href="https://classroom.google.com/c/NDA0NzExNTQ1Nzc4/a/NDY0MjM0MzZM5NDY2/details">https://classroom.google.com/c/NDA0NzExNTQ1Nzc4/a/NDY0MjM0MzZM5NDY2/details</a>