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) 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

(ii) Disaggregating of cells can be performed by
(a) Physical disruption (b) Enzymatic digestion
(c) Treating with chelating agents (d) All of the above.

(iii) At small substrate concentration Monod model behaves as reaction of the type.
(a) first order (b) pseudo first order
(c) second order (d) zero order 4

(iv) Perfusion system is used for the production of
(a) antibiotics (b) alcohol
(c) monoclonal antibody (d) single cell protein

(v) The approach in which genes are transferred into animals to obtain a large-scale production of the proteins encoded by these genes in the milk, blood, etc., is called
(a) In situ culture (b) Molecular pharming
(c) Gene therapy (d) Hybridoma technology.

(vi) Which one of the following techniques is generally used to produce transgenic animals?
(a) Processed mRNA containing only exons are introduced into blastocyst stage embryo.
(b) Entire foreign nucleus is introduced in enucleated unfertilized egg.
(c) Desired DNA is injected into fertilized eggs followed by implantation of embryo in foster embryo in a foster mother.
(d) cDNA of desired gene is introduced into animal embryos and implanted in a foster mother these

(vii) 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.

(viii) First transgenic animal to win US approval for food in 04 August 2017, is
(a) AquAdvantage' salmon (b) *Cyprinus carpio*
(c) Tilapia (d) Trout.

(ix) 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.

(x) Monoclonal antibodies can be modified for better research and therapeutic applications. Several such approaches are mentioned below in Group-I and Group-II.

Group-I	**Group-II**
(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.

Fill in the blanks with the correct word

(xi) In Monod Chemostat Model Ss_{terile} = _____is substrate concentration.

(xii) In microbial growth kinetics, Specific growth rate is defined as, μ = _____.

(xiii) FACS is a cell separation device based on _____ sorting.

(xiv) The colour change of animal cell culture media from Pink to _____ indicate the medium should be changed.

(xv) The principal of staining of animal cell with Trypan blue by dye _____ method.

Group - B

2. (a) Write the names of different parameters required for optimal growth of animal cell culture. *[(CO3)(Analyse/HOCQ)]*

   (b) What are basal media and complete media? Give one example of each. *[(CO4)(Remember/LOCQ)]*

   (c) Write the names of different serum components and their role in animal cell culture. Why heat inactivation of serum is done before using in the media? Why more than one freeze-thaw cycle after heat-inactivation is not recommended*?* *[(CO2)(Apply/IOCQ)]*

   (d) Write the names of three animal cell lines. Write three suitable suppliers name for getting animal cell lines. *[(CO2)(Apply/IOCQ)]*

2 + (2 + 1) + (3 + 1 + 1) + (1 + 1) = 12

3. (a) Write the names of three sources from where you can get the animal cell. Write about character of HeLa and $_3T_3$ cells. *[(CO1)(Remember/HOCQ)]*

   (b) Write three differences between adherent culture and suspension culture. *[(CO1)(Analyse /IOCQ)]*

   (c) Explain density dependent contact inhibition and anchorage dependency of primary cultures. *[(CO1)(Explain/IOCQ)]*

   (d) Write the different methods to transform a primary cell into transformed cell. *[(CO2)(Apply/IOCQ)]*

(1.5 + 1.5) + 3 + 3 + 3 = 12

Group - C

4. (a) Derive the following relation from Monod Model chemostat operation.
   D = μ, D = dilution rate, μ = sp. Growth rate. *[(CO4)(Analyse/LOCQ)]*

   (b) Animal cells are growing in a synthetic medium in a chemostat operation. Given data: Reactor volume V = 10 m^3 , with a sterile feed , flow rate F = 5 m^3 /hr., S_0 = 10 kg / m^3, $Y_{X/S}$ = 0.7, μ_{max} = 0.935 hr^{-1}, K_S = 0.70 kg/m^3 . (i) What will be the doubling time? (ii) What will be the exit concentration of cells and substrate? (iii) If one more 10 m^3 chemostat is connected, what will be the exit concentration of X and S from the second chemostat? *[(CO4)(Evaluate))/HOCQ)]*

3 + (3 + 3 + 3) = 12

5. The following data were obtained in a steady state chemostat operation of a specific animal cells (X) Producing antibody (P) from glucose.

D, (I/hr.)	0.084	0.10	0.16	0.198	0.242
S_0 , Kg/m^3	21.5	10.9	21.2	20.7	10.8
S, Kg/m^3	0.054	0.079	0.138	0.186	0.226
P, Kg/m^3	7.97	4.7	8.5	8.44	4.51
X, kg/m^3	2.0	1.2	2.4	2.33	1.25

(i) Find the rate equation for cell growth. (ii) Find out the dilution rate for maximum cell output (D_{max}) for the set –I and the dilution rate for washout ($D_{washout}$) for S_0 = 21.5 kg/m^{-3}. (iii) Find the rate for product (antibody, P) formation from the first set of data. *[(CO4)(Evaluate)/IOCQ)*

(4 + 4 + 4) = 12

Group - D

6. (a) Explain and write the steps of creation of KO mouse using ES cells and through classical HR method with diagram. *[(CO5)Explain/HOCQ)]*
   (b) Explain in details how ES cell carrying KO gene was selected by positive and negative selection. *[(CO5)(Remember/IOCQ)]*
   (c) Explain and write the steps of creation of KO mouse using cre-loxP method with diagram. *[(CO5)(Apply/IOCQ)]*

4 + 4 + 4 = 12

7. (a) Describe the steps of IVF with label diagram. *[(CO5)(Analyse/HOCQ)]*
   (b) What is chimera? Explain gene targeting and gene trapping. *[(CO5)(Remember/LOCQ)]*
   (c) Explain the mechanism of control gene expression in animal expression vector system by Tet Off and Tet On system. *[(CO5)(Apply/HOCQ)]*

4 + (1 + 3) + 4 = 12

Group - E

8. (a) Explain generation of human induced pluripotent stem cells for use in cell therapy. *[(CO6)(Analyse/HOCQ)]*
   (b) Explain the application of stem cells for the treatment of hemophilia and diabetes mellitus. *[(CO6)(Remember/LOCQ)]*
   (c) Explain the process of cell fusion by virus mediated and by electrofusion method in the production of monoclonal antibody, with label diagram. *[(CO6)(Apply/IOCQ)]*

4 + 4 + 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. *[(CO6)(Apply/IOCQ)]*

5 + 3 + 4 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	17.71	51.04	31.25