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) Cell lines that can divide a particular number of times until they reach _____ are called _____.
(a) pluripotency, continuous cell line (b) senescence, continuous cell line
(c) pluripotency, finite cell lines (d) senescence, finite cell lines

(ii) Which of the following gene editing tool is not a nuclease?
(a) ZFN (b) TALEN (c) Cre-Lox (d) CRISPER/Cas9.

(iii) In cell culture, the extracellular matrix coating on a substrate is required for
(a) Adherent cells (b) Non-adherent cells
(c) Immune cells (d) All of the above.

(iv) DNA vaccine offer several advantages over other existing vaccine approaches. Which one of the following statements related to DNA vaccine is not correct?
(a) The immune response is directed to the antigen encoded by the DNA and able to induce both humoral and cell mediate immunity
(b) DNA vaccine can induce prolonged expression of the antigen, enhancing the induction of immunological memory
(c) DNA vaccine could remain stable and potent for long time without refrigeration, eliminating the challenges of storage and transportation
(d) DNA vaccine construct can be engineered to carry several antigens to infect host and replicate in neuronal cells.

(v) Presence of which of the following will disqualify a culture medium as defined
(a) known quantities of growth factors (b) Phosphate buffered saline
(c) Serum (d) Vitamin A.

(vi) If I take 10 µl of animal cell suspension and mix it with 10 µl of trypan blue. Of this I load 10 µl into the hemocytometer. I get 25, 26, 22, 27 cells in 4 corner squares (indicated with red boundary in image), the cell count is
(a) 10,000 cells/ml (b) 50,000 cells/ml
(c) 100,000 cells/ml (d) 500,000 cells/ml.

(vii) The technique used in animal biotechnology for the rapid multiplication and production of animals with a desirable genotype is
(a) protoplast fusion and embryo transfer
(b) hybrid selection and embryo transfer
(c) in vitro fertilization and embryo transfer
(d) all the above.

(viii) Graphical plot of Boyle's law (PV = const.) will be a
(a) parabola (b) hyperbola
(c) rectangular hyperbola (d) straight line.

(ix) Which of the following complete set(s) of products are commercially produced using bioreactors?
(a) Insulin, milk, petrol, bio-oil (b) Insulin, mAbs, bio-ethanol, yogurt
(c) mAbs, milk, bio-oil, curd (d) mAbs, insulin, petrol, bio-oil.

(x) Match the chemicals in Group-A with their purpose in Group-B

Group-A	Group-B
1. Liquid N_2	(i) Cryo-protectent
2. DMSO	(ii) pH indicator
3. Phenol red	(iii) Longtime preservation
	(iv) DO estimation

Which one of the following options represents correct match of Group-A and Group-B?
(a) 1-i, 2-iv, 3-iii (b) 1-iv, 2-ii, 3-iii (c) 1-ii, 2-iii, 3-i (d) 1-iii, 2-i, 3-ii.

Group–B

2. (a) What are the different types of methods used for disaggregation of animal tissue or organ fragment? What are different enzymes used for disaggregation of animal tissue or organ fragment? [(CO1)(Remember)/LOCQ)]
(b) Describe briefly the procedure involved in warm and cold trypsinization for the preparation of primary culture from animal tissue or organ fragment. Write three important advantages of cold trypsinization over warm trypsinization. [(CO2)(Understand)/IOCQ)]
(c) Explain the principle of MTT assay for testing of cytotoxicity of chemical X for animal cell culture. [(CO5)(Explain)HOCQ)]
(2 + 2) + 5 + 3 = 12

3. (a) Write names three techniques for authentication of animal cell line. Explain principle and steps of the cell line authentication technique based on CD markers, with diagram. [(CO2)(Remember/LOCQ)]
(b) Explain the principle and steps of viable cell counting by the following: (i) viable cell assay by methylene blue (ii) viable cell assay by dye uptake method. [(CO3)(Understand/IOCQ)]
(c) (i) Explain the application of flow cytometry to know the stages of mammalian cell cycle like G_0, G_1, G_2 and M with standard experimental results.

(ii) Explain the application of flow cytometry to analyse human blood cells based on their size, with standard experimental result. [(CO2)(Explain)/HOCQ]

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

Group – C

4. (a) Monod model is an empirical model – justify the statement. [(CO4)(Evaluate)/IOCQ]

(b) A cell mass growth model is given by the following equation:

$dx/dt = [\mu_{max}. S. X]/ [K_s + S + i. K_s/K_i]$

Where, S_0=10 gm/L, X_0=0, Ks=1 gm/L. K_i= 0.01 gm/L, i (inhibitor concentration) = 0.05 gm/L, $\mu_{max} = 0.5\ hr^{-1}$, $Y_{X/S}$ = 0.1 gm cells/ gm substrate.

Do the following graphical plot:

(i) Plot X and S vs D

(ii) Plot X and S vs D when I = 0. [(CO4)(Evaluate)/HOCQ]

2 + 10 = 12

5. (a) Why structured model is better to understand the cells growth? [(CO4)(Explain/IOCQ)]

(b) A chemostat study was performed with the help of a hybridoma cell lines by varying the medium flow rates. The following data were obtained with initial glucose concentration (S_0 = 100 gm/lit) and the volume of the fermenter was 500 ml. The inlet stream was sterile:

F, flow rate, ml/hr.	31	50	71	91	200
X, gm/lit.	5.97	5.94	5.88	5.76	0.0
S, gm/lit.	0.5	1.0	2.0	4.0	100.00

(i) Find the rate equation for cell growth.

(ii) What should be the range of flow rate to prevent wash-out of the cells? [(CO4)(Evaluate/HOCQ)]

2 + 10 = 12

Group - D

6. (a) Describe the steps of IVF with labelled diagram. [(CO5)(Remember)LOCQ)]

(b) What are chimera? Explain gene targeting and gene trapping. [(CO5)(Define/Explain)/IOCQ)]

(c) Explain four types of Inhibitory "Anti-Gene" expression strategies as therapeutic agents. [(CO5)(Explain)/HOCQ)]

4 + (1 + 2 + 2) + 3 = 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 Parkinson'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/HOCQ)]
   (b) Write three differences between 3-D and 2-D cell culture. [(CO6)(Analyse/IOCQ)]
   (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)(Explain/IOCQ)]

5 + 3 + 4 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	27	32.3	40.6

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