University of Sadat City	Final Exam:	Methods in Immunology II	Course Code:	C-63	Percentage	60%	GEBRI
	Academic Year:	2018/2019	Academic Program:	Molecular Biology	N. of Exam Paper	2	
	Level:	Master	Department:	Molecular Biology			
	Course Name:	Methods in Immunology II	Total score:	60	Time allowed:	3 hrs	

Instructions of Exam:

1. Answer the obligatory questions.
2. Use the blue pen and pencil in answer sheet
3. Allow one sheet answer for every student
4. Is not allowed to borrow the tools (pen, pencils, drawing tools, calculator …etc)
5. Is not allowed to use the cell phone or any of its application during the time of exam

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Answer the following questions illustrating your answers with drawings

1. You inject a rabbit on several occasions with pure human serum albumin (HSA), collect some blood from the rabbit and allow it to clot. The yellowish liquid remaining is your antiserum. **(15 degree)**

a. Why did you give several injections of antigen?

b. Are all the antibody molecules in the serum specific for HSA?

c. How can you separate the anti-HSA from the other molecules (purify it)? How could you check the reactivity of your separated antibody?

d. After purification, you measure the binding of your antiserum to human serum albumin (HSA), human immunoglobulin (Hlg), rabbit serum albumin (RSA), and chimpanzee serum albumin (CSA). The results are shown

Antigen	Binding units
HSA	100
Hlg	1
RSA	1
CSA	90

e. Why should an antibody to HSA bind CSA? From amino acid sequencing, HSA and CSA are about 90% homologous (have the same amino acids at about 90% of the sequence) and HSA and RSA are about 45% homologous. Why does the antiserum show so little binding to RSA? Are

all epitopes of an antigen equally immunogenic? Predict the results if the antiserum had been made in a chimp.

f. If you need a monoclonal not a polyclonal antibody how could you prepare it?

2. Give a hand to help your friend to: (25 degree)

2.1. Prepare 100 ml of 0.1M Tris-buffer (Stock concentration is 2M).

2.2. Prepare 100 ml RPMI-1640 containing: 2% L-glutamin and 5% FBS

2.3. Dilute 50 ml 10% SDS to 3%

2.4. Prepare 100 ml 1M HCl (M.Wt. 36.5 and bottle concentration 37%).

2.5. Total number of cells if you have 10 ml and count on haemocytometer 150 cells (in 16 square)Adjust the count at 50,000 cell/well (100 µl cells /well)

2.6. How could you separate $CD4^+$ and $CD8^+$ cells from a blood sample? How could you examine their response to any drug? If you have only one antibody but coupled with magnetic beads how you could separate both types of cells? If you want to be sure from your separation and you have fluorescent -anti-CD4 Ab and anti-CD8? what you can do.

2.7. Concentrate a protein using dialysis bag

Give short notes about the following: (15)

a. Detection of cytokines by ELISA

b. PBMC preparation using Ficoll hypaque

c. Dialysis and Gel filtration as simple methods for protein purification.

d. Preparation on Monoclonal Ab