PhD. Entrance Examination, June 2017

Time: 2 hrs Code No – P-65 (PhD – Biochemistry) Max Marks: 80

Please read the following instructions carefully before answering:

1. Enter Hall Ticket number in the space provided above and also on OMR sheet.
   Part A contains 40 questions on biology, each question carries one mark. Part B contains 25 questions on research methodology. Questions number 41 to 50 carries one mark each. Questions number 51 to 65 carries two marks each.
3. There is no negative marking in any of the parts.
4. Answers have to be marked on the OMR sheet as per the instructions provided.
5. Apart from OMR sheet, the question paper contains 17 (Seventeen) pages including the instructions.
6. Please return the OMR answer sheet at the end of examination.
7. No additional sheet will be provided.
8. Rough work can be carried out in the question paper itself in the space provided at the end of the booklet.
9. Non programmable calculators are allowed.

PART A

[Each question has only one right answer. Mark the right answer. Each question carries one mark. There is no negative marking.]

1. Which of the following pair are unsaturated fatty acids?
   A. Oleic acid and stearic acid
   B. Palmitic acid and linoleic acid
   C. Myristic acid and oleic acid
   D. Oleic acid and linolenic acid

2. The relation of D-Glucose with D-Galactose and D-Mannose are respectively
   A. C-4 anomer and C-2 anomer
   B. C-4 epimer and C-2 epimer
   C. C-2 epimer and C-4 epimer
   D. C-2 anomer and C-4 anomer
3. The difference in structure between dCMP and CMP is
   A. CMP has 2'-OH group while dCMP does not
   B. dCMP has 2'-OH group while CMP does not
   C. CMP has a purine base while dCMP has pyrimidine
   D. dCMP has one phosphate group while CMP has three

4. Assuming the oligopeptide PLVEANDWHITE forms one continuous α-helix, the carbonyl oxygen of the asparagine residue is hydrogen bonded to the amide nitrogen of which of the following residues?
   A. Leucine.
   B. Alanine.
   C. Isoleucine.
   D. Histidine.

5. Which of the following is true about enzymes
   A. All enzymes contain a cofactor
   B. Without the cofactor, the enzyme is called a holoenzyme
   C. Enzymes increase the rate of a reaction
   D. There are five main classes of enzymes

6. Which of the following is incorrect about the function of the corresponding cofactor
   A. NAD⁺ as an oxidizing agent
   B. Pyridoxal phosphate helps in transfer of amino group
   C. Tetrahydrofolate transfers a two carbon unit
   D. Biotin helps in carboxylation

7. The first component of Complex I of electron transport chain that receives electrons from NADH + H⁺
   A. Fe-S cluster
   B. FAD
   C. FMN
   D. CoQ

8. How many ATP molecules are generated for one complete rotation of motor subunits of FoF1 ATP synthase?
   A. 2.5
   B. 4
   C. 3
   D. 3.5

9. Which of the following statements is correct?
   A. Phosphorylated glycogen synthase is the active form of the enzyme
   B. Unphosphorylated glycogen synthase is the active form of the enzyme
   C. Glycosylated glycogen synthase is the active form of the enzyme
   D. Monomeric glycogen synthase is the active form of the enzyme
10. Pyruvate decarboxylase and pyruvate dehydrogenase both contain
   A. Biotin
   B. Pantothenic acid
   C. Thiamine pyrophosphate
   D. Pyridoxal phosphate

11. Conversion of ribonucleic acid to deoxyribonucleic acid requires primarily
   A. Hydrolysis
   B. Reductase system
   C. Replacement of ribose by deoxyribose
   D. Oxidase system

12. Which of the following sequence of products formed in urea cycle is correct
   A. Citrulline----Arginosuccinic acid-----Arginine----Urea
   B. Arginosuccinic acid----Citrulline-----Arginine ----Urea
   C. Homocitrulline---Arginosuccinic acid---Arginine---Urea
   D. Citrulline---Arginine--Argino succinic acid---Urea

13. The activity of which of the following DNA polymerase is template independent?
   A. Reverse Transcriptase
   B. Telomerase
   C. DNA Polymerase I
   D. Terminal transferase

14. Which of the promoter in lambda phage expresses genes for recombination as well as
    antiterminator N and the cIII Protein?
   A. \( P_{RM} \)
   B. \( P_R \)
   C. \( P_R' \)
   D. \( P_L \)

15. Polynucleotide phosphorylase is an enzyme that has been used primarily to
   A. Synthesize artificial DNA
   B. Synthesize artificial RNA
   C. Hydrolyze DNA
   D. Hydrolyze typically tRNA

16. The energy for the addition of an incoming monomer to a growing polypeptide comes from
   A. ATP hydrolysis
   B. GTP hydrolysis
   C. From the hydrolysis of the incoming monomer
   D. From the last monomer of the growing polypeptide
17. Which of the following techniques can not be used to study the loss of gene function?
A. RNAi technology
B. CRISPR-cas9 technology
C. Homologous recombination mediated gene knock in
D. Zinc Finger Nuclease (ZNF) technology

18. Which of the following method is best suited to study the de novo synthesis of mRNA?
A. Real-time RT-PCR
B. Nuclear run-on assay
C. Nuclear run-off transcription assay
D. Northern hybridization

19. Which of the following techniques is best suited to study transcription?
A. DNase foot print
B. EMSA
C. Luciferase assay
D. S1 nuclease mapping

20. Hybridization of single-stranded RNA or DNA is facilitated by
I. high temperature
II. low temperature
III. high salt
IV. low salt
A. Options I and III are correct
B. Options I and IV are correct
C. Options II and III are correct
D. Options II and IV are correct

21. Fragmented DNA from a bacterial strain of genotype trp* leu* arg* str* ura* was transformed into a trp' leu' arg' str' ura' bacteria. Transformants of the following genotypes could be recovered.
I. trp* str'
II. arg' ura'
III. str' leu'
IV. trp* ura'
The most likely order of genes on the bacterial chromosome is:
A. str' -leu- arg -trp- ura
B. trp- str' -arg- ura- leu
C. trp-ura-arg- str'- leu
D. arg- ura- trp- str'- leu

22. In cattle, heterozygous condition for Black (C^B) and White (C^W) produces a grey coat colour. If two grey cattle are mated, what is the expected frequency of parental coat colour?
A. 25%
B. 50%
C. 75%
D. 100%
23. A transition mutation in a gene X introduced a premature stop codon. In order to obtain wild
    type product, additional mutagenesis was carried out and wild type products of gene X were
    obtained. Which of the following is most likely to have occurred to produce the wild type
    product?
    A. Insertion of a single base pair
    B. Deletion of a single base
    C. tRNA suppressor mutation
    D. Deletion of two nucleotides

24. In coffee plants, red berry colour (R) is dominant over yellow colour (r) and round (W) is
dominant over wrinkled (w). The two traits are on different chromosomes. What are the types of
gametes produced by a heterozygous plant that produces round red berries?
    A. RW, Rw, wr in equal proportions
    B. RW, Rw, wr in the ratio of 2:1:1
    C. Rw and wr in equal proportions
    D. RW and wr in equal proportions

25. In electrophoresis, the electrophoretic mobility (μ) determines the characteristics of
migration of different biomolecules. Which of the following is not having any influence on μ?
    A. Size of molecules
    B. Stereochemistry of molecules
    C. Shape of molecules
    D. Net charge of molecules

26. Select the true statement about fluorescence spectroscopy of molecules in the UV-visible
region.
    A. Emission usually occurs at energies that are greater than the energies of excitation
    B. Emission usually occurs at energies that are less than the energies of excitation
    C. Emission usually occurs at energies that are equal to the energies of excitation.
    D. None of the above is accurate

27. Which of the following is not a multiple sequence alignment tools?
    A. CLUSTAL OMEGA
    B. MUSCLE
    C. T-COFFEE
    D. AUGUSTUS

28. A research scholar raised antibodies against the idiotypic determinants of a human IgG. She
then checked for the specificity of this antibody in an ELISA based assay. She observed that
some of the wells of ELISA plate showed cross reactivity. Given below are the antigens that she
used to check cross reactivity of this antibody preparation with. With which all of the following
this antibody may show cross-reactivity?
    A. Whole IgM produced by the same plasma cell that produced the IgG.
    B. The Fc part of papain digested IgG produced by the same plasma cell that produced the IgG.
    C. The kappa chain of IgM produced from another plasma cell
    D. The Fab part of papain digested IgG produced from another plasma cell
29. The tool used to perform sequence alignment of a protein amino acid sequence against a protein database is called:
A. BLASTp
B. BLASTn
C. TBLASTn
D. None of these

30. Which two of the following statements are true?
   I. PAM100 and BLOSUM100 amino acid substitution matrices are comparable
   II. PAM100 and BLOSUM90 amino acid substitution matrices are comparable
   III. Smith-Waterman algorithm is used for global alignment
   IV. Smith-Waterman algorithm is used for local alignment
A. I and III,
B. II and IV
C. I and IV
D. II and III

31. The sedimentation coefficient of a particular DNA molecule is 22 S. How far will DNA molecule move at 40,000 rpm in 20 min at a distance of 6.0 cm from the axis of rotation?
A. 1.75 cm
B. 0.28 cm
C. 0.38 cm
D. 2.80 cm

32. Which of the following component(s) of complement could be missing and still with the remainder of the complement proteins the alternative pathway of the complement system can be activated?
A. C1, C2, and C3
B. C3 only
C. C2, C3, and C4
D. C1, C2, and C4

33. You injected H-2\textsuperscript{k} mouse with LCM virus and collected the cytotoxic T cells (Tc-cells) and macrophages from spleen. The macrophages (MΦ), either treated with IFN-γ or IL-10, were then co-cultured with the isolated Tc-cells in combination with (i) uninfected and LCM virus infected fibroblast cells from H-2\textsuperscript{k} mouse and (ii) uninfected and LCM virus infected fibroblast cells from H-2\textsuperscript{b} mouse. Which combination of co-cultured cells will have the \textit{highest} target cell lysis by Tc-cells?
A. MΦ treated with IFN-γ + Tc-cells co-cultured with LCM virus infected fibroblast from H-2\textsuperscript{b} mouse.
B. MΦ treated with IFN-γ + Tc-cells co-cultured with LCM virus infected fibroblast from H-2\textsuperscript{k} mouse.
C. MΦ treated with IL-10 + Tc-cells co-cultured with LCM virus infected fibroblast from H-2\textsuperscript{b} mouse.
D. MΦ treated with IFN-γ + Tc-cells co-cultured with uninfected fibroblast from H-2\textsuperscript{k} mouse.
34. If you require to test for congenital absence of a complement component, which of the following screening tests will be most suitable?
A. Quantitation of serum opsonic activity
B. Quantitation of serum hemolytic activity
C. Quantitation of C4 content of serum
D. Electrophoretic analysis of patient's serum

35. The following feature does not represent apoptosis
A. Pycnosis
B. Activation of PARP protein
C. Cytochrome C release from the mitochondria
D. Bax translocation to mitochondria

36. Increased levels of human chorionic gonadotropin hormone (hCG) during first trimester of pregnancy is to
A. Control the pregnancy by regulating estrogen secretion
B. Maintain progesterone synthesis
C. Diagnose the pregnancy
D. Enhance the oxygen supply to the fetus

37. All the following physiological stress conditions stimulate vasopressin release into blood circulation EXCEPT for
A. Increased osmolality in the plasma
B. Hypovolemia
C. Decreased body temperature
D. Fall in blood volume

38. Aging men is associated with the following physiological manifestations EXCEPT for
A. Increased bone resorption
B. Sarcopenia
C. Muscle degeneration
D. Increased androgen secretion

39. The brown color of faces is due to which pigment
A. Stecobilirubin
B. Stercobilinogen
C. Stecobilin
D. Stercobilin

40. Inappropriate ADH secretion can be associated with
   I. Diabetes mellitus
   II. Diabetes incipidus
   III. Hyponatremia
   IV. Water intoxication
PART B

[Each question has only one right answer. Mark the right answer. Questions from 41 to 50 carry one mark each and 51 to 65 carry two marks each. There is no negative marking.]

41. P and Q together can complete a job in 8 days and 16 days respectively. They work on alternative days with Q starting the job. In how many days will the job be completed?
A. 9
B. 10
C. 11
D. 12

42. If,
   I. ‘min fin bin gin’ means ‘trains are always late’;
   II. ‘gin din cin hin’ means ‘drivers were always punished’;
   III. ‘in cin vin rin’ means ‘drivers stopped all trains’; and
   IV. ‘din kin fin vin’ means ‘all passengers were late’.

   ‘Drivers were late’ would be written as:
A. min cin bin
B. fin cin din
C. fin din gin
D. gin hin min

43. A clock is set right at 5 am. The clock looses 16 minutes in 24 hours. What will be the right time when the clock indicates 10 pm on the 3rd day?
A. 11:15 pm
B. 11 pm
C. 12 pm
D. 12:30 pm

44. Find the missing number in the following figure.

A. 36
B. 38
C. 40
D. 42
45. A large cube is painted on all six faces and then cut into a certain number of smaller but identical cubes. It was found that among the smaller cubes, there were eight cubes which have no face painted at all. How many small cubes have exactly two faces painted?

A. 6  
B. 12  
C. 18  
D. 24

46. What is the standard deviation for the following series of numbers?

\[4, 5, 2, 6, 4, 3, 4\]

A. 10  
B. 1.66  
C. 1.20  
D. 1

47. How many triangles are there in the following figure?

A. 10  
B. 11  
C. 12  
D. 13

48. The angle sum of all interior angles of a convex polygon having seven sides is

A. 180°  
B. 540°  
C. 630°  
D. 900°

49. If \(y=x^e\) then \(\frac{dx}{dy}\) is ______; \(e^x\) means \(\text{exp}(x)\)

A. \(x/y\)  
B. \(1/x\)  
C. \(1/\ln x\)  
D. \(1/y\)

50. Which number is not the mean, median, or mode of the data set 4, 3, 15, 11, 3, 8, 7, 5?

A. 7  
B. 3  
C. 6  
D. 5
51. You attempted to sequence a DNA fragment with the Sanger dideoxy method and obtained a gel as depicted below. You observed that the A sequence lane contains few bands at the bottom of the gel and a large “blob” of radioactivity at the top of the gel. The other three lanes (G, T and C) appeared normal.

What could be the reason behind the abnormal A lane?
A. The processivity of DNA pol is high.
B. The fidelity of DNA pol is low.
C. The concentration of ddATP is too low.
D. The concentration of ddTTP is too high.

52. You wanted to knockout your favourite gene (YFG) by two sites homologous recombination that would allow you to replace gene YFG with a selectable marker KANMX. After generating the knockout strain you wanted to confirm it by Southern hybridization using the entire KANMX DNA fragment as the probe. For Southern blot you have digested the genomic DNA isolated from the KO strain by a restriction enzyme (RE) whose sites are indicated as vertical bars in the accompanying figure. You have noted that there is one RE site within YFG and two RE sites flanking YFG. There is a RE site in the KANMX selectable marker gene. The spacing between the RE sites and the sizes of YFG and KANMX are given.

Which of the following banding pattern would you expect on the Southern blot?
A. 5 kb and 3 kb bands.
B. 4.5 kb and 3.1 kb bands.
C. 4.1 kb and 3.5 kb bands.
D. 1 kb and 0.6 kb bands.

53. DNA sizes can be determined by gel electrophoresis. A DNA size standard and a DNA of unknown size are run in two lanes of a gel, depicted schematically. The migration of DNAs of known size in the standard is graphed to create a standard curve.
Which of the following graphs is most suitable for determining the size of DNA?

A.  
\[ \text{bp} \quad \text{Migration distance (cm)} \]

B.  
\[ \text{bp} \quad \text{Migration distance (cm)} \]

C.  
\[ \log(\text{bp}) \quad \text{Migration distance (cm)} \]

D.  
\[ \log(\text{bp}) \quad \text{Migration distance (cm)} \]

54. DNA repair pathways can be activated when an organism is subjected to mutagen which causes DNA damage. The type of repair pathway activated depends upon the kind of DNA damage caused. The preliminary idea of which mutagen leads to activation of which DNA repair pathway can be elucidated by quantifying the change in expression of gene known to be involved in that particular pathway. So to find out which mutagen is responsible for activation of which repair pathway, organism was subjected to three different mutagen and the expression level of gene G1 involved in repair pathway R1 and G2 involved in repair pathway R2 was monitored through real time PCR.
Given below is the \( C_t \) value obtained though real time PCR for gene involved in repair pathway in control and experimental conditions.

<table>
<thead>
<tr>
<th>Gene (G1)</th>
<th>Control</th>
<th>Mutagen (M1)</th>
<th>Mutagen (M2)</th>
<th>Mutagen (M3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>26</td>
<td>31</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Gene (G2)</td>
<td>16</td>
<td>8</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>House Keeping gene</td>
<td>24</td>
<td>18</td>
<td>26</td>
<td>20</td>
</tr>
</tbody>
</table>

On basis of above value predict which of the following statement made is correct
A. M1 leads to activation of repair pathway R2 since expression of G2 is up-regulated 4 folds.
B. M1 leads to activation of repair pathway R2 since expression of G2 is up-regulated 2 folds.
C. M2 leads to activation of only repair pathway R1 since expression of G1 is up-regulated 8 folds and pathway R2 is not activated as expression of G2 remains unchanged.
D. M3 leads to activation of repair pathway R1 since expression of G1 is up-regulated 2 folds.

55. Protein moon (M) and protein night (N) are known to co-localize into nucleus upon cellular stress. In silico studies also provided hint pointing that they may interact with each other. Co-immunoprecipitation (CoIP) was used to check interaction between protein M and protein N. In order to perform CoIP protein M and protein N was tagged with 6X-HA and Ty respectively and transfected into the cells. CoIP was done with cell lysate (I) in the presence of anti-HA antibody and (II) in the presence of pre-immune IgG (that acts as a negative control). Blots were probed with anti-Ty and anti-HA antibody as shown in the accompanying figure.

![Co-IP figure]

Which is the correct inference drawn on the basis of above western blot and description?

A. Protein N gets precipitated with protein M when pull down was done using Anti-HA antibody, proving that they interact with each other.
B. Protein M gets precipitated with protein N when pull down was done using Anti-Ty antibody, proving that they interact with each other.
C. Protein M and protein N do not interact with each other.
D. Interaction between the two proteins is not strong enough to be detected by Co-IP.
56. In a particular group of butterfly it was observed that most of them have bright orange coloured wings but few among them are unable to develop the orange colour in their wings and have white coloured wings. When their gene expression profile was compared it was found that butterfly with white wings failed to express the gene CLR known to be involved in pigment synthesis. Then it was hypothesized that differential epigenetic modification of that gene might be the possible cause. To test this hypothesis Chromatin Immunoprecipitation (ChIP) was done using following antibodies:

1: H3K9me3 (known repressive mark)
2: H3K27me (known repressive mark)
3: H3K9ac (known activation mark)
4: IgG (control)

Given below is the gel picture of PCR done using primer specific to CLR promoter from IP DNA of listed antibodies. On basis of it following statements are made. Which of the statements is correct?

A. Absence of trimethylation on H3K9 at UAS CLR might be the possible cause for repression of CLR expression in white winged butterfly.
B. Methylation of H3K27 at UAS CLR is possibly not responsible for repression of CLR expression in white winged butterfly
C. Presence of methylation on H3K27 and absence of acetylation on H3K9 might be reason for euchromatinisation of CLR promoter leading to repression of CLR in white winged butterfly
D. Presence of methylation on H3K27 and absence of acetylation on H3K9 might be reason for heterochromatinisation of CLR promoter leading to repression of CLR in white winged butterfly.

57. The gel electrophoresis mobility shift assay (EMSA) is used to detect protein complexes with nucleic acids. EMSA was performed to map at which motif of Promoter X is bound by protein A. Analyze the autoradiogram below and select the correct conclusion give below.
[Figure legends: - NE-nuclear extract; Probe: $^{32}$P labeled polynucleotide sequence; A binding competition was performed using competitive probe (cold probe) or mutated competitive probe ($\Delta$ cold probe); Protein A Ab; antibody against Protein A]

A. Protein A binds to both the motif of promoter X
B. Protein A binds to motif 1 of promoter X not on motif 2
C. Protein A binds to motif 2 not on motif 1 of promoter X
D. Protein A does not bind to the promoter X

58. For an enzyme which follows simple Michaelis-Menten kinetics, the following parameters were measured:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_2}{\rightarrow} E + P$$

$k_1$ and $k_{-1}$ are very fast, $k_2 = 100 \text{ sec}^{-1}$ and $K_M = 10^{-2} \text{ M}$ at 280 K.
For $[S] = 0.1 \text{ M}$ and $[E_0] = 10^{-5} \text{ M}$, what is $v_0$ at 280 K?

A. $10^{-1} \text{ mM sec}^{-1}$.
B. $10^{1} \text{ mM sec}^{-1}$.
C. $10^{-2} \text{ mM sec}^{-1}$.
D. $10^{0} \text{ mM sec}^{-1}$.

59. In case of an enzyme inhibition study for an enzyme, the following data were obtained for a competitive inhibition in which the $[I] = 3 \mu\text{M}$ for each determination of $v_0$ in the presence of inhibitor and $V_{\text{max}} = 200 \mu\text{M}$ of Product/min for both data sets. What will be the $K_i$ for this enzyme?
A. 1.5 μM  
B. 10 μM  
C. 30 μM  
D. 3.0 μM

60. One hundred Units of a protein contained in a crude extract are chromatographed on an ion exchanger. Twenty, 5-ml fractions are taken. Fraction 3 has 1.5 unit enzymatic activities. No other fraction has any enzymatic activity and no enzymatic activity was found by extensive washing of the column. When Fraction 8 and 12 are mixed together yielded a solution having 250 units of activity. What was in fractions 8 and 12?
A. The fractions 8 and 12 contain two degraded part of a protein.  
B. The protein contains two different polypeptide chains that are separated into fraction 8 and 12.  
C. The protein contains two different domains that are separated into fraction 8 and 12.  
D. The same protein eluted at different rates into the fraction 8 and 12.

61. The following experimental data have been obtained for the fluorescence intensity (F) and circular dichroism intensity (CD) of a protein solution at different temperatures.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>F (arbitrary units)</th>
<th>CD (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>65.0</td>
<td>-1310</td>
</tr>
<tr>
<td>30</td>
<td>65.0</td>
<td>-1310</td>
</tr>
<tr>
<td>40</td>
<td>64.7</td>
<td>-1304</td>
</tr>
<tr>
<td>46</td>
<td>58.8</td>
<td>-1186</td>
</tr>
<tr>
<td>50</td>
<td>40.0</td>
<td>-810</td>
</tr>
<tr>
<td>56</td>
<td>17.8</td>
<td>-366</td>
</tr>
<tr>
<td>60</td>
<td>15.5</td>
<td>-320</td>
</tr>
<tr>
<td>70</td>
<td>15.0</td>
<td>-310</td>
</tr>
<tr>
<td>80</td>
<td>15.0</td>
<td>-310</td>
</tr>
</tbody>
</table>
What fraction of the protein might be unfolded at 46 °C?
A. 0.124  
B. 0.248  
C. 0.214  
D. 0.482

62. A researcher used fluorescein labeled anti-CD4 and rhodamine-labeled anti-CD8 antibodies to stain thymocytes and lymph node cells from normal mice and from RAG-1 knockout mice. He then subjected these to FACS analyses. However, by mistake, he forgot to label the tubes. The FACS plots for tube 1, 2, 3 and 4 are shown below.

![FACS plots](image)

With the FACS plots shown above, select the correct match of the tubes with their respective possible FACS profile.

A. Tube 1: Thymus of RAG-1 KO; Tube 2: Lymph node of Normal mice; Tube 3: Thymus of RAG-1 KO; Tube 4: Lymph node of Normal mice  
B. Tube 1: Thymus of normal mice; Tube 2: Lymph node of RAG-1 KO; Tube 3: Thymus of Normal mice; Tube 4: Lymph node of RAG-1 KO  
C. Tube 1: Thymus of normal mice; Tube 2: Lymph node of RAG-1 KO; Tube 3: Thymus of RAG-1 KO; Tube 4: Lymph node of Normal mice  
D. Tube 1: Thymus of normal mice; Tube 2: Lymph node of Normal mice; Tube 3: Thymus of RAG-1 KO; Tube 4: Lymph node of RAG-1 KO

63. You wanted to develop a conventional sandwich ELISA diagnostic kit to test for the presence of protein from a new strain of pathogenic bacteria in infected cattle sera. You started with using a monoclonal antibody specific for a single epitope of the bacterial protein, both to sensitize the wells of the ELISA plate and as the enzyme-labeled detecting antibody. The ELISA failed to detect the antigen despite the use of a wide range of antibody concentrations. What is the most probable cause of this problem?
A. The antigen is too large.
B. The antibody has a low affinity for the antigen.
C. The enzyme-labeled antibody used should have been a different isotype than the sensitizing antibody.
D. The monoclonal antibody used to sensitize the wells is blocking access to the epitope, thus when the same antibody is enzyme-labeled, it cannot bind to the antigen.

64. A salicylic acid (pKa = 3) solution with was found to have pH=5. The ratio of salicylic acid to its conjugate base i.e. salicylate is (salicylic acid: salycylate)
   A. 0.01: 100
   B. 1: 100
   C. 100: 1
   D. 100: 0.01

65. Absolute configuration of the following two mirror images of threonine (I, II) are respectively:

```
  O  O
 /\ /\  
H3C NH2 OH  HO OH
  \  \  
   I   II
```

   A. (2S, 3R) and (2R, 3S)
   B. (2R, 3S) and (2S, 3R)
   C. (2R, 3R) and (2S, 3S)
   D. (2S, 3S) and (2R, 3R)

Rough work