SCHOOL OF BIOSCIENCES AND TECHNOLOGY
CONTINUOUS ASSESSMENT TEST - II
WINTER SEMESTER 2025-2026

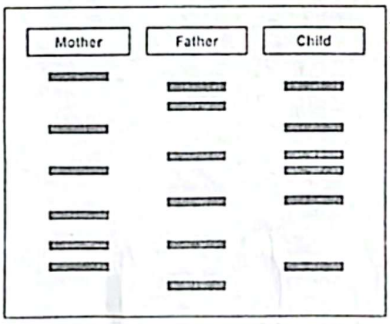
Programme Name & Branch	: B. Tech. biotechnology
Course Code and Course Name	: BBIT206L, Analytical Techniques in Biotechnology
Slot(s)	: A2+TA2
Faculty Name(s)	: Shanthi C and Jitendra Satija
Class Number(s)	: VL2025260502442, VL2025260502521
Date of Examination	: 15/03/2026
Exam Duration	: 90 minutes Maximum Marks: 50

General instruction(s):

Answer All Questions

- CO – Course Outcome; CO – Course Outcome;1. Illustration of solution preparations and application of different tools on quantification. 2. Enumerate the usage and maintenance of analytical instruments. 3. Distinguish the analytes by using appropriate tools. 4. Interpret the data to predict unknown compounds. 5. Suggest analytical technique/instrument to solve real-life problem.

BL – Blooms Taxonomy Level (1 – Remember, 2 – Understand, 3 – Apply, 4 – Analyse, 5 – Evaluate, 6 – Create)

<table>
<tr><th>Q. No</th><th>Question</th><th>M</th><th>CO</th><th>BL</th></tr>
<tr><td>1.</td><td>(a) In ^1H NMR spectra, how many H peaks would you get for the compounds given in the following table? Also, find out the splitting of the ^1H peak for the underlined protons. (8 Marks)
<table>
<tr><th>Compound</th><th>No of H Peaks</th><th>Spin-Spin Splitting</th></tr>
<tr><td>Br-C<u>H</u>$_2$CH$_2$CH$_2$-Br</td><td></td><td></td></tr>
<tr><td>Br-C<u>H</u>$_2$CH$_2$-Br</td><td></td><td></td></tr>
<tr><td>(CH$_3$)$_2$C<u>H</u>-O-CH$_3$</td><td></td><td></td></tr>
<tr><td>(C<u>H</u>$_3$)$_3$C-O-CH$_3$</td><td></td><td></td></tr>
</table>
(b) Three methyl halides, A, B, and C, have the formula CH_3X, where X = Cl, Br, or I. Their ^1H NMR spectra show a singlet corresponding to the three equivalent protons at the following chemical shifts: A = δ 3.05 ppm, B = δ 2.70 ppm, C = δ 2.20 ppm. (2 Marks)
(i) Assign the correct halogen (Cl, Br, I) to compounds A, B, and C.
(ii) Arrange the compounds in order of increasing shielding (upfield shift).</td><td>10</td><td>CO4</td><td>BL4</td></tr>
<tr><td>2</td><td>(a) Suppose the parents of a newborn child suspect that a mistake has occurred at the hospital and that their baby may have been swapped. DNA samples from the mother, father, and child were analyzed using agarose gel electrophoresis, and the band pattern is shown in the figure.


(i) Explain the principle of DNA separation by gel electrophoresis. (3 Marks)
(ii) Compare the banding patterns and determine whether the child belongs to the given parents. Justify. (3 Marks)</td><td>10</td><td>CO4</td><td>BL3</td></tr>
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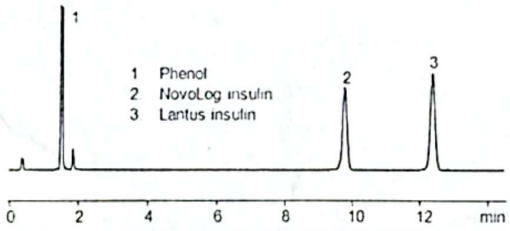
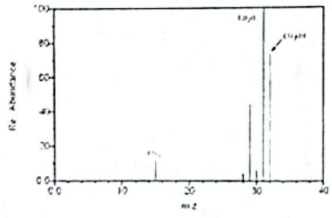
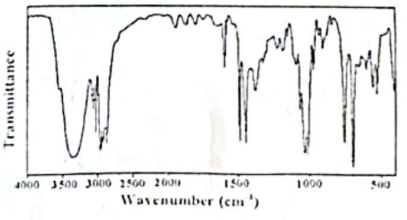
	(b) A mixture of proteins was applied to a gel-filtration (size-exclusion) chromatography column. The fractionation range of the gel is 25,000 Da to 120,000 Da. Predict the order of elution of the proteins (i) Urease (molecular weight = 482,700 Da), (ii) Catalase (molecular weight = 242,500 Da), (iii) Hemoglobin (molecular weight = 64,500 Da), and (iv) Lactoglobulin (molecular weight = 37,100 Da) from the column with suitable justification. (4 Marks)			
3.	A mixture containing five amino acids, i.e., arginine, histidine, glycine, tyrosine, and leucine, was separated by thin-layer chromatography (TLC) using silica gel as the stationary phase and butanol: acetic acid: water as the mobile phase. The observed Rf values were 0.265, 0.279, 0.533, 0.789, and 0.812, respectively. (i) Comment on the structural features and polarity of these amino acids. (4 Marks) (ii) Explain the principle of separation. (2 Marks) (iii) State whether the chromatographic technique is normal-phase or reverse-phase chromatography and justify your answer. (2 Marks) (iv) The Rf value of a compound is reproducible only when specific experimental conditions are maintained constant. Identify three such variables and explain their significance in TLC separation. (2 Marks)	10	CO3	BL4
4.	(a) Why does HPLC require small column packing particles? (2 Marks) (b) What is gradient elution, and how does this differ from an isocratic elution? Write the advantages of gradient elution over isocratic separations. (2 Marks) (c) The chromatogram given below of insulin variants was obtained using a reverse-phase HPLC C-18 column. What can you infer about the properties of the separated proteins? (4 Marks) (d) From the chromatogram shown below, calculate the selectivity factor (α) of peak 3 relative to peak 2 if the mobile phase dead time (t_M) is 1.8 min. (2 Marks) 	10	CO3	BL3
5.	(a) The mass spectrum of methanol is given below in fig. 5a. (i) identify the molecular ion peak, (ii) base peak, and (iii) M+1 peak. Justify your answer. (5 Marks) (b) What are the functional groups and fingerprint region in an IR spectrum (fig. 5b), and how are they useful in analysis? Observe the given IR spectrum and identify the possible functional groups. (5 marks)  Fig. 5a  Fig. 5b	10	CO4	BL4