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MID SEMESTER EXAMINATION-2015

B.Tech VIth Sem

COURSE CODE: 10B11BT615 **MAX. MARKS: 30**

COURSE NAME: DIAGNOSTICS AND VACCINE MANUFACTURE TECHNOLOGIES

COURSE CREDITS: 04 **MAX. TIME: 2 HRS**

Note:- All Questions are compulsory

Section A (1 X 6 = 6)

Answer the following **with reasons** (not more than 2 sentences).

1. Zone of inhibition formed in a disk-diffusion experiment is irregular instead of circular, how can zone be quantified in such a scenario.
2. Which of the following is generally used as secondary antibody in Immunofluorescence based experiments – IgA, IgG, IgD, IgM. Explain with reason.
3. If causal organism for an infection has been identified, would you prefer a broad spectrum or a narrow spectrum antibiotic for treatment of such an infection?
4. Draw a curve for formation of precipitate when increasing concentration of a monovalent antigen is added to constant concentration of a monoclonal antibody.
5. Rocket Immunoelectrophoresis is considered to be a better technique than Immunoelectrophoresis, although it can be used only for negatively charged antigens.
6. Agglutination Inhibition has better sensitivity for detection of an antigen in comparison to agglutination techniques.

Section B (3 X 3 = 9)

Q1. Discuss the conditions for non-formation of precipitation bands in a double diffusion experiment. Why is occurrence of false negative considered as the biggest disadvantage of the technique? (3)

Q2. What is the role of a blocking agent? List the properties which should be present in a reagent to be used as blocking agent in ELISA or western blot methods. (2)

Q3. What is Zeta potential? How does it influence agglutination experiments when concentration of the antibody is low? (2)

Q4. Agglutination based methods are preferred over Precipitation based methods for pathological diagnostics. (2)

Section C (5 X 3 = 15)

Q1. Ethanolic extract from leaves of an endangered plant from north-western Himalayas was prepared and tested against Salmonella sps causing diarrhea, by microbroth dilution method. Following observation was recorded after 14h of incubation:

A	1	2	3	4	5	6	7	8	9	10	11	12
Visible growth [- absent; + present]	-	-	-	-	-	-	+	+	+	+	+	+
Culture dilution	-1	-2	-3	-4	-5	-6	-7	-8	-8	-8	-8	-8
No. of colonies	0	24	23	18	15	12	29	12	14	16	19	27

(Concentration of the extract was 2000µg/ml in column No. 1 and was diluted two-fold in following columns; 100µl of the culture was plated on agar media for CFU count.)

i. Calculate Minimum Inhibitory Concentration of the leaf extract. (1)

ii. Calculate Minimum Lethal Concentration of the leaf extract. (2)

iii. Predict the mode of action of the extract with reasons. (1)

iv. Draw growth curve of the bacterial cells in column No. 2 in comparison to column No. 12. (1)

Q2. With respect to Sandwich ELISA answer the following with suitable explanation:

i. Merits of tagging of enzyme molecule to the secondary antibody. (1.5)

ii. Merits of using two antibodies for binding to the antigen. (1.5)

iii. Need of intermediate washing steps after removal of each reagent. (1)

iv. Significance of using a combination of colourless substrate and enzyme. (1)

Q3. With respect to Kirby-Bauer Test give reasons for the following:

i. Only log phase bacterial cultures should be used for testing. (1)

ii. KB test cannot be used for antimicrobial testing of fastidious organisms. (1)

iii. Incubation temperature should not exceed 35°C, although optimum temperature for bacterial growth is 37°C. (1)

iv. Increase or decrease in the incubation time can lead to false negative or false positive results. (2)