

OLLSCOIL NA hEIREANN, GAILLIMH
NATIONAL UNIVERSITY OF IRELAND, GALWAY

SUMMER EXAMINATION 2009 (MAY 19TH 2009)
3BS9, 3MR3

MICROBIOLOGY – PRACTICAL EXAMINATION

EXTERNAL EXAMINER: PROFESSOR G. McMULLAN

INTERNAL EXAMINERS: MICROBIOLOGY ACADEMIC STAFF

TIME ALLOWED: 3 HOURS

ANSWER QUESTION 1 AND ANY OTHER 3 QUESTIONS

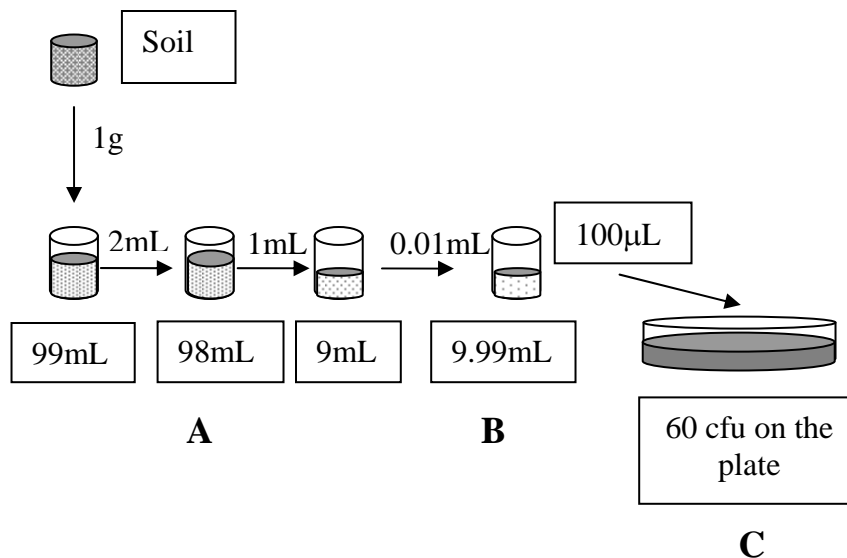
PLEASE USE A SEPARATE ANSWER BOOK FOR QUESTION 1

Q1.

(a) Convert the following terms to the unit indicated.

- (i) 10,000mM = ? M
- (ii) 0.1 mM = ? μ M
- (iii) 1000mL = ? L
- (iv) 100 μ L = ? mL
- (v) 1g = ? mg
- (vi) 0.01 mg = ? μ g

(b) A soil sample was analysed for a total bacterial count by serial dilution according to the following schematic.



Please answer the following questions.

- (i) What is the serial dilution at **A**?
- (ii) What is the serial dilution at **B**?
- (iii) What is the total dilution at **C**?
- (iv) How many bacteria are present per g of soil?

- (c) Describe how you would prepare 200mL of a 0.8% agarose solution in 1 X TAE buffer starting with a 50 X TAE stock solution and agarose powder.
- (d) If 100mL of a 0.5M EDTA solution was diluted to 500mL with distilled water.
- (i) What is the Molarity of the new solution?
 - (ii) How many g of EDTA would be present in the stock solution and in the diluted solution?
 - (iii) Express the EDTA in % (w/v) terms in both solutions.
(Mol.Wt of EDTA = 292)
- (e) What is the Oxytetracycline content in Units/mL of a Nutrient Agar (vol = 20mL) that contains 100 μ L of a 0.2 % (w/v) OTC solution?
Each mg of antibiotic powder contains 5000Units of Oxytetracycline.
- (f) What volume of a 1% (w/v) stock solution of Tryptophan would you add to 1000mL of Minimal Media to give a final concentration of 100 μ g per mL of Tryptophan?
- (g) Mussel tissue and liquor (100mL) was transferred to 400mL of sterile diluent and blended at low speed in a Waring blender for 1 minute. A one in one-hundred dilution was prepared by adding 1mL of the tissue to 99mL of sterile diluent. This was used to inoculate a series of Most Probable Number (MPN) tubes containing MacConkey Broth Purple and a Durham tube. The tubes were scored for the presence (+) or absence (-) of acid and gas formation after 24hours incubation at 44.5°C. The following results were obtained for presumptive faecal coliforms.
- | | | | | | |
|-----------------------------|---|---|---|---|---|
| Series 1 (10mL vol) | + | + | + | + | - |
| Series 2 (1mL vol) | + | + | - | - | - |
| Series 3 (0.1mL vol) | - | - | - | - | - |
- Using the MPN tables provided, calculate the most probable number of faecal coliforms per mL of mussel tissue.
- (h) An assay of 0.5mL of a 1/200 dilution of a supernatant(vol 150mL) obtained by autolysis of 50g of dried yeast cells indicated that it contained 4.5IU of Invertase activity. Calculate the number of IU/g of yeast cells.

- Q2. Imagine that you are hired as a consultant microbiologist to check the microbiological quality of the seawater at a popular beach resort near Galway city. Specifically, you are asked to count the number of faecal coliforms and the total coliforms in the seawater. You decide to perform a water filtration analysis of the water. You collect three separate 100 ml samples and prepare 10^{-1} , 10^{-2} , and 10^{-3} dilutions of each. Then 30 ml of each undiluted and diluted water sample is filtered onto sterile filters in duplicate. The filters are placed in Petri dishes onto filter paper soaked in Membrane Lauryl Sulphate (MLS) medium. After incubating these plates for 24 h at 37°C and 44.5°C the following counts were obtained:

| Temp | 37°C | 37°C | 37°C | 37°C | 44°C | 44°C | 44°C | 44°C |
|----------|------|-----------|-----------|-----------|------|-----------|-----------|-----------|
| Dilution | Neat | 10^{-1} | 10^{-2} | 10^{-3} | Neat | 10^{-1} | 10^{-2} | 10^{-3} |
| Sample 1 | 345 | 31 | 1 | 0 | 22 | 1 | 0 | 0 |
| Sample 2 | 299 | 46 | 2 | 0 | 32 | 0 | 0 | 0 |
| Sample 3 | 309 | 40 | 4 | 0 | 36 | 3 | 0 | 0 |

- Calculate the number of faecal coliforms and the total coliforms in the seawater. Express your answer as CFU per 100 ml.
 - How would you ensure that this measurement was not affected by contamination during the procedure?
 - Explain the difference between faecal coliforms and total coliforms.
 - Describe the details of another method that you could use to count the faecal coliforms and explain any limitations associated with this method.
- Q3. (a) The cup plate assay was used to generate the following data for a standard curve and an unknown test concentration of Chloramphenicol.

| Chloramphenicol ($\mu\text{g/mL}$) | Zone Diameter (mm) |
|---|-----------------------|
| 12.5 | 10.8, 10.7, 10.9 |
| 25.0 | 11.6, 11.6, 11.6 |
| 50.0 | 12.0, 12.4, 12.8 |
| 100.0 | 13.2, 13.2, 24.4 |
| Unknown | 12.8, 12.8, 12.8 |

With the aid of a semi-log plot of the data and using your judgement, calculate the concentration of Chloramphenicol in the unknown sample.

OR

- Describe how the Kelsey – Sykes capacity use dilution test for disinfectants is carried out. In your answer list at least five characteristics of an ideal disinfectant.

Q4. **Given the following Bacterial strains:**

Enterococcus faecalis : contains the plasmid PAM 180 that confers Erythromycin resistance. The plasmid also carries a copy of the transposon TN916 which confers tetracycline resistance.

Bacillus subtilis ; This bacillus strain contains a chromosomal encoded Amylase gene, a Casinase gene and also a gene which confers resistance to Ampicillin. The strain is auxotrophic for tryptophan and will utilise glucose as a carbon source.

1. How would you select for *B.subtilis* TN916 transconjugants?
2. Describe the procedure you would carry out to isolate a *B.subtilis* TN916 transconjugant that has an inactivated Amylase gene. Explain the order of plates in replica plating procedure.
3. Can this procedure be used to isolate a tryptophan mutant strain? Explain your answer.
4. How would you determine if TN916 alone, or TN916 + PAM180 was transferred to *B.subtilis*. ?
5. Once you have isolated the *Bacillus subtilis* Tn916 mutant of interest (i.e. an amylase mutant). What procedures would you carry out to maintain the transposon in the inactivated gene?

Q5. Write a short critical essay on any **one** of the topics covered in the video/seminar tutorials.

Q6. (a) Define the terms

- (i) International Unit of Enzyme Activity
- (ii) Specific Activity

(b) In preparing a crude extract of alkaline phosphatase from *Bacillus cereus* an initial extract of 1200mL volume was obtained from 80g (dry weight) of cells. When 0.1mL of a 10^{-2} dilution of the extract was assayed at 37°C in a 10 min incubation using p-nitrophenyl glycoside as substrate, 0.0015mMoles of p-nitrophenol was found to be produced during the assay.

Calculate the alkaline phosphatase activity of the extract in IU/mL and the amount of enzyme obtained per g (dry weight) of bacterial cells (IU/g).

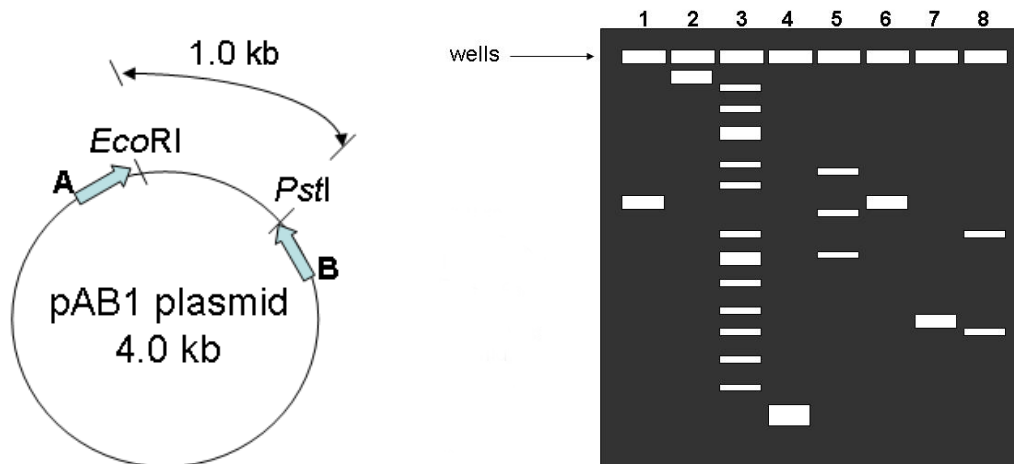
(c) If the protein concentration of the enzyme extract is 0.25%. Calculate the Specific Activity of the alkaline phosphatase extract.

- Q7. You have cloned a 1 kb DNA fragment into the unique *EcoRI* and *PstI* restriction sites of a 4 kb (now 5 kb) plasmid called pAB1, as shown below. In order to screen your cloning, you (i) digest plasmid DNA from an *E. coli* cell with *EcoRI* and *PstI*, both individually and in a double digest, and (ii) you attempt to amplify the 1 kb fragment using primers that anneal just outside the cloning region – as shown at A and B.

Unfortunately, your samples become mixed up with another labworkers so you load, in random order, the following 8 samples on an agarose gel:

- (i) molecular weight marker
- (ii) undigested pAB1 plasmid
- (iii) pAB1 digested with *EcoRI*
- (iv) pAB1 digested with *PstI*
- (v) pAB1 digested with *EcoRI* and *PstI*
- (vi) the 1 kb insert amplified from pAB1 using primers 1 and 2
- (vii) an *E. coli* genomic DNA prep
- (viii) an *E. coli* mRNA prep.

In the picture of the agarose gel, therefore, identify the samples electrophoresed in lanes 1-8. Explain your answer in 1-2 sentences in each case.



- Q8. (a) Using appropriate diagrams, describe in detail the technique of SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In a similar manner, discuss in detail, the theoretical basis of immunoblotting and how immunoblotting is performed. Give a clinical application of the technique.
- (b) Describe the functions and appearance, with suitable illustrations, of monocytes, and granulocytes in a blood smear, stained with Wright's stain, when viewed under the microscope.

MPN TABLES

| Number of tubes giving positive reactions | | | | |
|---|---------|-----------|------------------|-------------------|
| 5 x 10ml | 5 x 1ml | 5 x 0.1ml | MPN per 100ml | MPR* per 100ml |
| 0 | 0 | 0 | none found | |
| 0 | 0 | 1 | 2 | |
| 0 | 1 | 0 | 2 | |
| 1 | 0 | 0 | 2 | |
| 1 | 0 | 1 | 4 | |
| 1 | 1 | 0 | 4 | |
| 1 | 2 | 0 | 5 | |
| 2 | 0 | 0 | 4 | |
| 2 | 0 | 1 | 5 | |
| 2 | 1 | 0 | 5 | |
| 2 | 1 | 1 | 7 | |
| 2 | 2 | 0 | 7 | 7-9 |
| 2 | 3 | 0 | 11 | |
| 3 | 0 | 0 | 7 | |
| 3 | 0 | 1 | 9 | |
| 3 | 1 | 0 | 9 | |
| 3 | 1 | 1 | 13 | |
| 3 | 2 | 0 | 13 | |
| 3 | 2 | 1 | 16 | 14-16 |
| 3 | 3 | 0 | 16 | 14-16 |
| 4 | 0 | 0 | 11 | 11-13 |
| 4 | 0 | 1 | 14 | 14-16 |
| 4 | 1 | 0 | 16 | 14-16 |
| 4 | 1 | 1 | 20 | 18-20 |
| 4 | 2 | 0 | 20 | 18-22 |
| 4 | 2 | 1 | 25 | 23-27 |
| 4 | 3 | 0 | 25 | 23-27 |
| 4 | 3 | 1 | 31 | 29-34 |
| 4 | 4 | 0 | 32 | 29-34 |
| 4 | 4 | 1 | 38 | 34-41 |
| 5 | 0 | 0 | 22 | 20-23 |
| 5 | 0 | 1 | 29 | 25-34 |
| 5 | 0 | 2 | 41 | 36-50 |
| 5 | 1 | 0 | 31 | 27-36 |
| 5 | 1 | 1 | 43 | 36-50 |
| 5 | 1 | 2 | 60 | 50-70 |
| 5 | 1 | 3 | 85 | 70-95 |
| 5 | 2 | 0 | 50 | 40-55 |
| 5 | 2 | 1 | 70 | 60-80 |
| 5 | 2 | 2 | 95 | 80-110 |
| 5 | 2 | 3 | 120 | 105-135 |
| 5 | 3 | 0 | 75 | 65-90 |
| 5 | 3 | 1 | 110 | 90-125 |
| 5 | 3 | 2 | 140 | 120-160 |
| 5 | 3 | 3 | 175 | 155-200 |
| 5 | 3 | 4 | 210 | 185-240 |
| 5 | 4 | 0 | 130 | 110-150 |
| 5 | 4 | 1 | 170 | 150-200 |
| 5 | 4 | 2 | 220 | 190-250 |
| 5 | 4 | 3 | 280 | 240-320 |
| 5 | 4 | 4 | 345 | 300-390 |
| 5 | 5 | 0 | 240 | 200-280 |
| 5 | 5 | 1 | 350 | 290-420 |
| 5 | 5 | 2 | 540 | 450-600 |
| 5 | 5 | 3 | 910 | 750-1100 |
| 5 | 5 | 4 | 1600 | 1350-1900 |
| 5 | 5 | 5 | >1800** | |

*MPR gives counts which are at least 95% as probable as the MPN in being the correct number.

**There is no discrimination when all tubes show growth; the theoretical MPN is infinity. The true count is likely to exceed 1800.