Study Guide for Microbiology

Laboratory Exam II

Determination of Microbial Numbers: Be familiar with the quantitative plating method (viable cell count), know what is meant by serial dilution, and how to calculate individual and total dilution factors. Be able to count colonies and determine the number of viable cells present in a given volume of broth culture.

Use of Selective and Differential Media & Replica Plating Techniques: Be familiar with the types of differential and selective media used in this laboratory. Know why they are selective and on what basis they are differential (know pH indicators, etc.). Be able to recognize and interpret growth patterns visible on these media. Know how replica plating is accomplished and what the advantages of this technique are.

Bacteriological Examination of Water - Standard Methods Testing: Know the steps involved in the Standard Methods test series, their names, the media used, what factors would indicate a positive test in each case, and which organism type is the indicator of fecal contamination. Be able to explain why the presence of *E. coli* in water is an indication of fecal contamination, and why such water would be unsafe to drink.

Physiological Characteristics of Bacteria (three sections): Be able to recognize and interpret the results of the biochemical (enzymatic) tests used in the identification of physiological unknown #1 including O/F test, carbohydrate deeps, TSI, SIM, citrate, urea, amino acid decarboxylation (lysine & control), MR-VP, catalase, oxidase, esculin hydrolysis, coagulase, and hemolysis reactions. Know what is being tested for in each case, how the results are indicated (pH indicators, test reagents, bubbles, etc.) and what positive and negative reactions look like (i.e., be able to distinguish between the two).

Application of the PCR in the Identification of Unknown Bacteria: Know what the polymerase chain reaction is, how it works and what it is used for. Know what *Taq* polymerase is, where it was originally found, and why a thermostable polymerase enzyme is essential to the PCR. Know what oligonucleotide primers are and why they are necessary to the PCR (what their functions are). Know what gene was targeted during our PCR & why.

Nucleotide Sequencing – Genomics, Proteomics and Bioinformatics: Understand the basic mechanism involved in Sanger chain termination nucleotide sequencing. Know what dideoxynucleotides are, and what they are used for. Know what an electropherogram is. Be able to define genomics, proteomics and bioinformatics. Know what the acronyms NCBI and BLAST stand for. Be able to analyze data obtained from the NCBI BLAST algorithm.

The Miniscreen - Rapid Isolation of Plasmid DNA: Know what plasmids are and why these are often extracted from bacterial cells. Know which plasmids we were working with, what their characteristics are, and why they are often used as cloning/expression vectors.

Restriction Endonuclease Digestion of DNA and RFLP: Know what restriction endonucleases are, where they were originally found, how they are named and what they are used for. Know what a recognition sequence is, and what types of termini are formed when restriction enzymes cut DNA. Be familiar with *Eco*RI and its recognition sequence. Know what modification enzymes are and why they are significant. Know what RFLP patterns are, how they are generated and how they relate to DNA fingerprints. Be able to recognize RFLP patterns generated by gel electrophoresis and use these to identify unknown bacteria.

Gel Electrophoresis of DNA Samples: Be able to recognize the equipment used in our gel electrophoresis exercise and know how it is used (how the gels are positioned and why). Be able to recognize the restriction fragments of our lambda standard, pUC19, pGEM, pGLO and PCR product DNA. Know how to determine the approximate size of fragments using the lambda standard as a reference.

Calcium Chloride Procedure for Making Competent Cells & Transformation of *E. coli* with Plasmid DNA: Know what competent cells are and how they are prepared (i.e., why growing to log phase and treating with calcium chloride is significant). Know what transformation is. Be able to recognize and interpret the results obtained on the transformation exercise plates (TSA, TSA-AMP and TSA-AMP-Arab). Know how the operon controlling expression of the GFP gene works, and what the inducer is.

Introduction to Viruses - Phage typing & Isolation of Coliphage from the Environment: Know what phage typing is and how it works. Be able to use the variation in plaque formation with θX-174 and θT2 to determine the identity of unknown bacterial strains. Know how to calculate degree of dilution in a serial dilution (individual and total) and how to determine the concentration of phage particles in a 1ml sample of KCl broth.

Bacteriophage Reproduction and Plaque Formation: Know what a bacteriophage is and what evidence of cytolytic bacteriophage activity looks like on a bacterial lawn. Be familiar with the life cycle of a cytolytic phage such as θX-174 or θT2. Know what is meant by burst size (burst number) and latent period (burst time), and how to determine these given data on semi-log paper.

Microbial Control (Effects of Temperature, Ultra-Violet Light and Chemicals): Review the written information about the effects of temperature, pH and osmotic pressure on microbial growth. Be familiar with the results we obtained with *Serratia marcescens*. Know what prodigiosin is and under what circumstances it is formed. Be able to recognize and interpret the effects of ultra-violet light on bacterial growth. Know what antiseptics and disinfectants are and how their effects on microbial growth can be demonstrated.

Antimicrobial Sensitivity Testing - Agar Diffusion Method or Kirby-Bauer Test: Know what antimicrobial agents and antibiotics are, what a zone of inhibition is, what is meant by minimal inhibitory concentration and where on a plate this concentration is found. Be able to interpret results to determine the sensitivity or resistance of microorganisms to given antimicrobial agents. Define broad spectrum & narrow spectrum drugs.

Composition of Blood and White Cell Study: Know what plasma is and what it is composed of. Know what formed elements are and be able to identify five different types of white blood cells using the 45x lens and prestained blood smears.

Diagnostic Immunology: Define serology and be familiar with the serological tests we used (precipitation and agglutination). Know what an Ouchterlony test is and be able to interpret the results obtained with a test of this type. Be familiar with the factors involved in hemagglutination (agglutinogens and agglutinins) and be able to interpret the results of a blood typing test (be able to determine blood type from smears on a glass slide). Know how the terms genotype, allele, homozygous, heterozygous, homologous DNA, phenotype, genotype, universal donor and universal recipient relate to blood type. Know why an ELISA is a much more sensitive test than precipitation or agglutination.