

Laboratory Exam 2, Spring 2013

1. Define:

Serial dilution – A serial dilution is the stepwise dilution (decrease in concentration) of a sample of material (e.g., bacteria or virus particles suspended in liquid), such that the degree of dilution at each step can be calculated. Making a serial dilution requires a series of containers (tubes, bottles, etc.) filled to a known volume, and a means of transferring specified quantities of liquid from one container to the next.

Confirmatory test (Bacteriological Examination of Water) – The confirmatory test is the second step in the Bacteriological Examination of Water, and involves transferring a sample of culture medium from a positive presumptive test onto the surface of an agar plate (e.g., EMB agar), incubating the plate for 24 hours at 37°C and observing colonies that develop. Dark colonies with a metallic green sheen are indicative of *Escherichia coli*.

Oligonucleotide primer – An oligonucleotide primer is a small segment of single-stranded DNA (usually around 18-20 nucleotides in length) capable of annealing (forming hydrogen bonds) with a set of bases on a much longer section of DNA (one or more genes in length). The primers used in this laboratory were bacteria 8-forward and 1530-reverse, and were designed to bind with nucleotide sequences toward the ends (front and back) of the 16S r-RNA genes of bacteria.

2. Viable/ Only living cells can grow, reproduce and form colonies that can be seen with the “naked” eye and counted.
3. The answers here are variable and will require calculations. Always begin by determining the individual dilutions created at each step. Make sure you express these as exponents (e.g., 1×10^{-3} , 1×10^{-2} , etc.). To determine the total dilution achieved, add together the exponents from all steps./ To determine the number of cfu/ml, count the number of colonies visible on the plate surface and multiply that times the total dilution factor. Your answer must be expressed as a positive exponent.
4. Replica plating/ time and media/ Nutrient agar was used to make the replica master plates, so all the organism types used could grow on it. If all the organism types appeared on the last plate, this indicated the transfer was successful and therefore all plates were inoculated with all the organism types. Variations in growth on the other medium types could then be attributed to their being selective.
5. Halophiles/ differential/ The answer is variable here and will require observation of the materials provided. Organisms that can ferment mannitol and form acid will cause the pH indicator (phenol red) in the medium to turn yellow, those that cannot will grow without causing a change in the medium color (it will remain red).

6. Selective medium
7. Gram-negative/ lactose/ Answers are variable here and will require observation of the materials provided. Lactose fermenting colonies will appear bright pink on MAC and yellow on T-7, while non-fermenting forms will appear pale tan on MAC and blue on T-7.
8. Oxidation/fermentation/ This medium is used to determine the type of metabolism used by the bacteria being tested, but it can also provide information about the gas requirements of the organisms./ bromthymol blue/ The answer will be variable here, and will require observation of the materials provided. Fermentative organisms will produce acids that will cause the pH indicator in the medium to turn yellow, while organisms not capable of fermentation (respiratory forms), will allow the medium to remain green under the vaspar seal. Respiratory bacteria may produce aerobic acid causing the surface of the green medium (in the unsealed tube) to turn yellow, but some can also produce alkaline end products, causing the surface of the medium to turn blue./ Fermentative cultures are facultative anaerobes, while most respiratory forms are considered obligate aerobes with respect to their gas requirements.
9. The answer is variable here and will require observation of data. In the tube containing fermentative organisms the pH indicator in the medium will be yellow in color due to acid production and the medium will show evidence of gas production as split or lifted agar, bubbles, or cracks. Organisms not capable of fermenting the carbohydrate will grow (using the peptone in the medium) but will not cause a color change, so the medium will remain red. Gas may or may not be formed.
10. Acid/ Methyl red/ acetoin/ This answer is variable depending on the data provided. Red-colored medium in an MR-test, and pink to wine-red colored medium in a VP-test indicate positive results. If liquids remain yellow or tan, the results are negative.
11. Amino acid/ hydrogen sulfide/ iron sulfide/ The answer is variable here and will require observation of data. Tubes containing black precipitate are positive for H₂S production.
12. Decarboxylation/ an amine/ The answer is variable here and requires observation of the data. If both tubes contain yellow-colored broth, there is no cadaverine present. If the control tube contains yellow-colored broth and the lysine tube contains purple-colored broth, cadaverine is present./ The pH indicator is bromcresol purple.
13. The control tube is necessary because the medium used is initially purple, and a positive test result (in the lysine tube) is also purple. The control tube must be inoculated, and must turn yellow to insure that the culture being tested is capable of fermenting glucose. If the culture is not fermentative, the test is invalid.

14. The vaspar seal is applied to create an anaerobic environment so that fermentative bacteria will ferment the glucose present. The seal also keeps volatile amines in solution.
15. Citrate permease/ urease/ ammonia
16. Oxidase/ cytochrome C
17. Coagulase/ The answer is variable and will require observation of data. If the rabbit plasma has solidified, the results are positive and coagulase enzymes are present. If the plasma remains liquid, the organisms present are not capable of making coagulase enzymes.
18. Alpha-hemolytic (α -hemolytic)
19. The answer is variable here and will require the observation of data. Know how to interpret enzymatic test data and use an identification chart to determine the identity of bacteria.
20. Polymerase chain reaction/ The PCR is used to amplify (increase the concentration of) DNA samples in vitro.
21. *Thermus aquaticus*/ The enzymes found within hyperthermophiles are heat stable (not denatured when exposed to boiling water), and could therefore remain functional during the PCR, where the temperature used to denature the DNA is typically 94°C and is applied around 35 times.
22. At 94°C, the DNA will be denatured; the hydrogen bonds holding complementary bases together will be broken, and the two strands of the DNA double-helix will separate.

At 55°C the primers will anneal (hydrogen bond) with single strands of DNA. Each primer can anneal (bind or hybridize) with only specific regions of the DNA strand. The 8-forward primer binds near the front ends of 16S r-RNA genes, and the 1530-reverse binds near the back ends of the same genes, but on opposite DNA strands.

At 72°C the *Taq*-polymerase enzyme is activated, and DNA replication occurs (assuming dNTPs and the proper cofactor are available). Like other polymerase enzymes, *Taq*-polymerase adds nucleotides to the free 3' ends of existing DNA strands, so will begin adding nucleotides at the 3' ends of both primers. The process is called extension, because as nucleotides are added, the new (complementary) DNA strands being formed grow longer.

23. Before you begin drawing complementary DNA strands, look again at the answer provided for what occurs at 55°C. Then think about the position each primer must be placed in, if the DNA shown is going to be copied. Remember, *Taq*-polymerase can only add nucleotides to free 3' ends, so if your primers are backward, the gene shown will not be copied.

24. Primers determine what genes will be amplified during the PCR, because they can only anneal to specific nucleotide sequences. When primers anneal, they make the DNA a double helix, and this is essential because *Taq*-polymerase can only bind with double-stranded DNA. Primers provide the free 3' ends necessary for replication (*Taq*-polymerase can only add nucleotides to free 3' ends). Primer length and composition determine the temperature required for the annealing step.
25. Dideoxy/ phosphodiester/ The answer will be variable here and will require observation of data. Remember, sequencing reactions generate millions of DNA fragments of different sizes, and these are separated on the basis of size through gel electrophoresis. Align the strips according to their length, and then record the letter sequence generated.
26. Electropherogram
27. 16S r-RNA/ All bacteria have these genes (in fact, have multiple copies of them), and because these genes are essential to ribosome function, they have been highly conserved (have not changed very much) over time.
28. Define:

Expression vector – Expression vectors are segments of DNA capable of carrying genes into cells, replicating them and expressing their products (making m-RNA and proteins). Each vector has an origin of replication, and multiple cloning sites (nucleotide sequences recognized by specific restriction enzymes) allowing genes to be inserted behind promoter sites. Typically a vector is “cut” with a specific restriction enzyme, the gene to be copied is inserted into the gap formed (and bound there by ligase enzymes), and then the vector is placed into a host cell (e.g., by transformation). Once inside the cell, the vector DNA will be replicated by host cell enzymes and the genes associated with promoter sites will be expressed. The plasmids pUC19, pGEM and pGLO are all expression vectors and cells carrying them gain the ability to produce β -lactamase enzymes.

Latent period (burst time) – The latent period or burst time is the time required for the completion of a cytolitic bacteriophage life cycle, i.e., the time required from adsorption until new virions are released from the host cell. Because the adsorption, penetration, assembly and release phases of a bacteriophage life cycle are so short (require only a few minutes), most of the time required for the formation of new virions is taken up by the latent period (when replication, transcription and translation occur).

Alleles – Alleles are alternate forms of a gene, or homologous DNA segments. During the blood typing exercise, students were introduced to the alleles encoding membrane markers that determine blood type (A, B and O).

29. National Center for Biotechnology Information/ Basic Local Alignment Search Tool

30. The answer is variable here. Know how to use the NCBI BLAST algorithm to identify bacteria (as we did when completing PUNK2). Identify the organism type using the correct technical name (genus and specific epithet), and then record the taxonomic lineage from the data sheet provided.
31. Plasmids
32. Vectors/ origin of replication
33. β -lactamase/ promoter
34. Gel electrophoresis/ negative/ The answer is variable here and will require observation of data. Remember, DNA will travel toward the anode (positive electrode), so look at the gel to determine if or not the wells are located at the end of the chamber farthest from the anode. If they are, the gel is in the correct position; if they are not, then the gel is in the box backward.
35. The dye mixture contains sucrose, so is heavy and will cause the DNA to sink into the well during the loading process. As a loading dye, the mixture is easy to see, so students know where they are placing the DNA. The two dye samples used will travel toward the anode (like DNA), but because the bromphenol blue travels ahead of most of our DNA samples, and xylene cyanol travels behind them, the dyes allow us to keep track of where the DNA is as it moves down the gel, i.e., it serves as a tracking dye./ The small volume pipettes (white top) were used to mix DNA and dye.
36. The answers will be variable here and will require observation of data. Know how to determine the sizes of various DNA fragments using the bacteriophage lambda (cut with HindIII) as a ruler.
37. Restriction endonucleases/ viruses
38. Plasmid DNA is cut with restriction enzymes so that genes can be inserted (in the case of cloning vectors and expression vectors). DNA being used for “fingerprinting” is cut with enzymes (often more than one type) to produce fragments (RFLPs) that will create patterns when run in gels. These patterns can be used for identification purposes. Genomic DNA is cut into fragments for the preparation of gene libraries (the fragments are put into cloning vectors).
39. lamRIII
40. The technically name is cohesive termini, and these are 4 bases long.
41. Ligase
42. Restriction Fragment Length Polymorphism/ The answer is variable here and will require observation of data./ DNA fragments of similar size (in this case 310 and 286, also 209, 206, and 189 bp) will form one fat band (instead of two or three separate bands). Fragments less than 100 bp in length do not show in gels.

43. Transformation/ competent/ calcium chloride (CaCl₂)
44. a) No/ b) No/ c) All cells were able to grow on TSA plates, but only transformed cells (about 10% of the total population) were able to grow on the medium containing ampicillin. Only transformed cells make β-lactamase./ d) arabinose/ repressor
45. Plaques/ cytolytic bacteriophage
46. Phage typing/ The answer is variable here and requires that students observe the data presented.
47. The answers are variable here and require calculations. First determine the total dilution factor (each individual dilution is 1X10⁻¹), and then the plaques present must be counted and their number multiplied by the dilution factor expressed as a positive number (drop the minus sign on the exponent). Remember to write your answer in correct scientific notation.
48. The answers are variable here and require some calculations. Look for a sharp increase in the number of plaque-forming units (pfu) to determine the time required for the infected cells to be lysed. This indicates the end of the latent period. To calculate the burst size, divide the maximum number of pfu present by the initial number of pfu present.
49. Prodigiosin/ Answer is variable here and requires observation of data. The plate containing bright, red-orange colored colonies was kept at room temperature.
50. Endospore forming bacteria such as *Bacillus* species and any organism types that were thermophilic./ No!
51. Ultra violet/ UV radiation causes the formation of T-T dimers and when the DNA replicates these will be paired with one adenine instead of two, resulting in a deletion type point mutation. The resulting frame shift during translation is usually lethal to the cell./ UV radiation has very poor penetrating ability, so would not pass through the plastic.
52. Antiseptics/ Gram-positive
53. Answers are variable and require observation of data. The zones of inhibition must be measured (diameter in millimeters) and the values obtained compared to the chart provided to determine sensitivity.
54. Minimal inhibitory concentration/ At the outer-most edge of the zone of inhibition./ therapeutic
55. Answers here are variable and require observation of data. Broad spectrum drugs will control more different types of bacteria than will narrow spectrum drugs.

56. *Escherichia coli*/ They are easy to grow in vitro, and are easy to test for.
57. Presumptive test/ The answer is variable here and requires observation of data. Tubes containing medium that is cloudy, yellow in color and shows gas in the Durham tube are positive for lactose fermentation. These features are indicative of *E. coli*. Tubes containing clear, red-colored medium with no gas in the Durham tube indicate negative results.
58. Agglutinogens/ antibodies (immunoglobulins)
59. Homozygous/ heterozygous
60. The blood type is variable and requires observation of data. Remember that serum samples contain antibodies (agglutinins) that react with specific antigens (agglutinogens). Anti-A serum reacts with A antigens, anti-B with B antigens and anti-D with Rh antigens. Clumping (agglutination) indicates the antigen is present./ A person will not produce antibodies against “self” antigens (under normal circumstances)./ In order to receive blood from an AB Rh-negative donor, the recipient cannot produce either anti-A or anti-B antibodies, so their blood type would have to be AB. For any other blood types, the answer is no. The Rh-negative RBCs being donated would not carry Rh antigens, so the recipient could be either Rh-negative or Rh-positive (both could receive Rh-negative RBCs).
61. Ouchterlony/ precipitate/ The answer is variable here and requires observation of data. The presence of a precipitate band between the central well and any of the outer wells indicates that the serum sample being tested contains antibodies against the fungus.
62. The answers are variable and require observation of white blood cells under magnification.
Note - the five different types of white blood cells are illustrated on the web site.