

Laboratory Exam II (Example I) Fall, 2012

1. Define:

Differential medium – Differential media are those that allow multiple different types of organisms to grow, and cause them to look different, i.e., cause colonies of different organism types to appear unlike one another. In many cases differences appear as colony colors and are influenced by pH indicators in the media; however, in the case of blood agar, variation appears around the colonies as different organisms affect RBCs differently.

Oxidase test – The oxidase test is an enzymatic test (biochemical test) used to determine if or not organisms are capable of producing cytochrome C. In this laboratory, fresh culture material is transferred from an agar plate onto a piece of filter paper saturated with the oxidase test reagent (different formulations may be available). A clean toothpick is used for the transfer, and is used to rub the culture against a small section of paper making good contact. If a deep purple color appears within 30-60 seconds, the culture is oxidase-positive and can produce cytochrome C. If there is no color change, the test result is negative.

Thermus aquaticus – *Thermus aquaticus* is a type of hyperthermophilic bacteria found within hot springs (e.g., in Yellowstone National Park), and was the initial source of the *Taq*-polymerase enzyme most commonly used to catalyze the Polymerase Chain Reaction (PCR). Other types of bacteria (e.g., *Escherichia coli*) that grow readily at room temperatures are now used to produce this enzyme.

2. Viable/ Only living cells can grow, reproduce and form colonies that can be seen with the “naked” eye and counted.
3. Serial dilution/ 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/ 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. Selective/ halophiles/ 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. 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.

7. Oxidation/fermentation/ 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.
8. 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.
9. 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.
10. 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.
11. Decarboxylate/ 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.
12. 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.
13. Catalase/ bubble vigorously
14. 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.
15. Citrate permease/ urease/ ammonia/ The answer here is variable and will require observation of data. Tubes containing media that is deep blue or bright pink are positive for ammonia formation, while tubes containing green, yellow or peach-colored media are negative.
16. 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.
17. Polymerase chain reaction/ *Taq*-polymerase
18. 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.

19. 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.
20. Bacteria 8-forward and 1530-reverse/ 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.
21. 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.
22. Dideoxy/ phosphodiester
23. 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.
24. Define:

Cloning vector – Cloning vectors are segments of DNA capable of carrying genes into cells and replicating them there. Each has an origin of replication, and multiple cloning sites (nucleotide sequences recognized by specific restriction enzymes). 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 and any genes it carries will be replicated by host cell enzymes. The plasmids pUC19 and pGEM are cloning vectors; pGLO is a pGEM vector carrying an arabinose utilization operon with the GFP gene added.

Cytolytic bacteriophage – A cytolitic bacteriophage is a virus capable of infecting bacteria and causing cell lysis when it has completed its life cycle. The coliphages X174 and T2 are examples and cause the formation of plaques (clear areas) in a bacterial lawn because they kill the bacteria they infect.

Agglutination – Agglutination is a type of serological reaction involving the clumping of cellular antigens due to their binding with specific antibodies. Hemagglutination involves the clumping of RBCs when they are mixed with specific anti-serum samples (agglutinins). The RBCs are bound together by antibodies in the isotype IgM that bind with epitopes called agglutinogens (agglutinating antigens). Anti-A serum will clump RBCs carrying A epitopes, anti-B serum will clump RBCs carrying B epitopes, and anti-D serum will clump RBCs carrying Rhesus factor D epitopes (Rh).

25. National Center for Biotechnology Information/ Basic Local Alignment Search Tool
26. 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.
27. Bioinformatics
28. Plasmids/ origin of replication
29. β -lactamase/ expression
30. 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.
31. 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.
32. Ethidium bromide binds to the DNA, and is visible (fluorescent orange/pink) when exposed to ultra violet light, so it essentially makes the DNA visible to the naked eye.
33. 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.
34. Restriction endonucleases/ viruses
35. XmeNII
36. They are palindromes, i.e., sequences that read the same forward and backward.
37. Restriction Fragment Length Polymorphism/ The answer is somewhat variable here, but some trends are consistent. DNA fragments of similar size (in this case 231 and 225 bp) will

appear in one fat band (instead of two). Fragments less than 100 bp in length are rarely visible in the gel, but will appear in student drawings.

38. The “cut” DNA will look like this:
- | | |
|------------|------------|
| 5'...G | AATTC...3' |
| 3'...CTTAA | G...5' |

Sticky ends are technically called cohesive termini, and in this case are 4 bases long. Ligase enzymes would be used to “glue” DNA fragments together.

39. Transformation/ The *E. coli* cells were grown to the mid-log phase (middle of their exponential or logarithmic growth phase) and then were treated with ice-cold CaCl₂ solution to make their cell surfaces more attractive to the DNA.
40. No/ No/ All cells are 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.
41. Arabinose/ repressor
42. Plaques/ The *E. coli* mixed with the top agar form the lawn culture that is necessary to make plaques visible. Plaques appear as “holes” in the lawn, so if the lawn is missing, the “holes” cannot be seen.
43. Phage typing/ Answer is variable here and requires that students observe the data presented.
44. The answer is variable here and requires the plaques present 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.
45. Latent/ 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.
46. Prodigiosin/ Answer is variable here and requires observation of data. The plate containing bright, red-orange colored colonies was kept at room temperature.
47. Pasteurized/ No!
48. 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.
49. Antiseptics/ disinfectants/ Gram-negative
50. 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.

51. Minimal inhibitory concentration/ At the outer-most edge of the zone of inhibition.
52. Answers here are variable and require observation of data. Broad spectrum drugs will control more different types of bacteria than will narrow spectrum drugs./ The pathogen showing resistance to the greatest number of drugs is the one you would least want to be infected by.
53. *Escherichia coli*/ These bacteria live in the gut, are easy to grow in vitro, and are easy to test for. *Giardia* will not grow in vitro, and *Vibrio cholera* would be much more dangerous to work with.
54. Presumptive test/ confirmatory 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.
55. Agglutinogens/ alleles
56. Yes/ heterozygous
57. 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 type, 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).
58. 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.
59. 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.