

INSTRUCTIONS FOR BACTERIAL UNKNOWNNS

1) Write your unknown number in a safe place. You will write this number in all material that is submitted for grading (not doing so will result in points being deducted). Conduct a Gram stain of your unknown culture to verify the presence of **only** one (in the case of single unknowns) or two (in the case of double unknowns) microbial organisms. Determine if you have a Gram positive or gram negative microbe. Keep in mind that some bacterial cells will produce proteins that will stain pink and that will aggregate around the bacterial cell, particularly in older cells. This does not mean that the bacteria themselves are Gram negative, but rather that protein products being exported outside the cell wall where they are under different staining regime. Record your results in your note book. If you are relatively sure your broth is contaminated, request another broth from your instructor or wait 48hrs until the results of the streak (step #2) confirm or refute your suspicion.

2) Streak your unknown for isolation on a total of two plates of T-soy agar (or other complex media). If isolated colonies are not possible, specialized media will be made available that will help differentiate Gram negatives from Gram positives (e.g., MacConkey's agar, Endo agar, Phenyl Ethyl Alcohol Agar (PEA agar), Mannitol salts agar). Place one streaked T-soy plate at 25 C° and another at 37 C°. **NOTE:** It is important that you streak your unknown culture as soon as possible to prevent any competitive exclusions of your organisms by a contaminant (or in the case of your double unknowns, other organisms). Note if there is a temperature optimum for your unknown(s).

3) The original tube given to you for isolations should be **returned to your instructor** for safe keeping in case something happens to your back up broth or streak plates. Your name will be checked off once you have returned the original to the instructor.

4) Incubate your initial streaks for two to five days. Some species take a bit longer to emerge on plates. Do not throw them away until you are sure that no other colonies will show up. Attempt to distinguish unknown(s) based on colony density, color, size, texture, or geometry (smooth, glossy, dull, gummy, circular, jagged).

5a) For single unknown: conduct a Gram stain on the most common colony type to confirm isolation of unknown. Compare to those microbes Gram stained during step #1. Are they the same? If not, you may have contamination.

5b) For double unknown: conduct a Gram stain on the two most common colony types to confirm isolation of two distinct types of microbe morphologies and groupings. Are they the same as those you observed in #1? If not, you may have contamination. Remember, older cells will often produce proteins that will stain pink and that will aggregate around the bacterial cell. This does not mean that the bacteria themselves are Gram negative, but rather that protein products being exported outside the cell wall where they are under different staining regime.

6) If yes, finish isolations by inoculating each of your isolated unknowns into enriched media (e.g., T-soy broth, heart infusion broth or nutrient broth). These **working broths** will supply you with a confirmed isolated unknown for subsequent tests or for use in subsequent rapid

identification kits you may wish to employ. If not, repeat steps 1 - 5, or try to isolate by re-streaking your unknown on different selective media.

7) I strongly recommend that you do **NOT** continue with your examination process of your unknown until you have constructed an identification scheme (flow chart). **ADVICE:** begin your preparation of your flowchart by first separating all the organisms into their Gram designations and morphological groups. Use positive tests you have the most confidence near the top of the flow chart. Depending on the semester and the list of unknowns available, I may or may not expect that you hand this flow chart in. Whatever the case, I will not be able to properly guide you unless I see at least a rough-draft of this flow chart, so it's a good idea to start on them early and have me review your work.

8) **Other suggestions on constructing a flow chart**--Construct flow chart similar to the partial flow chart posted on the microbiology web page:

<http://www2.truman.edu/~jherrera/Microbiology/flowchart.html>.

- a. flow chart should logically lead me to the conclusion that you have reached concerning the identity of your unknown(s).
- b. The list of microbial species under each branch should be serially reduced as the number of branches increases.
- c. If you are not sure of the results place species on both sides of the flow chart branch in question. You can always separate them later on in your scheme (i.e., include them on both sides of your flow chart).
- d. A list of all possible microbial species will be made available before your unknown is due.
- e. Highlight the species you believe to be your unknown throughout flow chart.

9) What will you have to hand in (depending on the semester)?

- A. Write a short (1 to 2 paragraphs in length) narrative that describes:
 - * The reasoning and logic for selecting the identity of your unknown.
 - * The one or two tests that were critical in determining the identity of your unknown. Please state these explicitly in your narrative.
 - *State **explicitly** the **other** most likely identity of your unknown microorganism and why you did not select this identity for your unknown.
 - *Italicize or underline scientific names
 - *Use correct spelling and include your unknown number, please

B. An appropriately filled Descriptive Chart (this can be found at: <http://www2.truman.edu/~jherrera/Microbiology/Descriptive-chart.pdf>)

C. If you've constructed one, a flow chart.

10) Remember, there is a fair amount of interpretation that goes into reading results of microbiological tests. Minimize the number of times you seek guidance from your instructor or from other students. You have been taught everything you need to know to complete this exercise on your own. Interpretation of results should have been practiced during earlier portions of the laboratory during the examination of known microbes. If guidance is sought out, come prepared.

12) Try to minimize the use of media, tubes, plates and other research material. Following your flow chart will go a long way toward accomplishing this goal. If too much material begins to be used up, I will initiate a media & materials requisition procedure.

Grading criteria (unknown grade sheet)

UNKNOWN GRADE SHEET (Single Unknown)

Name _____

1) Identification of unknown (how close were you to identifying your unknown?) (10 pts):

-If wrong, how close were you to identifying the unknown correctly (10 pts)

2) Write-up (did you discuss any alternate species identification? Grammar? Clarity? Discussion logic? (10 pts):

3) Presentation (how neat and orderly? Neatness?) (10 pts):

4) Descriptive chart (5- point scale, properly filled-out, neat; 10 pts):