LABORATORY SKILLS TEST: BACTERIAL UNKNOWNS

Principle and Purpose

This Skills Test will examine a student's ability to identify bacteria using pertinent diagnostic characteristics. This exercise will provide the opportunity to apply the techniques that should have been mastered already (e.g., aseptic technique, Gram staining, etc.) as well as help facilitate the development of a student's critical thinking and problem-solving skills.

To complete this Skills Test, the various diagnostic tests previously presented shall be available to all students. In addition, expected diagnostic test results of the various bacterial strains available in this laboratory have been provided in three tables available separately as downloadable PDFs at the following URLs:

- Diagnostic Features of Selected Gram-Negative Bacteria (http://crcooper01.people.ysu.edu/microlab/gram-negative-bacilli-chart.pdf)
- Diagnostic Features of Selected Gram-Positive Bacilli (http://crcooper01.people.ysu.edu/microlab/gram-positive-bacilli-chart.pdf)
- Diagnostic Features of Selected Cocci (http://crcooper01.people.ysu.edu/microlab/selected-cocci-chart.pdf)

Students will receive a broth culture containing two unknown bacterial species. These bacteria can be of any combination from among those bacteria listed on the attached tables. That is, the unknown organisms shall be two Gram-negative bacteria, two Gram-positive bacteria, or one of each. The mixture can consist solely of bacilli, or cocci, or one of each cell type.

Students must follow the initial instructions as described below to begin this Skills Test. In addition, students are expected to keep a log of your activities and observations throughout this exercise. Use the "Skills Test Log Sheet" attached to these instructions. The log sheets may be photocopied/printed as needed.

By the deadline established by the laboratory instructor, students must submit the following forms *stapled together*:

- the "Unknown Answer Sheet and Flow Chart" (also available attached to this exercise); and
- the "Skills Test Log Sheet".

NOTE: THE SUBMISSION DEADLINE IS FIRM! This Skills Test is a significant part of your overall laboratory grade. If not wholly finished, students should submit what has been completed for partial credit. LATE REPORTS SHALL NOT BE ACCEPTED! Not submitting a report by the deadline shall have a student's score recorded as "0 points".

IMPORTANT: Students should realize that this Skills Test exercise will likely require them to return to the laboratory to perform work outside their assigned laboratory session. Plan accordingly!!! Also, please only enter the laboratory during Open Sessions. These open laboratory periods have been posted elsewhere.

Moreover, students may assist one another so far as providing advice and encouragement. Any collaboration beyond this level this stands precariously close to the precipice of academic dishonesty. In short, each individual is to perform his/her own work.

Finally, laboratory instructors shall not provide assistance or advice to students other than to help guide them to develop their critical thinking and problem-solving skills. Focusing a microscope,

determining if a diagnostic test result is positive or negative, etc., shall not be in purview of the laboratory instructor. The laboratory instructor shall be available during the scheduled laboratory section.

Read the following instructions carefully. Begin working immediately.

Skills Test IV Procedure

Day 1 (Monday or Tuesday Lab Sections)

Materials Needed:

- Numbered Unknown Sample
- Tryptic Soy Broth (TSB) culture of *Escherichia coli* (ATCC 25922)
- Tryptic Soy Broth (TSB) culture of *Staphylococcus aureus* (ATCC 25923)
- MacConkey's Agar (MAC) plate
- Mannitol Salt Agar (MSA) plate
- Tryptic Soy Agar (TSA) plate

Procedure:

1) The course/laboratory instructor will provide a rack of mixed culture unknowns that have been randomly labeled with a code number. Select one (1) tube and immediately record the numbers of those unknowns on the report sheet that will also be provided by your instructor. *Be sure to record this number on your "Unknown Answer Sheet and Flow Chart".*

   Important: Do not lose this code number. Not recording this number or asking the laboratory instructor for this code will result in four (4) points being deducted from the final report score.

2) Obtain one MAC agar plate, one MSA plate, and one TSA plate. Appropriately label these plates with relevant information including name, date, and unknown code number.
3) Using the very best technique, separately streak a loopful of the mixed culture unknown onto the three different plate media. Incubate these plates at 35-37°C for 18-24 hours.

   Note: Streaking these plates using the appropriate techniques will be especially critical to properly isolate individual clones of the two different organisms. Be diligent in this effort!
4) [An optional, but recommended step] Prepare a heat-fixed smear from the mixed culture unknown on a glass slide, then Gram stain it. (*Escherichia coli* and *Staphylococcus aureus* will be provided as Gram stain controls.)

   Record any observations on the data report sheet attached to this document. These observations may provide an idea of what to expect upon isolating the unknown bacteria.

   Note: Heat-fixed smears can be stored, then stained at a subsequent time if necessary. Transport the heat-fixed slides in a slide box.

Day 2

Materials Needed:

- TSA slants
- TSB culture of *Escherichia coli* (ATCC 25922)
- TSB culture of *Staphylococcus aureus* (ATCC 25923)

Procedure:

1) Remove the three streak plates prepared in step 3 (Day 1) from the incubator and observe them for isolated colonies.

   Record any observations on the data report sheet attached to this document.

   Note: Remember, the MAC and MSA agars are *differential and selective* growth media. The very first clue as to the possible identity of the unknown bacteria shall come from these media.

2) Obtain two (2) TSA slants. These slants will receive separate unknown isolates and serve as stock cultures, so be sure to label them appropriately.

3) Use a microbiological loop and aseptic technique, select two different species of bacteria from the plate media and transfer these to the separate, labeled TSA slants. Incubate the slants at 35-37°C for 18-24 hours.

   Note 1: It is critical that that two different organisms be picked. *Knowing the selective basis for the MAC and MSA media as well as the non-selective nature of TSA shall be critical at this point.* Combine this knowledge with that gained from the Gram stain of the mixed culture. In addition, it is common sense that what grows (or does not grow) on the MAC and/or MSA plates will be growing on the non-selective TSA plate. Be sure to take all of this information into consideration so that two isolates of the same species are not selected.

   Note 2: As stated above, the slants inoculated in this step will serve as stock cultures. When not in use, keep them in the incubator. Make new TSA slant cultures as needed, but to avoid working with old cultures, be sure to prepare freshly inoculated stocks of these isolates on a weekly basis.

4) Prepare a heat-fixed smear from each of the isolated organisms, then Gram stain them separately. (As Gram stain controls, cultures of *E. coli* and *S. aureus* will be provided.)

   The Gram stains of these isolates can be performed by one of the following two options:

   Option A: From the location on the plates from which you isolated your unknown bacteria, remove some of the colony with a loop and make a heat-fixed smear on a glass slide. Gram stain your smear.

   Record any observations on the data report sheet attached to this document.

   Option B: Incubate your stock cultures at 35-37°C for 12-18 hours (i.e., until Day 3), then from each tube separately remove some of the growth of the unknown bacterium with a loop and make a heat-fixed smear on a glass slide. Gram stain your smear.

   Record any observations on the data report sheet attached to this document.

   Note: If you do not have time to fully complete the Gram stain, you can prepare heat-fixed slides for staining at a later time. Store/transport the heat-fixed slides in a slide box.

Day 3 and Beyond

Materials Needed:

- TSA agar slants
- All diagnostic media (commercial and in-house prepared) NOTE: If a particular medium or reagent is not available, please ask your instructor if it can be obtained. Most media/reagents employed during the semester should be available, but sometimes such items may be out of stock and not readily able to be replaced.

Procedure:

Note 1: Before proceeding to actually performing some of the diagnostic tests, students must realize that access to the microbiology laboratory over the weekend is not possible. Since some of the diagnostic tests require that they be examined after 24 or 48 hours of incubation, plan accordingly. Hence, students must be aware that certain test results may not be valid if left to incubate past the specified observation period.

Note 2: It is imperative that students develop a strategic plan (i.e., flow chart) to help identify unknown bacteria. This may help ensure that only those diagnostic tests deemed necessary shall be performed. Performing more tests than needed shall count against a student's score for this exercise.

Based upon your observations of the growth of the unknowns on the various agar plates (step 1, Day 2), select one or two diagnostic tests to begin to elucidate the identity of these bacteria.

NOTE: DO NOT PERFORM EVERY DIAGNOSTIC TEST AVAILABLE. Only perform the minimum number of tests needed to identify an unknown. *Excessive testing will result in a lower score*. *Be efficient*.

Also, begin to diagram a flow chart of the steps taken to identify each unknown bacterium. These flow charts should start with the type of media upon which the microbes were isolated.

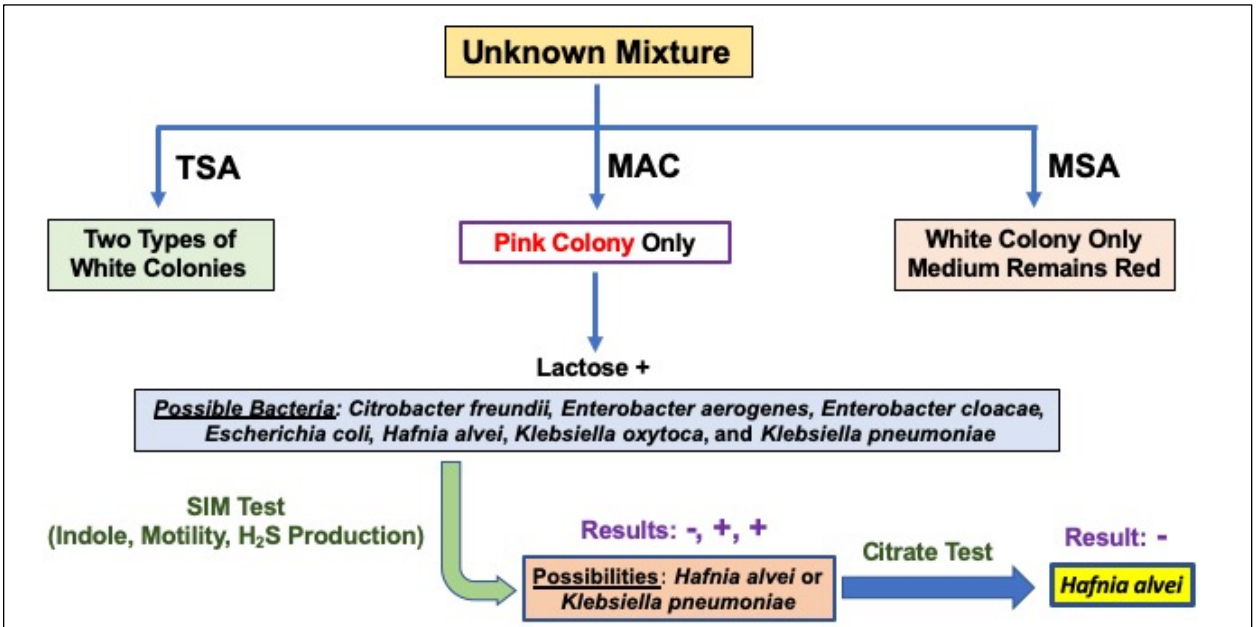

Figure 1. Example of a Diagnostic Flow Chart used to Identify a Bacterial Unknown.

By way of example, the flow chart in Fig. 1 was created using the following scenario. Suppose one of the unknown bacteria formed pink colonies on MAC agar. This might suggest that it is a lactose fermenter. This would eliminate a few of the possible bacterial unknowns found on the sheet entitled "Diagnostic Features of Selected Gram-Negative Bacteria". The possible bacterial unknowns are listed in Fig. 1. From this information, another diagnostic test should be chosen that will help eliminate others not having characteristics common to the unknown bacterium.

In the above scenario, one possible diagnostic test (though not necessarily the best or only choice of tests), might be the SIM test. which can provide data regarding motility, hydrogen sulfide production, and indole production, if performed correctly. From the data generated from the hypothetical scheme in Fig. 1, the results of the SIM test left two possibilities – *Hafnia alvei* and *Klebsiella pneumoniae*. A final test to different between two species is the citrate test. The hypothetical result shown in Fig. 1 indicates that the one unknown originally isolated from the MAC plate was *H. alvei*. Critical analyses and thinking skills should enable a student to determine if the colony formed on the MSA plate is *H. alvei* or a second, different species.

Grading

This Skills Test is worth 30 points towards the overall laboratory score.

This skills test will be graded based upon the following criteria:

Grade Component	Points Awarded if Complete or Correct	Points Deducted If Missing or Incorrect
On-Time Submission of Report	0 points	30 points
Unknown Number Included	0 points	4 points
Submission is Properly Stapled	5 points	5 points
Isolation and Correct Identification of Two Organisms	0 to 5 points each	5 points each
Limited Number of Relevant Tests Performed and Properly Interpreted	0 to 5 points	0 to 5 points
Completion of Log Sheets with Supporting Evidence	0 to 5 points	0 to 5 points
Quality Flow Chart(s) Included	0 to 5 points	0 to 5 points
Total Points	**30 points**	**0 to 64 points***

*The maximum number of points that can be deducted is 30.

Staple Here

Student Name: _____

BIOL 3702L: Microbiology Laboratory
Unknown Answer Sheet and Flow Chart

Mixed Unknown Culture Number: _____

Identification of Unknown #1: _____

Identification of Unknown #2: _____

In the space below and/or on the back of this sheet, prepare a flow chart of the identification scheme used for each isolate. Securely attach additional sheets if needed. Be sure to identify which flow chart corresponds to which isolate.

Staple Here

Student Name: _____

Skills Test Log Sheet

Mixed Unknown Culture Number: _____

Date: _____

Description of Gram Stain Results: _____

_____

_____

Date: _____

Observation of Primary Cultures on TSA, MSA, and/or MAC Agar Plates:

_____

_____

_____

_____

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

and proud.

Staple Here

Student Name: _____

Skills Test Log Sheet (cont.)

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

and proud.

Staple Here

Student Name: _____

Skills Test Log Sheet (cont.)

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

Diagnostic Test Performed: _____

Which Isolate Tested? (circle one) Isolate #1 Isolate #2

Date Performed: _____ **Date Results Read:** _____

Results:

and proud.