Micro Digital Media™
Third Edition

The quick and easy way to learn
VUMIE 2012

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Activity 1

The Virtual Lab

HINTS: To find the videos and more information on each of the topics from this Activity, visit the VUMIE 2012™ Online Help pages found here. From within VUMIE 2012™, you can access these videos and explanations from the dropdown list under Help.

The video links below come from the “Quick Start Tutorial” and “Show Me How To...” section of Help.

NOTE: Help files are located online and an Internet connection is required to access the content.

1. Open the VUMIE 2012™ software. Watch the videos “Before getting started...” and “Orientation to the Virtual Lab...” to get a glimpse of how the lab is laid out and the software operates. Once you get a feel for how the lab simulation works and what it provides, you are ready to try to work in the Virtual Lab.

Here are some hints on the mechanics of VirtualUnknown™ Microbiology software.

- To operate in the lab you will need to change your cursor to a tool. Use the dropdown list to choose the appropriate tool for the task you are doing.

- You will be using both left and right mouse buttons.

- **Right click** reveals options - light the burner, remove caps, record results, retrieve cultures from incubators. In the tutorial videos, a right click is indicated by a red circle. *Red for right*

- **Left click** selects options. It also is the basis for moving things around.
2. Note that initially there is nothing showing in the Unknown indicator box and that no media or reagents are available for use (as shown below). Take-home message #1: You must have an unknown selected before any work can begin.

3. Here is a video explaining how to get an Unknown. Below the process is explained step-by-step:

   a. Click on the “New” button (with biohazard symbol) to get an unknown organism assigned to work with.

   b. Select the lab exercise or type of microbe from the “Subgroup” the dropdown list. This list is of pre-created unknowns supplied by the software (and sometimes your instructor) for the MOC Lab Activities Manual. Selecting one limits the types and number of bacteria possible. A microbe is assigned to you, along with a case study fitting the microbe. For this exercise, please select “Enteric Bacillus”, indicating you want a specific pre-defined unknown microbe and case study randomly assigned from those fitting that description. For this exercise, check the box to permit auto-inoculation for this unknown.

   c. Provide an identifying name (“Label”) for the unknown. For this exercise, use the identifier “Lab1” [NOTE: Labels for labs cannot contain spaces!]. Inputting this identifier into the label field adds this unknown to the list of options available in the dropdown list of Unknowns found at left above the Virtual Lab. When you re-enter the Virtual Lab you can resume work on the same organism by selecting the name you entered in this field from the list in the Unknown dropdown box.

   d. Click on “OK” to have an unknown assigned based on the parameters you just selected.

   e. Read the Case Study. When you are done, exit the Case Study. [NOTE: Case studies are randomly assigned based on the microbe assigned, or are specifically assigned by your instructor.] It can be revisited by selecting “Case Study” from the options found under the “View” dropdown list.
f. **Review the Gram stain.** Your first test in identifying your unknown is performed when you record your interpretation of a Gram stain image that appears. The software asks for Gram reaction and cell morphology. **Try to exit the Gram stain image without making any selections.** What message do you receive?

g. Repeat steps 3a-f. **This time, record the Gram stain result “Spiral and Curved Bacteria”,** which is incorrect but which will show at the end of this activity just how such mistakes are handled by the software.

4. Once you have recorded a Gram stain result, the lab is opened up and you are ready to do some microbiology. Part of studying bacteria is doing metabolic tests using special media. This video shows how media are obtained for doing tests. A place to start is in detecting whether your unknown microbe can use glucose for carbon and energy. More info on how to get started can be found [here](#). For now, select “Phenol Red Glucose Broth with Durham Tube” from the Media dropdown list. You will be asked to provide an identifying label for the medium, just as you must label tubes and plates in the wetlab. Type in “prglucose” (no spaces) and click “okay”. Two tubes appear in the foreground on the lab bench. These are the tube of unknown organism at left (inoculum) and the sterile tube of phenol red glucose broth at the right.

5. With the selection of a medium for use in testing, the two traffic signals appear in the upper right above the lab change color; they are explained in this video. **What is the initial color of each and what might be the meaning of that color?**

   - Inoculation:

   - Contamination:
6. Move your cursor over the Bunsen burner and right click. Choose the option to turn the burner on. A flame will appear and begin flickering from the burner.

7. Above the lab is a field indicating the number of “Virtual Days” that have transpired in the lab. Click on the “New Day” button above the lab scene and note that time has advanced 24 hours (Virtual Day 1 ➔ Virtual Day 2). What warning do you receive? Why would this be important for working in the wetlab?

8. Go to the “View” dropdown list and select “Lab Report”. It is displayed initially in full-page format. Magnify the report so that the details may be read. Fill in the information below based on the content of the Virtual Lab Report:

   a. What information is given about the identity of your unknown?

   b. What is the first test recorded?

   c. How many organisms were eliminated by this result?

9. Exit the lab report for the time being.

10. Use the skills learned in the Tutorial to attempt to dispose of the medium by dragging the tubes to the Biohazardous Waste Bin and dropping them in. What message do you receive when you drop the tubes in the Biohazard bin?

11. Click ‘Yes’ and input results. Make a guess of “positive” for “acid from glucose” and “negative” for “gas from glucose”. The media will disappear.
12. Next, **guess at the identity** of your unknown by clicking on “Unknown” in the menu bar above the lab scene, and selecting “Identify” from the dropdown list. You will be cautioned that providing an identification will prevent you from further work in the Virtual Lab on this unknown. *That is fine – just guess.*

What information is given?

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13. When prompted about whether to open the lab report to review your work, do so. Review the results for the tests of “acid from glucose” and “gas from glucose”. **What information is given in the Test Detail (shown in red) for these two tests?**

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14. Exit the Virtual Lab Report and continue your tour of the Virtual Lab. Click on the yellow button marked “T”. This provides information on all of the common **identification tests** you can do using **VUMIE 2012**. Tests are listed in the pane at left and detailed information about each is listed in the pane at right. Any time you need information about how to conduct a test, it can be found here. **What are the first and last tests in that list (you may need to scroll down)?**

---

15. For info on media and bacteria found in the software, click on the blue button marked “M” and the green button marked “B”, respectively. Any time you need information about a **medium** or **bacterium**, it can be found here. These Help files are fully searchable to allow maximum effectiveness in providing useful information for this course. For now, close the Help browser and return to the software itself.
16. Click on “Help” and “Overview F1” and notice the icons above the topic index in the panel at left: “Contents”, “Index”, and “Search” (NOTE: The appearance of Help for your browser and operating system may differ from that shown at right). Let’s say a question in one of the exercises asks you for information about bacteria commonly found in cockroaches. Type the word “cockroaches” in the search field and click on the magnifying glass. What topic appears in the pane below the search window?

17. Click on the topic in the pane below the search window to bring up the information in the pane at right. Copy that information here:

18. In the same way, use of the Help Search feature will make finding information in VUMIE 2012 easy and convenient. Do not hesitate to go to “Show Me How To…” to get additional information on virtual lab skills or topics that are difficult to understand. For instance, to find out more about how aseptic transfers are done to streak plates in the Virtual Lab (and how the process closely approximates that undertaken in the wetlab), click on the “Show Me How To…” book under the Contents tab of Help and select the topic of interest from the “Do Tests” collection of files and videos.

19. When you feel comfortable with the Virtual Lab and believe you can navigate through its features sufficiently, print out the Virtual Lab Report containing a record of your activity in the lab (go to View → Lab Report and select the Print option). You may be surprised by the level of detail on your lab activities monitored by the software and reported to your instructor. Or, if your instructor is using the Admin Console to create and manage unknowns tailor-made for the students in your class, s/he may prefer to have you submit your report electronically. To do this, you must be enrolled in your instructor’s section for the course at your university. Simply use the “Submit Lab Report” option under “File”. By clicking this option, a pdf copy of the lab report is automatically emailed to your instructor.

20. To view the lab report at a later date, simply select the unknown “Lab1” (the name you gave to the unknown you just created) from the list appearing in the Unknowns dropdown box. This will reactivate that unknown. Now, going to View → Lab Report will bring it up into the lab report viewer.

Take-home message #4: The VUMIE 2012™ Help files are valuable for much more than just instruction on how to “work” the software.
Activity 2  Aseptic Tube-to-Tube Transfers

1. Open the software. Review the Tutorial to get to know your way around the Virtual Lab contents and how it functions. Once you feel comfortable with the workings of the lab, exit the Tutorial and enter the Virtual Lab. Remember the “Show Me How To…” feature in the Help files is always accessible to provide additional instruction on these topics.

2. Create a new unknown named “GPOS”, using “Gram Positive Coccus” as the “subgroup”. Be sure there are NO SPACES in a label for an unknown you create! For this exercise, DO NOT check the box permitting auto-inoculation. Autoinoculation allows the student to bypass the steps of an aseptic transfer and go straight to incubation of the medium – not something desired when the exercise is focused on teaching how aseptic transfers are done in the software! Your lab report indicates whether it is checked!

3. Read through the Case Study and interpret the Gram stain (feel free to make a guess if this topic has not been discussed in class). Obtain a tube of Phenol Red Glucose Broth with Durham tube from the “Media” dropdown list and provide an identifying label – prgb. Appearing will be two tubes – one at left which is your culture of unknown organism (the inoculum) and the one at right which is sterile phenol red glucose broth. The inverted Durham tube protruding up from the broth is used to capture any gas produced during metabolism of the sugar.

4. The purpose of aseptic technique is to control the conditions of the work environment so that sterile media remain that way until inoculated deliberately by the lab worker (you). The two tubes represent the “before” and “after” results when this is accomplished – a sterile medium is inoculated and grows to produce a pure culture of the unknown organism. List three precautions taken in the wetlab to reduce accidental and unwanted inoculation (also known as “contamination”) of sterile media.

5. Right click on the medium at right and select Record Results to make a guess about the result of this test. Select positive for Acid from glucose and no result for Gas from Glucose. Then click “OK” and the media will be automatically disposed of in the biohazardous waste bin (this happens every time you record results). Because you skipped vital steps, doing this will result in a note in the Lab Report that you made a guess.

Take-home message #1: The software can distinguish between a guess and a valid test attempt. Such information is hidden from the student until the ID of the unknown is finalized.

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6. Now we will go a few steps farther in completing an aseptic tube-tube transfer. Select Phenol Red Lactose Broth from the Media dropdown list. This medium differs only in the sugar contained (lactose instead of glucose), and the test is done exactly the same way. Label your tube “prlb”.

7. Note the traffic signals in the upper right corner. Be sure to have at your disposal a timekeeping device (watch or clock). Remove the caps from the tubes (by selecting that option following a right-click on the sterile tube at right) and begin timing. How long a time period is provided for users of VUMIE 2012 before the Contamination light turns red? (Hint: You are given a very generous time of well over 30 seconds!)

NOTE: The steps needed to complete an aseptic transfer with VUMIE 2012 mirror those used in the wetlab. To review how to accomplish these using the software, review the “Tutorial”, or “Show Me How To Do Aseptic Transfers” in “Help”.

8. Replace the caps on those tubes (intuitively, by the same right click on the tube at right), then drag the capped phenol red lactose broth tube and drop it in the 37o C incubator. Immediately retrieve the medium from the incubator by right-clicking on it and selecting the medium labeled prlb. Right click on the medium and select Record Results to see a closeup of the tube. Explain why a contaminated culture has not demonstrated any observable change in color or turbidity at this point?

9. Record a result for this test by making a guess. The medium will disappear as it is automatically dropped in the biohazard waste container.

Take-home message #2: Contamination is caused by any failure to follow aseptic transfer protocol to the letter. Shortcuts always lead to contamination. All mistakes are noted on the lab report.

10. Next, select a tube of Phenol Red Maltose Broth (again, only the sugar found in the medium has been changed) and repeat the process of exposing the medium to contamination by leaving the tube open to the air. However this time rather than recording a result and discarding the medium, place the contaminated tube into the incubator. Click “New Day” to incubate the tube for 24 hours. Retrieve the medium from the incubator. What does the turbidity and color change of the medium indicate? (You may need to click on the “T” icon and look up this test for the details.)
11. Do these results faithfully reflect what would be expected in the wet lab? Why does it make sense that contamination leads to a positive result in every case?

12. Record your results (positive is yellow, anything else is negative). Again, the medium is automatically disposed of once results are entered.

13. Obtain a tube of Phenol Red Fructose Broth. This time, watch the traffic signals and keep track of time as you complete the following steps:

   NOTE: The steps needed to complete an aseptic transfer with VUMIE 2012 mirror those used in the wetlab. To review how to accomplish these steps using the software, review the “Tutorial”, or “Show Me How To…” entry on aseptic transfers in “Help”.

   a. Select the inoculating loop from the Tool dropdown list. This is the tool you would be instructed to use in the wetlab.
   
   b. Remove the caps from the tubes (select with right click on sterile medium).
   
   c. Entering the left tube from the top, dip the loop down into the unknown culture to obtain your inoculum and remove it through the top of the tube for transfer to the sterile medium at right as demonstrated in the Tutorial.
   
   d. Once the sterile broth is inoculated, replace the caps on the tubes.

14. If the Contamination traffic signal turned red BEFORE you replaced the caps, you did not complete the transfer within the allotted time period. Drag the tubes to the biohazard bin to discard the medium. Select the “No results” option.

   Take-home message #3: If you contaminate a tube during aseptic transfers, you can dispose of the tube and start over using a fresh tube of the same medium.

15. Explain why the traffic signal indicating “Contamination” turned red even when the tubes were not open longer than allowed. HINT: Think about the process as it is taught in the wetlab and the important anti-contamination steps that were omitted. You may want to review the whole aseptic transfer process in the Tutorial to reveal the source for the contamination.

16. Once you have an aseptic transfer completed in the allotted period of time (with or without contamination), drag and drop the culture in the incubator and click “New Day”. Retrieve the medium and record the results.
17. Now, we will take care of all of the details involved in a successful aseptic transfer that is completed without contamination. Obtain a tube of Phenol Red Mannitol Broth. This time, watch the traffic signals and keep track of time as you complete the following steps:

a. Select the inoculating loop or wire from the Tool dropdown list.

b. Ignite the burner by right-clicking on the burner.

c. Flame your loop. What indication is there that the loop has been sterilized?

d. Remove the caps from the tubes. Drag them to the burner to quickly flame the mouths. The popup messages indicate when each has been successfully flamed.

[NOTE: The means for performing aseptic transfers is explained in the “Tutorial”. You may also review the fine points at any time by entering “Help” and using the “Show Me How To…” feature.]

e. Perform the aseptic transfer by dipping the sterilized loop into the culture at left and transferring it to the sterile medium at right. Be sure to enter and exit the tubes from their tops. Explain why the color change in the traffic signals makes sense from your knowledge of aseptic technique in a wetlab.

f. Once again drag the tubes and flame their mouths. Replace the caps. If you were able to complete this in the allotted time, tell what color changes now have occurred in the traffic signals. If not, discard the medium (drag and drop in biohazard bin without input of results) and try again.

g. To finish the aseptic transfer, flame your loop again – just as you would in the wetlab.

h. Place the inoculated culture in the 37° C incubator. Click “New Day”. Retrieve the medium and record the results. For information on the interpretation of the results, refer to the “Acid from Mannitol” test in the biochemical test reference book (accessed via the “T” button). How can you be sure the microbe being studied is responsible for the changes observed in the medium?
i. After input of results, media are automatically disposed of in the biohazard waste container.

*Take-home message #4: Using the traffic signals can insure aseptic transfers are completed successfully without contamination.*

18. **Perform without contamination the two tests done using the media listed in the table below.** Be sure to read up on how the test medium is inoculated, incubated, and test results interpreted, since you will need to record results in the software and in a table below.

<table>
<thead>
<tr>
<th>Medium Used</th>
<th>Appearance Following Incubation</th>
<th>Interpretation of Results (Positive &quot;+&quot; or Negative &quot;+&quot;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol Red Sorbitol Broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christiansen’s Urea Broth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Take-home message #5: Using the “M” button to learn about media and “T” button to learn about tests provides all information needed to do the tests and interpret the results.*

19. **Identify** the unknown bacterium by selecting that option from the “Unknown” menu above the lab. **Make a guess** from the list of bacteria that appears in the dropdown list. Click “Okay” to make that selection. A second warning will appear asking “Are you sure…?” Click on “yes”. A box will appear stating whether your guess was correct. The box asks if you wish to view the lab report and review your work. Click “yes”.

20. Once an ID is made, all information tracked by the software on your performance is revealed in the Virtual Lab Report. Read through the comments the software made for each attempt at an aseptic transfer and for the results you recorded. You will see that learning how to do aseptic transfers correctly is as important in the Virtual Lab as it is in the wetlab, and that the consequences for mistakes are just as severe. **What warnings or messages appear most frequently?**

What changes in your actions will you make to improve your aseptic technique next time you work in the Virtual Lab?

21. Submit your Virtual Lab Report and this completed MDM Exercise to your instructor.
1. Open the software. Review the **Tutorial** to get to know your way around the Virtual Lab contents and how it **functions**. Once you feel comfortable with the workings of the lab, exit the Tutorial and enter the Virtual Lab. Remember the “**Show Me How To…**” feature in the Help files is always accessible to provide additional instruction on these topics.

2. **Create a new unknown** labeled “GPOSNEW”, using “Gram Positive Coccus” as the “subgroup”. Be sure there are **NO SPACES** in a label for an unknown you create! For this exercise, **DO NOT** check the box permitting auto-inoculation. Autoinoculation allows the student to bypass the steps of an aseptic transfer and go straight to incubation of the medium – not something desired when the exercise is focused on teaching how aseptic transfers are done in the software! Your lab report indicates whether it is checked!

3. Read through the Case Study and interpret the Gram stain (**feel free to make a guess if this topic has not been discussed in class**).

4. **Obtain** a DNAse Agar plate with Methyl Green from the “Media” list and provide an identifying label – dna.

    Appearing will be a tube of your unknown culture for inoculating the sterile plate of mint green DNAse agar.

5. Read up on DNAse agar and its use by clicking on the green “M” icon and selecting the topic from the list of media included in VirtualUnknown™ Microbiology. You will see that this medium is used for testing whether unknown bacteria can digest DNA as a nutrient for growth. For this exercise, we will streak the plate and observe the isolated colonies to determine whether DNA has been digested. This information is useful for identification.

    **Take-home message #2: The Help files will provide all information needed related to a test, the medium used, and how to interpret results.**

6. The process for streaking plates in the Virtual Lab is a compromise from the actual process used in the wetlab. In the wetlab, the inoculating loop is used to dilute and mechanically spread the microbes over the surface of the agar in order to isolate bacteria from one another so that they might grow into genetically pure colonies arising as offspring of the original single cell landing at that point on the plate. This is done using a pattern repeated over and over as you move the loop from one sector to the next, removing a fraction of the cells in the previous sector and spreading them out in the new sector. Because the third sector represents a fraction of a fraction, and the fourth is a fraction of a fraction of a fraction, the numbers of cells picked up and distributed by the loop is reduced to the point where single cells are dropped off at some distance from one another and colonies consisting of millions or billions of the same microbe can arise, untouched by other colonies.

    In this version of VirtualUnknown™ Microbiology the streak plate process is simplified to simple “squiggling” of the loop across the plate surface until streak lines appear. A [video here](#) shows how it is accomplished:
a. If you have not already done so, select **DNase Agar plate** from the Media dropdown list. Label your plate “dna”.

**NOTE:** The steps needed to complete an aseptic transfer with VUMIE 2012 mirror those used in the wetlab. To review how to accomplish these steps using the software, review the “Tutorial”, or “Show Me How To Do Aseptic Transfers” in “Help”.

b. Select the inoculating **loop** from the dropdown list of “Tools”.

c. **Right click** on the Bunsen burner and **turn it on**. Place the loop in the **flame** until it turns red — a sign it is hot enough to have been sterilized by the flame. Note that the loop turns back to black as it “cools”

**NOTE:** The steps needed to complete an aseptic transfer with VUMIE 2012 mirror those used in the wetlab. To review how to accomplish these steps using the software, review the “Tutorial”, or “Show Me How To Do Aseptic Transfers” in “Help”.

d. **Right click** on the plate and select the “**Remove Cap/Lid**” option.

e. Now you must flame the mouth of the inoculum tube before starting the transfer. Do this by dragging the mouth of the inoculum tube through the flame and noting the popup box stating the inoculum has been flamed. **Failing to do this always results in contamination and is recorded on your Virtual Lab Report…not a good thing.**

f. Use the sterile loop to obtain an inoculum and transfer it to the plate of DNase agar. Again, be sure to enter and exit the tube from its mouth or inoculation cannot take place. Also, you can watch your progress by following the **traffic signals** in the upper right of the tool bar above the Virtual Lab.

g. Once the inoculum is transferred to the agar plate, squiggle the loop around the surface of the agar. This will approximate the mechanical dilution you would accomplish with the repeated streaking and flaming of the loop in the wetlab. You will know you have completed the streak plate when streak lines appear on your plate.

h. Before replacing the tube cap and plate lid, be sure to flame your inoculum tube as done previously, just as in the wetlab. Then, flame your loop.

i. If you complete this without contamination, drop the plate in the **incubator** and click **New Day**. This is the only test that is routinely incubated at 25 C. If there was contamination, dispose of the medium and **re-do**.
j. Remove the incubated plate from the incubator and observe for color changes and other indications of growth on the medium. Is it possible for there to be bacteria on the plate but for the DNAse test to be negative? Explain your answer.

k. Right click on the plate and select “Record Results”. This option brings up a closeup image. Use the information from the Test references and the image provided to determine whether the microbe is positive or negative for DNAse. Record the result by clicking either positive or negative. What result did you record?

Take-home message #3: Interpretation of test results involves connecting information from the “Tests” section of Help with the photographic results the software provides.

l. Open the Virtual Lab Report and view the results recorded so far. How many bacteria have been eliminated from consideration because of the Gram stain and DNAse test?

m. Close the lab report and open the Identification Matrix by clicking “View” and then selecting “Identification Matrix”, another option in the dropdown list through which you accessed the Virtual Lab Report. Here is an overview of how it is useful.

n. The Identification Matrix lists all tests used for Gram positive bacteria as rows and the bacteria included in the software as columns. Symbols are used that are defined below the matrix. What do these designations mean?

+  

[+]  

d  

[-]  

-
Note that in the first column (“Assigned Unknown”) the result that you recorded for DNAse at 25 C is shown. Let’s say you recorded a DNase result of positive (“+”). The only bacteria eliminated by recording a “+” result are those with a “-” result. The only bacteria eliminated when you record a result as “-” are those with a “+” result in the table. All others [ (+), d, and (-) ] remain in the matrix until they can be clearly eliminated by “+” vs. “-” results. Based on this information and the portion of the table displayed at the bottom of the preceding page, would “Acid from glucose” and “Arginine dihydrolase” be useful tests to perform now for identifying your unknown? Why?

Take-home message #4: Not all tests are useful for identifying bacteria. Choosing tests based on + and – results in the matrix helps minimize work and maximize effectiveness.

5. The tube-to-plate technique just used was performed to produce isolated, pure colonies. There are some tests that require you to streak plates to create bacterial lawns instead of isolated colonies. The goal is to cover the surface of the plate with bacteria, as is necessary for doing antibiotic sensitivity tests. Once the agar is seeded with the unknown bacterium, a paper disk impregnated with antibiotic is placed on the surface. The antibiotic diffuses away from the disk, with high concentrations nearby and weak levels farther away. If the antibiotic is toxic to the bacteria, the lawn will not grow where there is a high concentration, leaving a “zone of inhibition” (a clear zone) surrounding the disk. Resistant bacteria are not affected by the drug and growth covers the plate – no zones of inhibition! Antibiotic sensitivity testing is common in identifying unknown bacteria.

Below you will create a lawn and complete an antibiotic sensitivity test, as would be done in the wetlab.

a. Select Nutrient Agar plate from the Media dropdown list. Enter the identifier “na” for the medium. Appearing will be a tube of your unknown culture to use for inoculating the amber agar plate of Nutrient Agar.

b. From the Tool list, select a sterile swab. Light your Bunsen burner and right click on the Nutrient Agar plate to remove the cap of the tube of inoculum and the lid from the agar plate. In the wetlab, you would not remove the plate lid at this time, but in the Virtual Lab, both are removed simultaneously.

c. Flame the mouth of the tube of inoculum as you should for any aseptic transfer. Then, use your sterile swab to obtain inoculum and transfer it to the sterile plate. Be sure to enter and exit through the mouth of the tube. Squiggle the swab across the plate surface until a change in appearance occurs. This represents completion of the thorough swabbing of the surface to spread cells over the entire surface of the agar.

Take-home message #5: Antibiotic sensitivity tests require creation of lawns on plates using sterile swabs; the swab is not used for any other tests.
d. Right click on the plate and a new option appears - a list of antibiotics to choose from. **VUMIE 2012™** knows this is an antibiotic sensitivity test because you selected the correct medium (nutrient agar) and tool (swab) for creating a lawn on which to place the antibiotic disk.

Select **Bacitracin 2U** and a paper disk containing 2 units of Bacitracin will appear on the plate. Once the disk is on your plate, you will be able to again right click on the plate and replace the tube cap and plate lid. Place the plate in the 37 C incubator and click **New Day**.

e. Retrieve the Nutrient Agar plate with the Bacitracin disk from the incubator. Right click and select the option to **Record Results**. A closeup of the plate is provided for interpretation. If there is a zone of no growth surrounding the disk, the unknown is **sensitive** or **susceptible** to the antibiotic and growth was prevented at high concentrations. If **resistant**, the lawn grows up to the edge of the disk. **What result is shown on your plate?**

Would this antibiotic be effective for treatment of a wound infected by this unknown organism?

f. Record your results.

6. Open the Identification Matrix. Note that by completing these three (3) tests [Gram reaction and grouping, DNAsse test, and 2U Bacitracin sensitivity test], the unknown microbe assigned has been identified as **Streptococcus pyogenes**. No other bacterium listed among the 120+ in the software gave this collection of results.

a. Click on the “B” icon to find information about this microbe. **What are some of the health problems associated with infections by this organism?**
b. Review the Case Study again (View, Case Study). **Clues from the case study support which of the diseases caused by this microbe?**

7. Complete work on this unknown by selecting **Unknown → Identify**. The dropdown will indicate the only possible ID based on your results (not the same as shown in image at right). Click “OK”.

8. Review the **lab report** when you are done. You may note that many errors not revealed to students while work is in progress are now posted in the report for your instructor, since you are now unable to complete any more work on this unknown. **List below each separate error message (found in red) listed on your Virtual Lab Report.**

9. Is it fair to say the software is better able to provide feedback on your skill in the lab than would be an instructor watching the work of a lab with 20 students? Explain.

10. Submit the Virtual Lab Report and this completed exercise to your instructor.
Activity 4

Bacterial Cell Anatomy and Arrangement

1. Enter the Virtual Lab. Click the “New” button and select **Bacterial Anatomy 1** from the predefined unknown list found in the Unknown dropdown box above the Virtual Lab. Label the unknown “BactAnat1”.

   **Remember:** Labels for unknowns you create cannot have spaces!

   Read through the Case Study and observe the Gram stain. Use the image of the stain and information from your text, notes, and other resources to answer the questions below.

2. Circle the correct answer(s) in each instance below, to describe the unknown organism found in the Gram stain.

   **Gram reaction:**
   - Gram +
   - Gram –

   **Morphology:**
   - coccus
   - bacillus
   - spirillum
   - other

   **Arrangement:**
   - singles
   - pairs
   - chains
   - clusters
   - other

3. What is the terminology used for the cell morphology and arrangement observed for this organism?

4. When you do a Gram stain, you use these four chemicals. In the table below, indicate the color of cells in a smear of this organism after use of the listed chemical during completion of a Gram stain.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Bacterial Anatomy 1 unknown organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Violet</td>
<td></td>
</tr>
<tr>
<td>Gram’s Iodine</td>
<td></td>
</tr>
<tr>
<td>Decolorizer</td>
<td></td>
</tr>
<tr>
<td>Safranine</td>
<td></td>
</tr>
</tbody>
</table>

5. When bacteria are stained, it is because the stain ions react with cell structures that have the opposite ionic charge (+ and – are attracted to one another). Use outside resources to find out this information: What cell structure(s) is(are) thought to be stained by the dye to give this organism the color observed?

6. The difference in color between Gram + and Gram – cells is due to structural differences. Use your text or other resources to answer this question. Indicate which of the following cellular features would be found in an organism exhibiting this Gram reaction. (circle the correct answer[s] below)

   A. LPS layer present
   B. peptidoglycan present
   C. teichoic acids present
   D. flagella with only two rings in basal body
   E. endotoxin present
   F. periplasmic space present
7. Predict the cell wall structure of the unknown, based on the discussion of Gram + and Gram – cell walls found in your textbook. **Draw in the space below the cell wall structure expected for the unknown organism.** Label your drawing with all items listed above (A-F) that are appropriate for this type of wall structure.

8. Next, click the “New” button and obtain a different organism by selecting Bacterial Anatomy 2 from the predefined unknowns list found in the Unknown dropdown box above the Virtual Lab. Label the unknown “BactAnat2”.

   Read through the Case study and observe the Gram stain. Use the image of the stain and information from your text, notes, and other resources to answer the questions below.

9. **Circle the correct answer(s) in each instance below, to describe the unknown organism found in the Gram stain.**

<table>
<thead>
<tr>
<th>Gram reaction:</th>
<th>Gram +</th>
<th>Gram –</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology:</td>
<td>coccus</td>
<td>bacillus</td>
</tr>
<tr>
<td>Arrangement:</td>
<td>singles</td>
<td>pairs</td>
</tr>
</tbody>
</table>

10. **What is the terminology used for the cell morphology and arrangement observed for this organism?**

11. **When you do a Gram stain, you use these four chemicals. In the table below, indicate the color of cells in a smear of this organism after use of the listed chemical during completion of a Gram stain.**

<table>
<thead>
<tr>
<th>Crystal Violet</th>
<th>Gram’s Iodine</th>
<th>Decolorizer</th>
<th>Safranine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Anatomy 2 unknown organism</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. When bacteria are stained, it is because the stain ions react with cell structures that have the opposite ionic charge (+ and – are attracted to one another). Use outside resources to find out this information: **What cell structure(s) is(are) stained by the dye to give this organism the color observed?**
Take-home message #1: Gram stain color arises from ionic attraction between the chromophore (dye) and charged structures in the cell. Gram + cells retain the dyed complexes during the decolorizing step but they are washed away from Gram – cells. Gram – cells get their color from a second stain used to add a different, contrasting color.

13. The difference in color between Gram + and Gram – cells is due to structural differences. Use your text to provide info to answer this question. Indicate which of the following cellular features would be found in an organism exhibiting this Gram reaction. (circle the correct answer[s] below)

   A. LPS layer present  
   B. peptidoglycan present  
   C. teichoic acids present  
   D. flagella with only two rings in basal body  
   E. endotoxin present  
   F. periplasmic space present

14. Predict the cell wall structure represented here, based on the discussion of Gram + and Gram – cell walls found in your textbook. Draw in the space below the cell wall structure expected for the unknown organism. Label your drawing with all items listed above (A-F) that are appropriate for this type of wall structure.

Take-home message #2: Gram reaction is based on structure of the bacterial cell wall. Other structures of the cell can also vary in relation to the type of wall present.

15. Bacteria possess two sets of enzymes involved in peptidoglycan synthesis. One set is responsible for cell lengthening (elongation enzymes) and the other set is responsible for building the crosswall to separate daughter cells (crosswall enzymes) during cell division. Explain how the relative rates of activity of these two systems can influence the cell shape.
16. At the completion of cell division, autolytic enzymes split cell wall material to allow daughter cells to separate. Provide a guess on why some species of bacteria occur as pairs, chains, and clusters.

17. The arrangement of cells is based in part on the number of planes of division. Cells may divide in a single plane or in more than one plane. Bacteria in chains divide in a single plane so that the chain grows longer with each division. Would the unknown bacterium observed in the Gram stain for Bacterial Anatomy 2 most likely be the product of division in a single plane, in two planes, or in more than two planes? What evidence points to this answer?

Take-home message #3: Cell shape and grouping are due to (1) activity of enzymes involved in cell division, (2) the geometry of division planes, and (3) whether daughter cells fully separate.

18. Display the motility video clip for Bacterial Anatomy 2 by selecting Motility from the View options in the Main Menu bar.

Does the video clip demonstrate true motility or false motility (Brownian motion)? How can you tell?

Take-home message #4: Bacterial motility can be observed through the microscope, and is demonstrated via a video clip in VUMIE 2012™.
19. **Draw and label below how flagella are anchored in Gram + and Gram – cell walls and membranes.**

| Gram + | Gram - |

20. Even when flagella are present, there can be great variation in the number and arrangement of flagella found. However, each species is consistent among its cells for number and arrangement of flagella. **Draw below and label these arrangements of flagella possible in bacteria: monotrichous, lophotrichous, peritrichous.**

   - **Monotrichous:**

   - **Lophotrichous:**

   - **Peritrichous:**

21. When you have completed your work, submit this MDM lab report to your instructor. Exit the Virtual lab.
Activity 5
Bacteriological Media and Growth Patterns

1. Enter VirtualUnknown™ Microbiology’s Virtual Lab and select Growth from the list of predefined subgroups of unknowns. Label the unknown “growth”. Read the Case Study and record the Gram reaction and grouping. Use your textbook and the reference resources provided in the VUMIE 2012 Help files to complete the following questions. Your instructor will advise you on whether to check the box allowing autoinoculation.

2. Complex media are those that cannot be created using chemicals from a chemical stockroom. They contain nutrients that cannot be identified quantitatively or qualitatively (what or how much). Nutrient agar is an example, as its inclusion of “beef extract” cannot be faithfully recreated synthetically. Synthetic media (also called chemically-defined media) are made by mixing a recipe of chemicals such as you find in a chemistry stockroom. They CAN be described qualitatively and quantitatively (which exact chemicals to use and how much of each). Such media as glucose salts broth (glucose and prescribed amounts of mineral salts like potassium phosphate and magnesium sulfate) could be made synthetically, so it is a synthetic medium.

Take-home message #1: Complex media have nutrients that are “complicated” to explain, while synthetic media are chemically-defined and can be synthesized from scratch.

Selective media prevent the growth of some organisms while allowing others to grow. They contain an inhibitor, a chemical that interferes with growth for some microbes with sensitive structures or metabolism. Those not sensitive to the chemical grow normally; those that are sensitive do not. This allows isolation and selection of wanted bacteria without the appearance of colonies of unwanted species. An example would be EMB agar, which allows Gram – bacteria to grow well while preventing the growth of most Gram + bacteria. Differential media do not prevent growth of microbes, but those appearing may give one of at least two distinctly different appearances/outcomes based on their metabolic capabilities. For instance a bacterium using the sugar glucose would make the medium phenol red glucose broth look different from that of a bacterium not able to use that sugar. To make this obvious, differential media contain an indicator that changes color to alert the student to the use of the compound. Microbes not using the sugar would cause the medium to have a different color, and thus be easily distinguishable from the color seen for those that do. Tables with positive and negative results for a variety of bacteria when grown on differential media are very useful for studying the metabolism of unknown bacteria for the purpose of determining their identity. The ID matrix in VUMIE 2012 does just that!

Take-home message #2: Selective media have inhibitors that “select” what can grow on the medium. Differential media have indicators that cause microbes with different abilities to have a different appearance.

Click on the “M” icon to find reference material on the various media included in VirtualUnknown™ Microbiology, and complete the table below to provide descriptions for the following media:

<table>
<thead>
<tr>
<th>Complex or Synthetic?</th>
<th>Selective, Differential, Both, Neither?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malonate broth</td>
<td></td>
</tr>
<tr>
<td>Tryptone broth</td>
<td></td>
</tr>
<tr>
<td>KCN broth</td>
<td></td>
</tr>
<tr>
<td>OF glucose broth</td>
<td></td>
</tr>
<tr>
<td>Mannitol salt agar</td>
<td></td>
</tr>
<tr>
<td>Simmons’ citrate agar</td>
<td></td>
</tr>
</tbody>
</table>
3. The bacteria for this unknown are Gram negative rods. Compare the media in the table above with the dropdown list of media available for conducting tests with Gram negative rods. **Which of the media listed in the table above is/are used for isolating or identifying Gram positive cocci?**

4. **How does the selectivity of mannitol salt agar compare/contrast to the selectivity of malonate broth with regard to (1) Gram positive vs. Gram negative, and (2) among various Gram negatives?**

5. All life needs a source of carbon and a source of nitrogen for metabolism. However, different species have vastly different requirements for the forms those substances take. For instance, plants use carbon dioxide as their form of carbon. That would not support most other forms of life. Groups of microbes have their own preference for organic foodstuffs – from methane to crude oil to the same types of substances that support human life. Likewise, we must get our nitrogen in the form of amino acids obtained from food while some bacteria use the gas nitrogen straight out of the air. Such diversity in carbon and nitrogen sources is species-specific, meaning our knowing what is done by an unknown microbe can be compared to tables of test results to help determine its identity.

Click the “M” button to get to the Media resources and use the info provided to complete the table below:

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Nitrogen Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol red adonitol broth</td>
<td></td>
</tr>
<tr>
<td>Malonate broth</td>
<td></td>
</tr>
<tr>
<td>Christiansen’s urea broth</td>
<td></td>
</tr>
<tr>
<td>KCN broth</td>
<td></td>
</tr>
</tbody>
</table>

**Take-home message #3:** Although all life needs carbon and nitrogen, living things differ in how that carbon and nitrogen is supplied. Because of that, we can begin identifying microbes by discovering what carbon and nitrogen sources they can use and what ones they can’t.

6. Another substance of importance to many forms of life is **oxygen**. Cell metabolism falls into two basic categories – **respiration** (sometimes called oxidation when oxygen is involved) and **fermentation**. Oxidative organisms use the pathways of **glycolysis** and the **TCA (Krebs) Cycle** to oxidize foods down to carbon dioxide, electrons, and protons. The electrons are funneled through the cytochromes and added to protons and oxygen to make water (an electron and a proton make a hydrogen atom). In the process, ATP is made through **oxidative phosphorylation**. Thus, oxidative metabolism involves oxygen.
When oxygen is not available, an alternate pathway called fermentation is often used. ATP is still needed by a cell even if the cytochromes have no oxygen to put electrons into. So, the cell puts those electrons generated through glycolysis into cell compounds and in doing so generates modest amounts of ATP. **Fermenters do not use oxygen or cytochromes for generating energy.**

Some organisms are capable of **either oxidation or fermentation**, depending on whether oxygen is present. When it is, they use it through respiration and when it is not they perform fermentation. It is possible to test whether an unknown organism is an oxidizer or fermenter (or both) by using media with an oil barrier to keep oxygen out. These tests are called the **OF glucose tests**, testing whether glucose can be used in the presence and/or absence of oxygen. OF glucose tests use a differential medium with a pH indicator that changes from green to yellow if acidic products from glucose use are produced.

7. Complete the OF glucose tests for your unknown bacterium by inoculating, incubating, and interpreting the results using OF glucose broth and OF glucose broth with oil overlay. **Based on these results, circle below the type of metabolism that best fits your unknown microbe:**

   Oxidation of glucose  Fermentation of glucose  Both oxidation and fermentation  Neither

   **Take-home message #4: Not all forms of life need oxygen to survive. Some forms of life can use it if present (oxidation) or do just fine in its absence (fermentation). Fermentation technically refers to a process of oxygen-free metabolism, and one of many versions of the process is responsible for alcohol production.**

8. Another factor influencing growth is incubation temperature. Extremes of heat and cold can slow or halt growth altogether. **Provide examples from your own kitchen of the use of extremes in heat and cold to slow or prevent microbial contamination of foods.**

9. Restaurants and grocery stores have to take special precautions to prevent unwanted microbial growth to spoil foods or cause disease. Use Internet resources to find the required temperatures for these two situations:
   - Keeping dairy products cold enough to prevent spoilage
   - Keeping cooked foods hot enough to prevent spoilage

   **Take-home message #5: Microbes grow best in a temperature range close to that found in their natural habitat, and placing them in unnatural conditions retards their growth.**
10. The results of growth can be followed using a spectrophotometer to detect how much light can pass through a broth culture...the more cells, the less light passes through. Resulting growth curves are described in your text. In a growth curve, the number of cells increases logarithmically (exponentially) until a maximum number is achieved – the maximum number that can be sustained by the medium (called maximum stationary phase). Growth conditions that are not optimal result in slower rates of growth. **Using this info, predict the growth rates and graph below the expected results after 18 hours for six sterile tubes of an identical medium inoculated simultaneously with a human pathogenic microbe incubated at the temperatures indicated.**

**NOTE: Growth at 35C has already been plotted for this problem. Your job is to plot growth at the other temperatures.**
Activity 6

Controlling Microbial Growth

1. Much of this exercise is based on material from your lecture notes, text, and resources from the lab (including the Help files of VUMIE™ 2012). Feel free to use these resources to enrich your understanding of how microbial growth can be controlled.

2. Enter the Virtual Lab and select Controlling Growth from the list of predefined unknowns. Give this unknown the name “controlgrowth” (no spaces allowed in labels for unknowns). Check the box to allow autoinoculation. When you select your inoculation tool and right click to remove the caps/lid from the tube or plate, the option will appear to automatically inoculate your medium, bypassing the manual aseptic transfer process from inoculum to medium. This information is shown in the Virtual Lab Report, so only check this box if your instructor requests you do so.

3. Controlling microbial growth is of great importance in preventing food spoilage. Each year, more food is lost to spoilage and insect damage than is consumed by the world’s population. To meet the growing demands for food worldwide, we must find ways to prevent food loss. One of the oldest food preservatives is salt. How does salt control microbial growth and reduce spoilage?

4. Click on the Media references icon (designated by the letter “M”) to display the media for identifying bacteria. [Or, you might want to go to Help and search for “salt” or “NaCl”]. What two media include high concentrations of sodium chloride for preventing growth of most bacteria?

5. Adding salt to preserve foods is least likely to be effective against bacteria from which of the following? (Circle all correct answers)

   Bacteria found in your “gut”    Bacteria found on your skin    Bacteria found in your mouth

6. Nitrite is often used as a preservative in cured meats. Sodium nitrite keeps meat looking red and prevents the growth of anaerobic bacteria like *Clostridium botulinum*, as well as less dangerous spoilage bacteria.

   A test used in the micro lab detects the presence of nitrates as products of metabolism. Some bacteria form nitrates during a process called anaerobic respiration – electrons are added to nitrate instead of oxygen and the product is nitrates instead of water. The nitrate reductase test is used in the identification of many bacteria. Information on the nitrate reductase test can be found by clicking on the “T” icon.
a. Which microbes would be more likely to be inhibited by nitrites in food?

   Oxidizers                Fermenters

b. Based on your understanding of the nitrate reductase and nitrite reductase tests, why would the inclusion of nitrite to foods inhibit spoilage by anaerobic bacteria? (this is a hard question!)

7. The potent poison potassium cyanide (KCN) is lethal to humans when ingested. Yet, we find it as a growth medium for some bacteria. Why is cyanide toxic to humans but not to some bacteria? How is it metabolized by bacteria?

8. List below two culture media employing an inhibitor to prevent the growth of unwanted microbes. Explain how each inhibitor accomplishes its task and state what microbial group is inhibited.

   Take-home message #1: Altering growth temperature can control microbial growth rates, and altering the growth medium by adding inhibitory chemicals can do so also.

9. Sometimes microbial growth can be controlled by omitting key nutrients from a medium. Creating selective media like this is commonly done for making media useful in isolating and identifying target bacteria. For instance, omitting all carbon sources other than a single, hard-to-use nutrient can prevent growth of some common bacteria while allowing others of interest to the researcher to flourish. List below two media that are selective because they contain a sole source of carbon that is not used by all bacteria.

   Take-home message #2: Omitting key nutrients from a medium can also control microbial growth.

10. Complete all work as assigned by your instructor and close the Virtual Lab.
Activity 7  Antimicrobial and Chemotherapeutic Agents

1. Enter the Virtual Lab and select Antimicrobials from the list of predefined unknowns. Label the unknown “antimicrobials”. Your instructor will tell you whether to check the box permitting autoinoculation. Use the information in VirtualUnknown™ Microbiology and other resources to answer these questions.

2. Complete the following tests and fill in the table below with the results you recorded in your lab report for this organism. Remember to use a swab when creating your bacterial lawns on nutrient agar.

<table>
<thead>
<tr>
<th>Antibiotic or Chemical</th>
<th>Zone of inhibition observed? (yes/no)</th>
<th>Resistant or Sensitive? (yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novobiocin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacitracin – 2 units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacitracin – 0.04 units</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. What is the mode of action for each of these antibiotic chemicals?
   - Novobiocin
   - Bacitracin

4. Locate a table in a reference book that lists common antibacterial drugs and their modes of action. Fill in the table below to indicate whether representatives of each class of antibiotics might be effective for treating an illness caused by this microbe.

<table>
<thead>
<tr>
<th>Class</th>
<th>Macromolecular Synthesis (or Cell Process) Inhibited (mode of action)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins and Cephalosporins</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides and Tetracyclines</td>
<td></td>
</tr>
<tr>
<td>Macrolides and Streptogramins</td>
<td></td>
</tr>
<tr>
<td>Rifamycins</td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
</tr>
<tr>
<td>Sulfonamides</td>
<td></td>
</tr>
</tbody>
</table>
5. People also have 70S ribosomes in the mitochondria found in nearly every cell. **Why do antibiotics that interfere with 70S ribosome function (and thus protein synthesis) kill bacteria but not the patient?**

6. When bacterial DNA is replicated, the closed circles DNA of the two daughter genomes are interconnected, like links of a chain. **How are they separated into two daughter cells? What antibiotics exploit this process to control microbial growth?**

7. Some antimicrobial agents imbed in membranes producing unregulated channels between the cell and its environment. **Based on your understanding of semipermeable membranes, how could a chemical like this cause the death of the bacterial cell?**

*Take-home message #1: Antibiotics work to disrupt normal cell metabolism by a variety of strategies.*

8. Continue with testing to identify this unknown organism. **What is its identity?**

9. **What are some common diseases caused by this microbe?**

*Take-home message #2: It is not uncommon for a single pathogen to cause a variety of diseases.*

10. Complete and submit all work as assigned by your instructor and close the Virtual Lab.
Activity 8 Identifying Gram Negative Rods

Identifying bacteria is a common activity in the microbiology lab. The process is much like playing the game Clue™ – each time you gather a piece of information, it supports some identities and eliminates others from consideration. The process continues as you gather more information until only one microbe remains and all others have been eliminated as possibilities. Thus, identification of microbes is a process of elimination based on logic and carefully-performed tests to determine the capabilities of the unknown microbe.

The pieces of evidence used in the microbiology lab are your observations of the shape, morphology, Gram reaction, and the results from biochemical tests performed using a pure culture of the unknown organism. Results are compared to tables of known results for all bacteria with similar shape, morphology and Gram reaction to see where similarities and differences occur. Such tables in VirtualUnknown™ Microbiology can be accessed by clicking “Identification Matrix” under the “View” option in the lab. Displayed will be tests along the left and the microbes still possible (based on the results you have input) across the top. Every time you record a test result, the Identification Matrix shrinks as the columns with results that directly conflict with those you record for your unknown are eliminated from the table. Microbes with results that agree with those you record (or that are displayed as [+] , d, or [-]) remain possibilities and more testing is needed.

A way of gauging how much progress is being made is to observe the Virtual Lab Report to see how each test result reported increases the number of microbes eliminated. Ideally, every test conducted would reduce the number of possible microbe IDs in half (just as each round of the “March Madness” basketball tournament reduces the number of possible national champions in half). Smart testing reduces the number of tests needed to provide an identification. The results at right show useless tests!

The job of the microbiologist is to determine which tests are useful for skillfully reducing the number of possible identities until only a single name remains. That name is the identity of the unknown – the only microbe with test results identical to those you recorded.

1. Open the Virtual Lab and obtain a new unknown. Select “Enteric Bacillus” from the Exercise Topic or Subgroup dropdown box and provide the identifier “gramnegativerod”, since all enteric bacilli found in the software are Gram negative rods. Read the Case Study and record the Gram stain results.

2. Move the Virtual Lab to the right side of your screen. Select the Identification Matrix from the View dropdown box. Resize the matrix and place it to the left of the Virtual Lab.
3. It is not possible to see all tests or organisms in the matrix at this point - too many columns, too many rows! However, the number of possible IDs for your unknown microbe can be reduced significantly through the results of a few standard tests that are the starting point when tackling the identification of Gram negative rods. As you record results for these tests in the Virtual Lab, you will quickly see columns disappear as bacteria with conflicting results are eliminated from the matrix. These basic tests leave you with a manageable number of possibilities that can be pared down by a few obvious tests to give you a definitive ID. Consulting the ID Matrix and Help files to learn how to perform the tests gives you a fair shot at completing the ID quickly and easily.

4. A good starting point would be to use the Help Files, particularly the “Show Me How To…” files related to identifying bacteria. Work through the Help files and you will find a recommended approach to get started on the ID process for identifying your Gram negative enteric bacillus. What tests would you perform initially to start you on your way?

Take-home message #1: There are some standard tests to start with when identifying Gram negative bacilli that quickly whittle the number of possible microbes down to a manageable number.
5. Oxidase and O-F glucose help distinguish between “non-fermenters” (like the Pseudomonads) and the enteric organisms found in VirtualUnknown™ Microbiology. If you had a true unknown in a test tube rather than one generated by the software, these tests would be among the first attempted to help determine which tests to undertake next – those for non-fermenters or those for enteric organisms. Why are the Oxidase and O-F glucose tests of no practical value for identifying your virtual Gram negative enteric bacillus?

6. For fun, click on Unknown and select Record Results. We are going to assign results without doing the tests just to get a feel for the way the ID Matrix responds to your input of results. Find “Indole” in the list of tests and record the result “Positive”. Watch as the number of columns is reduced by the results you enter.

7. Next, repeat this process to record results: “+” for Methyl Red, “-” for Voges-Proskauer, “-” Citrate Utilization, “+” for Lactose Fermentation. What happens to the matrix?

8. How many unknown microbes remain? What test(s) would you do next to cut that number of possible organisms in half?

9. Try changing the result for Indole from positive + to negative -. What does this do to the matrix? How is this information helpful to you if you have recorded a test result incorrectly?

Take-home message #1: When you change a test result, the matrix is recalculated to display possible microbes based on the new information – anything eliminated by the changed result is added back in.

10. Complete and submit all work as assigned by your instructor and close the Virtual Lab.
Activity 9

Oxidase and O-F Glucose Tests

The oxidase test chemically detects the presence of the terminal cytochrome (also known as cytochrome oxidase) in the electron transport system (ETS). Cytochromes are complex proteins that participate in the disposal of electrons taken from molecules as they are oxidized in metabolism. Cofactors like NAD and FAD transport the electrons to the membranes where they are deposited in the cluster of cytochrome proteins. There, electrons are moved one at a time through the cytochromes until they are put into a final electron acceptor by the terminal cytochrome. This process generates energy for driving ATP production in the cell.

In aerobic respiration, the final electron acceptor is oxygen, which is combined with the electrons and protons to make water (two electrons and two protons and \( \frac{1}{2} \) O\(_2\) make H\(_2\)O). In anaerobic respiration, the final electron acceptor is another inorganic molecule – nitrate or carbonate or a sulfur compound – and the final product is something other than water. The reason humans need air to survive is so that the final electron acceptor (oxygen) is supplied to the terminal cytochrome of every mitochondrion in all our cells for aerobic respiration. The reason why anaerobic organisms can still participate in respiration is because they use one of the alternate final electron acceptors instead. The reason why some bacteria are able to live with or without oxygen is because they can switch in between final electron acceptors based on what is available at the time. In the oxidase test, the oxidase reagent serves as a substitute final electron acceptor and changes color when electrons are added by cytochrome oxidase.

O-F glucose represents a less specific, but equally important test for knowing the metabolic patterns of an unknown organism. The test is really quite simple. Bacteria are placed in a complex medium containing glucose as a carbon source and an indicator that changes colors based on pH of the medium. If the bacterium can use the sugar glucose, acid products accumulate and the pH will drop and the medium will turn from its neutral green color to yellow. Two tubes are used – one with the medium exposed to air and one with an oil overlay to prevent air from entering the medium. In this way, a microbe can be tested for its ability to use the sugar in the presence and/or absence of oxygen. The results label the organism as an oxidizer (with air), a fermenter (without), or both.

In this exercise, you will learn how to conduct these tests in the Virtual Lab. The mechanism for conducting them in the wetlab is very similar.

1. Open the Virtual Lab and click New to select Enteric Bacillus from the topic or subgroup list, then provide it with the name “OFOxidase”. Read through the case study and provide a Gram stain interpretation to enter the Virtual Lab.

2. Click on the “T” icon and select the Oxidase Test from the Help files. Read through the test material to familiarize yourself with how the test is performed. How does the method for performing this test differ from most other Virtual Lab tests?

3. Go to the “View” option above the lab and select Oxidase Video. Appearing will be a results input box and a popup video showing the Oxidase test being performed.
4. The test begins by collecting microbial growth using a non-metallic instrument. This is transferred to a paper strip impregnated with Oxidase reagent.

5. Use information from the Help files to guide your interpretation of the results. Record the results.

6. Open the ID Matrix and locate “Oxidase” in the list of tests. Note that the result you recorded is shown in the left-most test column and that no bacteria with directly conflicting results remain in the matrix.

7. Close the ID Matrix and open the Virtual Lab Report. Note the recording of the result you entered for the Oxidase test and mention of the number of microbes eliminated by this information. Was this test useful in your ID of the unknown? Explain.

8. Now we will take a look at the O-F glucose tests.
   a. Select OF glucose broth from the Media list. Label the tube “OFglu”. What pH indicator is responsible for its green color?

   b. Select the “wire” from the Tool menu. Using a loop would tear the agar added to the medium to reduce saturation with oxygen, preventing its use to follow sugar use in anaerobic conditions.
c. Perform an aseptic tube-to-tube transfer of inoculum from the culture tube of unknown to the sterile OF glucose medium. Be sure to flame the inoculating wire and the mouths of the tubes before and after the transfer is made. If you need help with this process, refer back to the exercise on tube-to-tube transfers, the Tutorial for the software, or the “Show Me How To…” Help files. Remember that you can monitor your success in performing a good aseptic transfer by following the progress of the traffic signals in the upper corner above the Virtual Lab.

d. Once the OF glucose broth tube has been inoculated, place it in the 37° C incubator.

e. Next, select OF glucose with oil from the Media list and label the tube “OFgluOIL”. The sterile medium that appears has an overlay of sterile mineral oil. Autoclaving drives all oxygen out of the agar and the oil prevents it from seeping back in. Only bacteria that can use glucose in the absence of oxygen will be positive for this test.

f. Inoculate this medium as you did the other OF glucose medium and place it in the 37° C incubator. Click New Day to complete incubation of the two tubes.

g. Remove the OF glucose broth tube from the incubator and record the result. Remove the OF glucose broth with oil overlay tube from the incubator and record the result. What results did you record for these two tests?

| OF Glucose | + | - | OF Glucose with Oil Overlay | + | - |

h. Based on these results, is use of glucose by this unknown dependent on the presence of oxygen?

Yes
No

i. Which of these best describes this unknown? (Circle)

Oxidizer
Fermenter
Oxidizer and Fermenter depending on presence of O₂

j. Complete and submit this as assigned by your Instructor. Then, exit the Virtual Lab.
One of the most informative media used for the identification of Gram negative bacilli is **Triple Sugar Iron Agar (TSIA)**. This medium contains three sugars (thus the name) and an iron compound for detecting hydrogen sulfide (H₂S) production. The medium is an agar dispensed into a test tube, sterilized, and tilted to solidify on a slant. It contains the indicator phenol red to detect pH changes resulting from sugar use. Thus, it is a differential medium. **The TSIA test will allow determination of whether glucose is used, whether gas is produced during the process, whether hydrogen sulfide is a product of metabolism, and whether either lactose or sucrose can be used (though it does not tell which disaccharide sugar is used).**

If **glucose** is used by the unknown bacterium, **acid products** are generated in the butt of the tube and change the pH indicator **phenol red** from its normal red color to **yellow**. The slant may remain red or turn hot pink. However, if both glucose AND either lactose or sucrose (or all three) are used, the entire tube may be changed to yellow. Such a condition would be described as **“acid over acid”** – yellow slant over a yellow butt. It would not be possible for a bacterium to use lactose or sucrose without also using glucose, so glucose use (and a yellow butt) represents the minimum typical basic sugar metabolism possible for bacteria that can use sugars.

Some bacteria produce gases like carbon dioxide during sugar digestion. **Gas production** results in either cracks in the agar or movement of the agar from the bottom of the tube toward its mouth. **Hydrogen sulfide** production results in the appearance of **black discoloration** of the agar due to the production of iron (II) sulfate (FeS) when the gas interacts with the iron compounds included in the medium. Thus, a variety of color changes and alterations of the media are possible, providing a wealth of information central to identification of unknown Gram negative bacteria.

A previous exercise included a discussion of the oxidase test and O-F glucose for studying oxidation and fermentation by bacteria. **Non-fermenters detected with the O-F glucose test would also be incapable of using glucose in TSIA, resulting in a hot pink butt** instead of the yellow that would appear if the glucose was metabolized to produce acid products. Thus, many labs prefer to **begin the identification** of an unknown Gram negative rod by inoculating TSIA. The color of the butt can help determine whether an enteric bacillus or a non-fermenter is present.

Practice with inoculation and interpretation of TSIA will help prepare students for identifying Gram negative bacilli.

1. Open the Virtual Lab and select **Media Tests 1** from the list of subgroups of unknown organisms. Provide the Unknown Identifier “MT1”. Read through the Case Study and interpret the Gram stain. This organism is **Escherichia coli (E. coli)**, one of the more infamous enteric organisms. Ask your instructor about allowing auto-inoculation.

2. Select **Triple Sugar Iron agar** from the Media list. Label the tube “tsia”. Appearing in the Virtual Lab will be a tube of inoculum and a TSIA agar slant.
3. **Right click** on the Bunsen Burner to light it. Then, select the *inoculating wire* from the Tool list (TSIA contains agar!). Flame your wire. You may review Tube-to-Tube transfers via Help or a look at a previous exercise.

4. **Right click** on the TSIA tube to remove caps. Flame the mouths of the tubes, then use the inoculating wire to transfer the inoculum from the unknown culture to the sterile TSIA medium. Be sure to monitor success of the transfer with the traffic signals at the upper right. Contaminated? Discard the tube and **repeat the test** with TSIA until you correctly complete the aseptic transfer.

5. **Place** the tube in the 37° C incubator and click “**New Day**”. Take the incubated TSIA tube out of the incubator and note its appearance. Right click on the tube and select “**Record Results**”.

6. A closeup of the TSIA tube is provided with fields for you to input results for (1) hydrogen sulfide production, (2) acid from glucose, and (3) gas from glucose. **Why does it make sense that no fields are provided for recording whether lactose and/or sucrose are also used?**

7. The image shows the red medium has been changed to uniformly yellow, and cracks have formed as the agar has been pushed up from the butt of the tube. Interpret these observations and record the results in the software. **Record the results observed in the software and in the table on the next page under “MT1”.**
8. Open the Identification Matrix and note that the results are displayed in the left-most column. If you have the ID Matrix open while you record your results, columns for bacteria with conflicting results will disappear as each result is recorded.

9. Once you have recorded your results in the table below, create three additional unknowns (Media Tests 2, Media Tests 3, and Media Tests 4) and repeat the TSIA test for each, adding the results to the table.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acid from glucose (+/-)</th>
<th>Gas from glucose (+/-)</th>
<th>H$_2$S production (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1 – <em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT2 – <em>Enterobacter agglomerans</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT3 – <em>Salmonella typhi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT4 – <em>Proteus vulgaris</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Which of the four microbes is/are not likely to be positive for lactose or sucrose use? Explain your reasoning.

11. Which (if any) produce H$_2$S?

12. Complete and submit all work assigned by your instructor and exit the Virtual Lab.
The IMViC series is a collection of four biochemical tests that provide important information for identifying enteric bacteria – bacteria that ferment glucose and are frequently associated with the gastrointestinal tracts of animals. The acronym is derived from the names of the four tests: Indole, Methyl Red, Voges-Proskauer, and Citrate.

The combination of the results of the four tests quickly reduces the number of possible identities to a manageable number of related organisms. It is not uncommon for the number to be reduced from the original 70+ to less than a dozen after these results alone are input into the software.

The **Indole test** looks for the production of indole, the chemical that gives feces its typical odor. Bacteria that are indole positive have enzymes that can digest the amino acid tryptophan to produce indole. The **Methyl Red test** detects the products of glucose metabolism, particularly whether a mixture of acids are produced that drop the pH of the growth medium to below pH 4.5 (known as **mixed acids fermentation**). Voges-Proskauer looks for production of a molecule called acetoin (acetylmethylcarbinol), a neutral fermentation product from glucose. Because acetoin has a neutral pH, enteric bacteria are typically positive for one or the other (Methyl Red or Voges-Proskauer but not both). **Citrate test** determines whether the unknown can use citrate as its sole carbon source for growth. The results of these four basic tests are central to identifying enteric organisms.

In the exercise below, these tests will be performed and the results studied.

1. Enter the VirtualUnknown™ Microbiology’s Virtual Lab and select Media Tests 1 (MT1) from the list of predefined unknowns (or MT1 from your list of unknowns that was created for the previous exercise). This organism is *E. coli*. Your instructor will advise you on whether to check the box permitting autoinoculation.

2. Complete these tests following the instructions provided.

**Indole Test**

a. Select Tryptone broth from the Media list and perform a tube-to-tube transfer from the unknown culture.

b. Inoculate and place the inoculated tube in the 37°C incubator and click "New Day" to incubate the culture. Remove it from the incubator.

c. This test requires added reagents to read results. Select the dropper from the Tool list and then locate Kovac’s reagent from the Reagent list. This chemical is added to the overnight culture of unknown to complete the indole test.

d. Remove the tube cap and position the dropper over the mouth of the tube. Click once to dispense the Kovac’s reagent into the tube. An instant change in the tube should occur, indicating a reaction between the reagent and indole in the tube.
e. Right click on the tube to select “Record Results”. A closeup of the culture appears to help with interpretation. If you need help understanding with this test, click the “T” icon and select Indole test from the list. **Record your results in the software and also in the table on page 47.**

f. There are several biochemical tests in this software (and in the wetlab) that are conducted in the same manner – **inoculate** the medium for the test, **incubate** overnight, and then **add reagents** that will react to the presence of the chemical being tested for with a color change that reveals its presence.

**Methyl Red Test**

a. Select **MRVP broth** from the Media list and perform a **tube-to-tube transfer** from the unknown culture.

b. Place the inoculated tube in the 37C incubator and click “**New Day**” to incubate the culture overnight. Remove it from the incubator.

c. This test also requires **added reagents** for color changes indicating the result. Select the **dropper** from the Tool list and then locate **Methyl Red reagent** from the Reagent list. This chemical is added to the overnight culture of unknown to complete the methyl red test.

d. Remove the tube cap and position the dropper over the mouth of the tube. Click once to dispense the Methyl Red reagent into the tube. An instant change in the tube should occur, indicating a reaction between the reagent and mixed acids in the tube.

e. Right click on the tube to select “**Record Results**”. A closeup of the culture appears to help with interpretation. If you need help in understanding this test, click the “T” icon and select Methyl Red test from the list. **Record your results in the software and also in the table on page 47.**

f. **What pH indicator and conditions (pH range) are responsible for the color change shown?**
Voges-Proskauer Test

a. Repeat the procedure just performed for the Methyl Red test, as the Voges-Proskauer (VP) test uses the same medium (MRVP broth) as the Methyl Red test. Inoculate the tube of MRVP broth and incubate overnight at 37°C. Remove the culture from the incubator.

b. Again, here is a test requiring addition of a reagent. Select the dropper from the Tool list. For the VP test, you must add two reagents – Barritt’s A and Barritt’s B. Select Barritt’s A and add it to the culture tube. Then select Barritt’s B and add it also. After addition of Barritt’s B, a color change to red signifies the presence of acetoin in the culture medium. In the wetlab, this color change may take half an hour. Here, it happens instantly.

c. Right click on the tube to select “Record Results”. A closeup appears to help with interpretation. If you need help understanding with this test, click the “T” icon and select Voges-Proskauer test from the list. Record your results in the software and also in the table on page 47.

Citrate Utilization Test

a. Perform a tube-to-tube transfer of inoculum from the unknown culture to a Simmons Citrate agar slant. Incubate the culture overnight at 37°C, and then remove it culture from the incubator.

b. There is no need to add reagents to this test. If the unknown microbe can use citrate as its sole carbon source, it will grow on the slant and the medium will turn from green to blue. Any other result is a negative result.

c. Right click on the tube to select “Record Results”. A closeup of the culture appears to help with interpretation. If you need help understanding with this test, click the “T” icon and select Citrate Utilization test from the list. Record your results in the software and also in the table below.

3. Repeat the above assignment by conducting these same four tests for the different microbes assigned for the following unknowns: Media Tests 2 (MT2, Enterobacter aerogenes); Media Tests 3 (MT3, Salmonella typhii); and Media Tests 4 (MT4, Proteus vulgaris). These can be selected from the list of predefined unknowns. Fill in the table with the results for all four microbes.

<table>
<thead>
<tr>
<th>Test</th>
<th>E. coli Media Tests 1</th>
<th>Enterobacter aerogenes Media Tests 2</th>
<th>Salmonella typhii Media Tests 3</th>
<th>Proteus vulgaris Media Tests 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl red test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voges-Proskauer test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate utilization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. What is the product being detected in a positive indole test? Where does it come from?

5. What is the chemical composition of Kovac’s reagent?

6. Why does the Kovac’s reagent float atop the broth culture?

7. What metabolic products are being detected in a positive methyl red test? Where do they come from?

8. Predict the expected result for the methyl red test of an organism that cannot ferment glucose. Explain.

9. What is the product being detected in a positive Voges-Proskauer test? Where does it come from?

10. What is the chemical composition of the reagents used for the VP test?
    Barritt’s A
    Barritt’s B
11. What is the metabolic ability being detected in a positive citrate test?

12. Why is a reagent unnecessary for completing the citrate test? How is citrate utilization detected?

13. The IMViC results are often presented as a “package” and seen as indicative of a particular group of bacteria. For instance, common enteric bacterium *E. coli* most often yields the results [++]-, where the tests are presented in the order of indole, methyl red, Voges-Proskauer, and citrate. **Give the patterns for the other microbes tested:**

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Indole</th>
<th>Methyl Red</th>
<th>Voges-Proskauer</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

14. Submit this MDM lab report and any Virtual Lab Reports requested by your Instructor before exiting the Virtual Lab.
One of the most useful types of metabolic tests for identifying bacteria is the carbohydrate utilization test. Bacteria are placed in a medium containing a sugar and pH indicator and incubated overnight. If the bacterium can use the sugar, acid products form that change the pH indicator color. Using this system, a variety of sugars can be substituted in the same simple medium to test the abilities of an unknown organism. It is not uncommon for several sugar fermentation tests to be included in the battery of tests conducted in an identification.

Below you will practice inoculating, incubating, and interpreting the results for sugar fermentation tests for bacteria (as done in the Virtual Lab).

1. Enter the VirtualUnknown™ Microbiology's Virtual Lab and select Media Tests 1 (MT1) from the list of predefined unknowns. This organism is Escherichia coli. Your instructor will advise you on whether to check the box allowing autoinoculation.

2. Complete the tests listed in the table on page 52 and record the results in the software AND in the table. Note that information about the media used can be accessed by clicking on the "M" icon, and information on the tests can be accessed by clicking on the “T” icon. Record the results you observe in the software, and answer these questions as you proceed:

   a. What is the main medium used for determining production of acid and gas from glucose?

   b. What is the medium’s initial color?

   c. What chemical is responsible for the color?

   d. What color change indicates a positive test result? Provide an explanation for the color change in a positive test.

   e. What is the initial pH of the medium?

   f. Estimate the final pH of the medium in a positive test.

   g. What is the purpose of the Durham tube?
3. **Repeat the above assignment** for the different microbes assigned for each of the following predefined unknowns:
   - Media Tests 2: MT2, *Enterobacter aerogenes*;
   - Media Tests 3: MT3, *Salmonella typhii*; and

For each, perform the tests listed below:

**Fill in this table with the results for all four microbes. Print out and attach the Virtual Laboratory Reports for all four microbes:**

<table>
<thead>
<tr>
<th>Test</th>
<th>E. coli Media Tests 1</th>
<th>Enterobacter aerogenes Media Tests 2</th>
<th>Salmonella typhi Media Tests 3</th>
<th>Proteus vulgaris Media Tests 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid from glucose:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas from glucose:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose fermentation test:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose fermentation test:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** For many of these tests, these four bacteria show more similarities than differences. Do not be alarmed if all bacteria share the same capability for using a sugar. Differences will emerge with more tests being performed over a broad range of sugars.

4. Use this table, the “T” Reference Library in VirtualUnknown™ Microbiology, and the information in your textbook and laboratory manual to answer the following questions:

   a. All four bacteria were able to metabolize glucose, but not all produced identical results for the other sugars. If all four bacteria were able to metabolize glucose, explain why all four were not able to metabolize lactose and sucrose.
b. How many carbohydrate fermentation tests are supported in VirtualUnknown™ Microbiology?

c. Negative tests indicate the sugar was not metabolized to produce acids. How can growth be accomplished in a carbohydrate test medium when the sugar is not metabolized?

d. Both phenol red glucose broth and OF glucose broth can be used to determine whether glucose can be metabolized. How are these two tests similar and how do they differ?

e. Predict the results for the TSIA slant for each of the four bacteria tested above, based on their sugar fermentation patterns.

<table>
<thead>
<tr>
<th></th>
<th>E. coli Media Tests 1</th>
<th>Enterobacter aerogenes Media Tests 2</th>
<th>Salmonella typhi Media Tests 3</th>
<th>Proteus vulgaris Media Tests 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color of TSIA Slant:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color of TSIA Butt:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cracks in (or Vertical Displacement of) Agar:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Complete and submit this exercise as assigned by your instructor.
Not all nutrients supporting microbial growth are sugars. Often, proteins supply the carbon and nitrogen needed for growth. They are digested extracellularly by proteases into amino acids that can be transported into the microbes and metabolized internally to enter common metabolic pathways for energy production (glycolysis and TCA cycle) and to provide amino acids and other small molecules for growth.

Several biochemical tests for identifying bacteria focus on the metabolism of amino acids. Below are those tests, presented to enable practice and further understanding of microbial metabolism.

1. Use the VUMIE 2012 reference library, your textbook, and your lab manual to provide information on the tests, media, and reagents required to complete this assignment.

2. Enter the VirtualUnknown™ Microbiology’s Virtual Lab and select Media Tests 1 (MT1) from the list of predefined unknowns. This is E. coli. Your instructor will advise you on whether to use autoinoculation.

3. Complete the tests listed in the table below, recording the results for E. coli in the software and the table below. Information on how the tests are accomplished and interpreted can be found by clicking the “T” icon above the lab.
   a. Arginine dihydrolase test, lysine decarboxylase test, and ornithine decarboxylase test use similar media and are performed in the same manner. Be sure to note that appearance of these tests must be observed at 24- and 48-hours in order to accurately interpret the results.
   b. Phenylalanine deaminase test uses a different type of medium and is performed in a different manner.

4. Repeat the above assignment for the different microbes assigned for each of the following Textbook Exercises:
   Media Tests 2: MT2, Enterobacter aerogenes;
   Media Tests 3: MT3, Salmonella typhii; and
   Media Tests 4: MT4, Proteus vulgaris.

Fill in the table with the results for all four microbes. Print out and attach the Virtual Laboratory Reports for each of the four microbes:

<table>
<thead>
<tr>
<th>Test</th>
<th>E. coli Media Tests 1</th>
<th>Enterobacter aerogenes Media Tests 2</th>
<th>Salmonella typhii Media Tests 3</th>
<th>Proteus vulgaris Media Tests 4</th>
</tr>
</thead>
</table>
Below are some questions relating to the amino acid tests used for bacterial identification:

5. **What ingredient is responsible for the initial color of lysine decarboxylase broth?**

6. **How does this ingredient allow detection of the metabolism of lysine by the microbe? Why must this test be observed at 24 and 48 hours?**

7. **Indicate the expected appearance for lysine decarboxylase (LDC) broth in a positive (+) LDC test:**

<table>
<thead>
<tr>
<th></th>
<th>Color of medium</th>
<th>Indicated pH of medium</th>
<th>Medium Ingredient Metabolized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated broth</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Observation at 24 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation at 48 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Below are the structures for lysine and ornithine. **Complete the chemical reactions for each, showing the products of the enzymes in positive decarboxylase tests and labeling the products:**

\[
\text{NH}_2 \quad \text{(Lysine)} \\
\text{H} - \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_2 \\
\downarrow \quad \text{COOH}
\]

\[
\text{NH}_2 \quad \text{(Ornithine)} \\
\text{H} - \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_2 \\
\downarrow \quad \text{COOH}
\]
9. In contrast to the decarboxylase and dihydrolase media just used, phenylalanine agar for the phenylalanine deaminase test is similar in appearance to routine culture media like nutrient agar. **How can this medium without a colored indicator be useful for detecting metabolism of phenylalanine?**

10. **Why must reagents be added to the phenylalanine agar slant after, rather than before, incubation?**

11. **List the two reagents added to complete the phenylalanine deaminase test, and explain how each contributes to the determination of phenylalanine deamination.**

12. Decarboxylases remove carboxyl groups. This enzyme, however, is a **deaminase**. With this in mind, complete the reaction below to show the structure of the product of phenylalanine deaminase, and provide its name.

\[
\begin{align*}
\text{NH}_2 & \quad \text{(Phenylalanine)} \\
H - C - CH_2 - \text{(C}_6\text{H}_5) & \quad \text{COOH} \\
\end{align*}
\]

13. **How might deaminases be central to the use of peptides or proteins as the sole source for carbon and energy for bacteria and other organisms?**

14. Complete and submit your results for this exercise as requested by your instructor.
As mentioned in Exercise 8, “Identifying Gram Negative Rods”, identifying bacteria is a common activity in the microbiology lab. Like the game Clue™, each time you gather a piece of information to solve the mystery, you gather some information that supports some identities and eliminates others from contention. In the lab, the process continues as you gather more information until only one microbe remains and all others have been eliminated as possibilities. Thus, identification of microbes is a process of elimination based on logic and carefully-performed tests to determine the capabilities of the unknown microbe.

The pieces of evidence used in the microbiology lab are your observations of the shape, morphology, Gram reaction, and the results from biochemical tests performed using a pure culture of an unknown organism. Results are compared to tables of known results for all bacteria with similar shape, morphology and Gram reaction to see where similarities and differences occur. Such tables in VirtualUnknown™ Microbiology can be accessed by clicking “Identification Matrix” under the “View” option in the lab. Displayed will be tests along the left and the microbes still possible (based on the results you have input) across the top. Every time you record a test result, the Identification Matrix shrinks as the columns with results that directly conflict with those you record for your unknown are eliminated from the table. Those microbes with results that agree with those you record (or that are displayed as [+], d, or [-]) remain possibilities and more testing is needed.

A way of gauging how much progress is being made is to observe the Virtual Lab Report to see how each test result reported increases the number of microbes eliminated. Ideally, every test conducted would reduce the number of possible microbe IDs in half (just as each round of the “March Madness” basketball tournament reduces the number of possible national champions in half). Smart testing reduces the number of tests needed to provide an identification. Careless testing includes tests that are useless – see the lab report at right!

The job of the microbiologist is to determine which tests are useful for skillfully reducing the number of possible identities until only a single name remains. That name is the identity of the unknown – the only microbe with test results identical to those you recorded. Here is how it is started for Gram + cocci.

1. Open the Virtual Lab and obtain a new unknown. Select “Gram Positive Coccus” from the Exercise Topic or Subgroup dropdown box and provide the identifier “gramposcoccus”. Read the Case Study and record the Gram stain results.
2. Move the Virtual Lab to the right side of your screen. Select the Identification Matrix from the View dropdown box. The matrix will fill the screen. In the upper right is an icon allowing you to either resize or minimize the ID Matrix to the tray below the lab. Resize the matrix and place it to the left of the Virtual Lab. Your setup should look like this:

![Matrix and Virtual Lab]

3. It is not possible to see all tests or organisms in the matrix yet - too many columns, too many rows! However, the number of possible IDs for your unknown microbe can be reduced significantly through the results of a few standard tests that are the starting point when tackling the identification of Gram positive cocci. As you record results for these tests in the Virtual Lab, you will quickly see columns disappear as bacteria with conflicting results are eliminated from the matrix. These basic tests leave you with a manageable number of unknowns that can be pared down by a few obvious tests to give you a definitive ID. Consulting the ID Matrix and Help files to learn how to perform the tests gives you a fair shot at completing the ID quickly and easily.

4. A good starting point would be to use the Help Files, particularly the "Show Me How To..." files related to identify bacteria. Work through the Help files and you will find a recommended approach to get started on the ID process for identifying your Gram positive coccus. What tests would you perform initially to start you on your way?
5. A key “first test” used in a clinical setting is evaluating growth characteristics on 5% sheep blood to detect presence of enzymes that destroy and digest blood cells and heme. Find the Help file information on blood hemolysis to learn more about this test. **What are the three possible results for the test?**

6. Just for fun, click on Unknown and select Record Results. We are going to assign results without doing the tests. Find Beta Hemolysis and record a result for this as positive (+). Watch as the number of columns is reduced by the results you enter. **Why would growth characteristics on blood agar be an important start for identifying bacteria in a hospital lab?**

7. Repeat the manual input of test results for the **bile esculin slant**. Record both as positive. Note the change in the number of columns as these results are entered. Look up “bile” to see where it is produced and what it is used for by the body. **Why are positive results with bile esculin important information toward understanding the normal ecology of the organism being tested?**

8. **What would be the next test you would perform on the unidentified Gram positive coccus to help provide a quick identification?**

9. Complete and submit this exercise as assigned by your instructor.

10. In the exercises to come, you will use the same logic with real test results to identify unknown Gram positive cocci.
One of the first tests often conducted with bacteria isolated from a hospital setting is determining the pattern for blood hemolysis. Initial isolation of bacteria is very often accomplished on blood agar to encourage growth of all organisms – many disease-causing organisms are notoriously fastidious and cannot be recovered from clinical samples without the especially rich nutrients supplied by 5% sheep blood added to a basic nutrient agar.

However, differences in abilities for bacteria growing on blood agar result in differences in appearance. Some bacteria possess a hemolytic ("blood splitting") enzyme that breaks the erythrocytes to release the heme. Colonies for bacteria with this hemolysin produce a discoloration of the agar (often greenish or brown). These bacteria are termed “alpha-hemolytic”, and the result seen in the agar is termed “alpha-hemolysis” (also sometimes shown as “α-hemolysis”).

Other bacteria are capable of lysing erythrocytes AND digesting the heme. The result is clearing of the agar under the colonies as the blood cells and their contents are digested completely. This is termed beta-hemolysis (“β-hemolysis”) and these bacteria are termed beta-hemolytic.

There is a third type of hemolysis termed gamma-hemolysis (“γ-hemolysis”). This term is something of a misnomer, since in gamma-hemolysis, NO change is seen in the agar – no discoloration, no clearing.

In the exercise to follow, you will gain some firsthand experience in streaking blood agar plates (virtually) and interpreting the types of hemolysis that result.

1. Enter the Virtual Lab and click “New” to obtain a new unknown. Select “Blood Hemolysis 1” from the “Exercise Topic and Subgroup” list. Give the identifier "hemolysis1".

2. Select 5% sheep blood agar from the list of media. Label the plate “ba”.

3. A plate of blood agar and a tube of the unknown culture appear in the lab. Use the tube-to-plate aseptic technique for preparing isolated colonies and streak the plate, as shown in an earlier exercise. A plate with streak marks (as shown at right) will appear. Be sure the traffic signals indicate no contamination. If contaminated, discard the medium and get a fresh plate to start over.

4. Place the plate in the incubator and click “New Day” to give the plate 24 hrs of growth. Retrieve the plate and right click to “Record Results”. A closeup of the plate appears.
5. Using the information provided above (and elsewhere if so instructed) record the type of hemolysis present in the software and here (Circle):

   Alpha   Beta   Gamma

6. Click the “New” button and repeat this process with the “Blood Hemolysis 2” unknown from the “Exercise Topic and Subgroup” list. Give the identifier “hemolysis2”.

7. Streak the plate and incubate it at 37C overnight. Retrieve the plate and right click to “Record Results”. Are the halos transparent or a discoloration of the medium?

   Transparent   Discoloration

   Record your results in the software and here (Circle).

   Alpha   Beta   Gamma

8. Use the Help files and Identification Matrix to provide answers to the following…

   a. Which form of hemolysis is most frequently associated with these microbes?

      *Staphylococcus aureus*:    *Streptococcus pyogenes*:

      *Streptococcus pneumonia*:    *Streptococcus mutans*:

      *Enterococcus faecalis*:    *Staphylococcus epidermidis*:

   b. How is blood agar related to chocolate agar?

   c. How might the presence of hemolysins enable a microbe to be more virulent than one without hemolysins?

9. Follow your instructor’s directions concerning submission of the MDM exercise and/or Virtual Lab Report.
The catalase test is traditionally one of the first test performed on Gram positive cocci. It provides a quick means for distinguishing between major groups: *Staphylococcus* and *Micrococcus* (generally catalase positive) and the Streptococci – *Streptococcus*, *Enterococcus*, and *Lactococcus spp.* (generally catalase negative).

Catalase is a protective enzyme produced by many aerobic bacteria. The enzyme breaks down hydrogen peroxide produced during respiration. You may recall that the cytochromes pass along electrons to a final electron acceptor (FEA). In aerobic respiration that FEA is oxygen, in a process that adds two electrons and two protons to ½ O₂ to make water. However, if only half the diatomic oxygen (O₂) is used, that leaves a very dangerous substance around – singlet oxygen (O⁻). Singlet oxygen is very reactive and often reacts with diatomic oxygen (O₂) to make ozone (O₃⁻) or with water (H₂O) to make hydrogen peroxide (H₂O₂). All three substances – singlet oxygen, ozone, and hydrogen peroxide – are very toxic to cells and can disrupt metabolism or cause catastrophic mutations. Vitamins and dietary supplements often are advertised to be “free radical scavengers” to accomplish the removal of these and other reactive substances from our systems.

Aerobes typically have one or more enzymes that detoxify these products of metabolism. Catalase is one of them. It has the ability to convert hydrogen peroxide into diatomic oxygen and water. You may have placed peroxide on a cut and noted the bubbly foam produced as it interacts with catalase found in blood and our cells. It is administered as a liquid, but as the enzyme splits peroxide to generate oxygen gas, bubbles flow out of the liquid.

In the Virtual Lab, a video shows the catalase test for your unknown organism; the method is described below.

1. Open the Virtual Lab and click the “New” button. Select the “Media Tests 6” unknown from the “Exercise Topic and Subgroup” list. Give the identifier “MT6”. Review the Case Study and record the Gram stain results. Based on shape and grouping, what would you predict to be the genus of this organism?

2. As done in a previous exercise, open the Identification Matrix and reduce its size to place it side-by-side with the Virtual Lab. This is done so you can see the changed occurring as you conduct the catalase test.
3. From the “View” list, select “Catalase Video”. Appearing will be a viewer where you can watch the addition of 3% hydrogen peroxide to a smear of fresh growth on a microscope slide. The video can be replayed if needed. Based on the information provided above, is this organism catalase positive or negative? (Circle)

   Positive    Negative

   What evidence supports your interpretation?

   What would the video look like if the result was opposite to that portrayed in the video?

4. Record the results in the software and watch the Identification Matrix for changes. Click on View and select Lab Report to determine how many species of bacteria were unknown based on the result you recorded. How many were eliminated?

5. Would strict aerobes or strict anaerobes be more likely to possess catalase? Explain your reasoning.

6. Follow instructions from your instructor about submission of this MDM exercise and the Virtual Lab Report.
Activity 17  Bile Esculin Test

Bile salts are substances produced by the digestive system to emulsify fats, making them digestible in the aqueous environment of the human body. Because the bacterial membrane and Gram negative cell wall also contain fat-like lipid materials, they also are subject to disruption by bile salts. However, some bacteria are resistant to bile salts. Bile esculin agar is used to test the ability of an unknown organism to grow in the presence of bile salts. Enteric organisms, such as Gram negative bacilli typically found in the gut, would be expected to grow on bile esculin. Also growing would be Gram positive cocci such as the enteric cocci – Enterococcus spp. And, some other bacteria such as the Staphylococci often are able to grow on bile.

Also present in the medium is esculin, a glucoside compound. Some bacteria can digest esculin to produce esculentin, which gives the agar a brown-to-black discoloration. Thus, bile esculin agar is useful for two tests – ability to grow on bile, and ability to metabolize esculin to esculentin. A positive result for growth on bile is obvious growth. A positive result for esculin hydrolysis is a very dark discoloration of the medium. Below you will see how this test is performed.

1. Enter the VirtualUnknown™ Microbiology’s Virtual Lab and select Media Tests 5 from the list of predefined unknowns. Label it MT5. This organism is Staphylococcus aureus. Your instructor will advise you on whether to check the box permitting autoinoculation.

2. Select Bile Esculin from the Media dropdown list. Inoculate this medium and place in the 37C incubator. Click “Next Day” to complete its 24-hour incubation period.

3. Remove the medium from the incubator and record the results in the software for the Virtual Lab Report. Also record the results in the table below. If you need help interpreting the test, click on the “M” icon and to read up on how the medium is used. You can look up the tests for which it is used by clicking on the “T” icon and reading up on those tests.

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Staphylococcus aureus Media Tests 5</th>
<th>Staphylococcus saprophyticus Media Tests 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on Bile:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esculin Hydrolysis:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Once the test is completed, review the Virtual Lab Report and the Identification Matrix to see how the results from the bile esculin agar have reduced the number of possible identities from the original 50+.

5. Repeat the above assignment using Media Tests 6 (MT6, Staphylococcus saprophyticus) and record the results in both the software AND in the table above.

6. Use the table, the Help files in VirtualUnknown™ Microbiology, and the information in your textbook and laboratory manual to answer the following questions.
7. Which of the following bacteria would be most likely to encounter bile salts in their normal habitat? (Circle all correct answers)

- Staphylococcus aureus
- Enterobacter aerogenes
- Enterococcus faecalis
- Streptococcus pyogenes
- Escherichia coli
- Staphylococcus epidermidis

Why would those you selected be expected to grow on bile esculin agar?

8. Where in the digestive system are lipids digested? Is there any evidence that these bacteria are more inclined to cause infections in those organs than are bacteria incapable of growing on bile?

9. It is possible to have an organism that is positive for both “growth on bile” and “esculin hydrolysis”, an organism that is negative for both, and an organism that is positive for one but not the other. Explain the only possible way an organism could be positive for one but not for the other when using this medium.

10. Follow your instructor’s directions about submission of this MDM exercise and your Virtual Lab Report.
Activity 18  

Mannitol Salt Agar

Often, the first step taken to isolate a Gram positive coccus is the use of Mannitol Salt Agar (MSA). MSA is a nutrient agar that contains a few additional substances. It contains the sugar mannitol and the pH indicator phenol red to show whether the sugar mannitol has been used to produce acids. It also contains 7.5% sodium chloride (NaCl) as an inhibitor to prevent the growth of unwanted bacteria. Thus, it represents a medium that is both selective (because of the salt) and differential (because phenol red reveals whether an unknown organism has the ability to use mannitol).

Use of salt is one of the oldest methods for preserving foods. The salt draws water out of spoilage organisms, stopping their metabolism. This is proof of the saying that “water follows salt” and is an excellent example of osmosis in action – water leaving cells to dilute the salt in the surrounding vicinity. Some bacteria are very resistant to drying due to modifications in their cell membranes. Among them are many skin bacteria.

An unknown specimen thought to contain such bacteria is streaked for isolation on MSA. Those that form colonies will either turn the medium yellow underneath (if mannitol is fermented) or hot pink (if mannitol is not fermented, the non-sugar nutrients used yield alkaline products). Thus, two tests are conducted using MSA: (1) growth of the unknown in the presence of 7.5% NaCl, and if growth occurs (2) whether the microbe can ferment mannitol. Below is some virtual practice in the use of the medium.

1. Open the Virtual Lab and click “New”. From the dropdown list of unknowns, select “Media Test 5”. This organism is Staphylococcus aureus.

2. Read through the Case Study and interpret the Gram stain. Once in the Virtual Lab, select Mannitol Salt Agar from the Media list. Label the plate “msa”.

3. Inoculate the plate following the tube-to-plate streak process for producing isolated colonies. Incubate overnight at 37C (you know how this is done!).

4. Remove the culture from the incubator and observe the growth. Provide results for these two tests:

   Growth on 7.5% NaCl:  +  -

   Mannitol Fermentation:  +  -

5. What result would be expected in the “growth in 6.5% NaCl test” for bacteria capable of growth on mannitol salt agar? Explain.

6. Follow your instructor’s directions pertaining to submission of this MDM exercises and the Virtual Lab Report.
There is a huge inventory of possible tests to use for identifying bacteria. Through experience and statistical analysis, clinical microbiologists have determined which tests are most helpful for identifying the majority of isolates sent their way in a hospital setting. Just as certain bacteria occur more frequently than others in specimens taken from patients, certain tests more frequently provide reliable and useful help in identifying the cultures. It would make sense for the lab technician to inoculate these tests routinely when a specimen is received in order to provide a more rapid answer to what is causing the infection. At the same time, performing the tests can be costly, and providing a means for doing tests “in miniature” would save the expense of using greater volumes of media in large numbers of plates and tubes. For these reasons, commercial products have been developed that bundle commonly used tests in miniaturized systems that have been rigorously tested to provide reliable and rapid identifications for the clinical lab.

Most rapid ID systems use conventional biochemical tests bundled into a single container (often called a “panel”) with 12-30 small wells of various media. A mechanism has been developed to quickly and easily inoculate all wells and secure the panel for incubation. Some panels require incubation overnight, while others are ready to be interpreted in as few as four (4) hours. Interpretation of the results usually does not require the addition of reagents to “develop” the chemical transformation detected by the test. This makes the results particularly easy to obtain for a speedy identification.

The results of the tests are interpreted using a code number that is generated from the pattern of test results and compared to code numbers recorded through the years for thousands of cultures of bacteria identified by conventional tests and observations. When the code number of an unknown microbe matches a code number for an identified species of bacteria, the identity of the unknown is revealed – its biochemical fingerprint matches that for others of the same species. The 12-30+ tests included in a rapid ID panel each have an assigned numerical value for a positive result, and the tests are bundled together for creation of a code of reasonable length. For instance one digit in a code number can be derived from the results for three tests, with one test being weighted at 4 points, one at 2 points, and the other at 1 point. If positive for all three tests, the value for that bundle of tests would be 7 (4+2+1). If all tests were negative, the bundle value would be 0. It would be possible to have values of 7, 6, 5, 4, 3, 2, 1, or 0, based on the outcome of the three tests bundled for each number. Other tests are bundled together similarly to generate other digits on the way to creation of a 5-10 digit code number.

Panels are often specific to a particular group of organisms, because tests appropriate for one group might not be useful for identifying another. For instance, it is not uncommon for rapid ID systems to have several products with separate panels for Gram negative enteric rods, Gram negative non-fermenters, Gram positive cocci, anaerobic microbes, and other groups as appropriate. A panel relying heavily on carbohydrate fermentation tests would not be of any value in identifying bacteria that are non-fermenters – all test results would be negative!

### Rapid ID Systems Used in Teaching

One of the most commonly used rapid ID systems for microbiology teaching labs is the collection of BBL- Enterotube products (Enterotube II™ and OxiFerm™) from Becton-Dickinson, or BD-Diagnostics. These are simple to inoculate and base their 5-digit code numbers on results from 12 wells of agar media inoculated by a sterile wire that runs through the media. It is touched to a pure colony of the unknown and drawn out of the container, passing through (and inoculating) all 12 media. Most of the tests require no addition of reagents after incubation, meaning easy determination of results.
Another system commonly used is the Analytical Profile Index, or "API" system from bioMérieux Clinical Diagnostics. Their products include the API-20e™ panel (also called API strips) for identifying enteric bacteria and a wide number of specialized panels for other bacterial groups and even yeasts. There are 20 wells containing dehydrated media that are reconstituted when a broth culture of the unknown organism is added. After incubation, color changes reveal the pattern of results used to develop the code number for the unknown.

Even newer and more sophisticated rapid ID systems using new technologies and different approaches and types of information used for obtaining identities are being developed on a frequent basis.

Use web and software resources to answer the questions below:

1. Do a web search to find the tests used in the Enterotube II™ and API-20e™ test systems for identifying enteric organisms. Use that information to fill in the table below. Be sure all tests from both systems are included in your table. Then, check which are included in the VirtualUnknown™ Microbiology software.

<table>
<thead>
<tr>
<th>Test</th>
<th>Included in Enterotube II™ (check if yes)</th>
<th>Included in API-20e™ (check if yes)</th>
<th>Also included in this software (check if yes)</th>
<th>Results for E. coli in software (+/-)</th>
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</tbody>
</table>

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2. Open the Virtual Lab and click “New”. Select “Enteric Bacillus” from the list of predefined unknowns and provide the identifier “RapidID” as the label for this unknown. Read the Case Study and interpret the Gram stain.

3. Once in the lab, open the Identification Matrix and locate the column for the microbe *Escherichia coli*. Record in the right-most column of the table on the previous page the results expected for *E. coli* for all tests found in the Enterotube II™ and API-20e™ panels, based on the Identification Matrix results. If a test is not provided in the software that is found in one of the two panels, record that result as a zero (0).

4. Next, use the manufacturers’ product information to determine how test results are used to generate a digital code number for comparison to its databases for supplying an identity for the unknown microbe.

5. Then, using the results for *E. coli* provided by the software, create the digital code for those test results in the blanks below each of the panel illustrations below.

*Enterotube II*

- 4 + 2 + 1 - 4 + 2 + 1 - 4 + 2 + 1 - 4 + 2 + 1 - 4 + 2 + 1

- __________ - __________ - __________ - __________ - __________

*API-20e™*

- 1 2 4 - 1 2 4 - 1 2 4 - 1 2 4 - 1 2 4

- __________ - __________ - __________ - __________ - __________

6. Follow your instructor’s directions about submitting this MDM exercise and the Virtual Lab Report.
In the teaching lab, identifying an unknown bacterium may take place over a matter of days or weeks. In the medical world, such patience would not be possible. Causes of microbial disease must be found and treatments begun as swiftly as possible to reduce the chance of lethal complications for patients suffering from infections. This often involves rapidly identifying the causative agent so that appropriate strategies for treatment can be undertaken.

In a previous exercise, the concept of rapid ID systems was introduced as a way to package tests for swiftly and methodically identifying unknown organisms. That same strategy can be used in the Virtual Lab with conventional tests by inoculating multiple tests and placing them in the incubator before clicking “New Day” and moving forward in virtual time.

In many of the upcoming exercises you will be asked to identify an unknown microbe recovered from a medical setting. In each case, your goal should be to do so within no more than three virtual days. Your instructor is alerted to the number of virtual days that have expired by the Virtual Lab Report. Be ready for your instructor to specify the need to “save the patient” through ID within three days. Strategies provided and practice with tests in previous exercises should be helpful in planning the tests necessary for identifying the microbes in each upcoming exercise. Here is a plan many microbiologists might follow:

**Day One:**
- Collect background information for clues to the infection (your Case Study)
- Perform Gram stain to get shape, grouping, and Gram reaction. (done when entering the Virtual Lab)
- Conduct initial tests for Gram positive cocci or Gram negative bacilli

**Day Two:**
- Read results of tests from previous day, add any reagents needed, and record the results.
- Consult the Identification Matrix to see what possible identities remain.
- Create a dichotomous tree for the remaining organisms to reveal what additional tests you need to conduct in order to have an ID on Day Three. Then, conduct those tests.

**Day Three:**
- Read results of tests from the previous day.
- Provide the physician with the name of the organism responsible for the disease.

**Creating the Dichotomous Tree**

A dichotomous tree, or decision tree, is a schematic representation of the tests and results necessary to sort through a group of possible identities so that planned experiments can result in a definitive ID. Take, for instance, the following organisms and tests in a hypothetical Identification Matrix:

<table>
<thead>
<tr>
<th></th>
<th>E. chevri</th>
<th>E. fordii</th>
<th>S. hondae</th>
<th>P. toytaii</th>
<th>J. dodgi</th>
<th>R. nissanii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid from glucose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gas from glucose</td>
<td>-</td>
<td>-</td>
<td>D</td>
<td>+</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Xylitol fermentation</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Adonitol fermentation</td>
<td>(-)</td>
<td>D</td>
<td>(-)</td>
<td>(+)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Maltose fermentation</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>D</td>
<td>(-&gt;)</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNAse</td>
<td>(-)</td>
<td>-</td>
<td>+</td>
<td>D</td>
<td>(-&gt;)</td>
<td>+</td>
</tr>
</tbody>
</table>
1. **Looking at these test results, which test(s) would be totally useless for eliminating any of the six microbes?** (Remember that eliminations only occur when there is a clear + or – conflict, not when (+), (-), or D are indicated – these reflect tests where more ambiguous and unreliable results are encountered)

2. Here is a decision tree that uses five (5) of the tests to completely sort out the six microbes.

   ![Decision Tree]

   + Motility
   -
   
   E. chevyii
   E. fordii
   S. hondae
   P. toyotii
   
   + Maltose
   -
   
   E. fordii
   S. hondae
   
   + Xylitol
   -
   
   S. hondae
   

3. This is not the only approach that would work to separate these organisms. **Draw below an alternate approach using these or other tests from the matrix that would also sort out the six microbes.** Remember that results of (+), D, or (-) do not eliminate a microbe – in such instances the microbe would need to be continued on BOTH outcomes for the test.

4. Now, it's your turn! **Obtain your virtual patient** by clicking on the New the red cross button to have the software assign a “Save the Patient!” case. You will have three virtual days to find the ID and help the medical professionals save the patient! When done, submit your Virtual Lab Report to your instructor.
Activity 21  Infectious Disease and Epidemiology

1. Enter the Virtual Lab and follow your instructor’s directions to select one of the Epidemiology options from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study and use the information provided and other resources to answer the following questions. Identify the organism using VirtualUnknown™ Microbiology to provide information a to the complete understanding of the disease in the Case Study.

2. Which Epidemiology unknown did you identify? 1 2 3 4 5 (circle one)

3. Case Study #___________. What the identity of this microbe?

NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies re assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

4. Is this a microbe commonly associated with infectious diseases? Yes No (circle one)
   If so, which ones?

5. Would this best be described as a systemic infection? Yes No (circle one)
   Explain your answer.

6. Would this best be described as a nosocomial infection: Yes No (circle one)
   Explain your answer.

7. Was there an animal reservoir for the microbe? Yes No (circle one)
   Explain your answer
8. **Which of the following would describe the disease?**
   a. bacteremia  
   b. toxemia  
   c. viremia  
   d. septicemia  
   e. none of these

   **What evidence supports this decision?**

9. **Were predisposing factors involved in this case study?** If yes, list them.

10. **What are “notifiable diseases”? Is this one?**
Pathogenicity refers to the capacity to cause disease. Virulence is a measure of pathogenicity. Many bacteria are pathogenic but not particularly virulent – it takes a huge number of organisms to cause disease. Others can cause horrible infections with only a handful of organisms entering a vulnerable area of the body. The number of cells needed to cause disease is called the Infectious Dose and is often referred to as the ID$_{50}$ – the number of organisms required to cause disease in 50% of a test population. The higher the ID$_{50}$, the lower the virulence, since it takes more microbes to cause disease than for a different pathogen with a low ID$_{50}$. Virulence and infectious dose are inversely proportional.

What makes one microbe more virulent than another? Some bacteria possess enzymes and cell structures that make them better able to resist the normal defenses of the body. In such a case, they would have a greater chance of establishing a disease-causing infection. These virulence factors include toxins and invasive factors that are commonly discussed in microbiology courses.

Complete the following exercise in the Virtual Lab to learn more about pathogenicity and virulence.

1. Enter the Virtual Lab and select Pathogenicity from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study carefully and proceed to identify the organism responsible. Use your textbook and VirtualUnknown™ Microbiology Help resources to answer the following questions. You may also need to review the Case Study for important details. Case Study # ____________.

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

2. Conduct the biochemical tests necessary to identify this microbe. What is the identity of this microbe?

3. Which of the following would describe the toxin most likely produced by this organism? (circle)

   Enterotoxin  Cytotoxin  Neurotoxin  Endotoxin

   What evidence supports your selection?

4. What is the likely portal of entry in diseases caused by this organism?

5. Did this organism most likely originate in the food or in the creek? Explain your answer
6. Which of the following would best predict symptoms observed in an infection caused by this organism? (circle your answer)

- red skin rash, sore throat, and fever
- blurred vision, vomiting, and watery diarrhea
- uncontrolled muscle contraction, fever, and convulsions
- fever, cramps, and diarrhea
- coughing, sneezing, and headache

What evidence leads you to this conclusion?

7. Which of the following describe the toxin produced by this organism? (circle all that apply)

- heat stable
- low LD$_{50}$
- impact frequently lessened by immunization
- can lead to septic shock
- not released until cell death
- prevents function of neurotransmitter chemicals

What evidence leads you to this conclusion?

8. What is the most notorious member of this genus? What disease does it cause?

9. Follow your instructor’s directions for submitting this MDM exercise and your Virtual Lab Report.
The body possesses two defense systems to protect it from potential pathogens: innate (or nonspecific defenses) and adaptive (or specific) defenses. First encountered are the innate defenses, consisting of the barriers (skin, mucosal membranes) and secretions (saliva, tears, earwax, mucous, among others). And if these are breached by damage to a barrier, cell damage releases chemicals that trigger additional innate responses from the body. The inflammatory response at the site of injury results in dilation of blood vessels to allow phagocytic white blood cells (leukocytes) to pass through capillaries to the point of injury. This inflammatory response is typified by redness, inflammation, pain, and swelling. It accomplishes the overall flow of fluids to the injury site and allows leukocytes to accomplish phagocytosis – capture and destruction of any foreign material entering the point of injury.

These responses are considered innate or nonspecific because they occur in the same manner no matter the source of the injury or the content of the infiltrating material. Whether pollen or splinter or bacteria or spider bite – the approach and result are the same. Complete the exercise below to learn more about these nonspecific defenses to disease.

1. Enter the Virtual Laboratory and select Nonspecific Defenses from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read carefully the Case Study and proceed to identify the unknown organism using VirtualUnknown™ Microbiology. Case Study # _______.

   Use the information from your textbook, data obtained during the identification, and any other resources necessary to answer the following questions.

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

2. What is the identity of this unknown bacterium?

3. List and briefly explain differences defining innate immunity, as opposed to adaptive immunity.

4. What causes each of the following changes observed in the inflammatory response?
   - Redness
   - Inflammation
   - Pain
   - Swelling
5. **What role does phagocytosis play in the process of innate immunity?**

6. **Which of these elements of innate immunity (barriers, secretion, inflammatory response, phagocytosis) would be involved in fighting the infection described in this case study? Explain.**

7. Some microbes possess defensive enzymes that protect them from the elements of nonspecific immunity. Read through the list of tests supported by *VirtualUnknown™ Microbiology* and identify three that detect presence of such defensive enzymes. **List those, and describe how each would allow its possessor to persist and cause disease.**

8. Follow your instructor's directions for submitting this MDM exercise and your Virtual Lab Report.
More advanced species of animals possess an adaptive or specific immune system. Adaptive immunity uses white blood cells (leukocytes called “lymphocytes”) to fight off invading organisms by a response targeting that organism and nothing else. Specific immunity consists of billions of clones of lymphocytes, each with its own specific purpose and use. Unlike barriers and secretions and inflammatory response and phagocytosis (all of which respond equally to any invader), specific immunity relies on lymphocytes to provide a response tailored specifically to the invading organism. For instance, lymphocytes programmed from before you are born to respond to an infection caused by chickenpox are of no use to any other invaders or infections. And, each other type of invader has its own specific clone lying in wait, should it get into the body.

In the exercise below, some aspects of specific immunity will be explored. In many instances, you will be required to look to your textbook and the web to find additional information.

1. Enter the Virtual Lab and select Specific Immunity from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study and perform the tests necessary to identify the bacterium. Case Study #________.

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

2. What was the bacterium’s identity?

3. Is there a vaccine currently in use for this organism? If so, how is it produced and administered?

4. Which branch of adaptive immunity (cellular or humoral) would play the most significant role in defeating this illness? Explain.

5. Would recovery from this infection normally lead to lasting immunity from a second infection? Explain how this protection is provided.
6. Describe the expected immunological course of events during the infection, development of disease, and recovery of this patient, including the following information:

   a. the means by which specific clones are formed and activated,

   b. the role of macrophage in the process,

   c. the classes of immunoglobulins responsible for the first response and main response to the pathogen,

   d. the way immunological memory is generated.

6. Follow your instructor’s directions for submitting this MDM exercise and your Virtual Lab Report.
Activity 25  Infections of the Cardiovascular System

1. Enter the Virtual Lab and follow your instructor’s directions to select one of the Cardiovascular options from the list of predefined unknowns. **Your instructor will tell you whether to check the box permitting autoinoculation.** Read the Case Study carefully, and use the information provided to answer the following questions (consult your textbook for additional information). Identify the organism using VirtualUnknown™ Microbiology to provide information necessary to the complete understanding of the disease in the Case Study.

2. Which unknown did you select?  
   Cardiovascular 1  Cardiovascular 2  (circle one)

   Case Study # ___________.  What was the identity of this microbe?

   **NOTE:** If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

3. Based on the signs and symptoms observed, and the agent identified, what would be the diagnosis for this patient?

4. If not treated, what complications might be seen as this disease progresses?

5. **Draw and label a cross-section of the human heart.** Indicate on drawing where the following infections would occur:
   - myocardial infarction
   - endocarditis
   - pericarditis
   - murmur caused by infection
6. **Provide a likely scenario by which this victim would have been exposed to this organism.**

7. **How does the growth of this organism in the host lead to the signs and symptoms associated with this disease?**

8. **What precautions do oral surgeons routinely take to avoid complications such as this?**

9. **Follow your instructor’s directions for submitting this MDM exercise and your Virtual Lab Report.**
Activity 26  
Infections of the Respiratory System

1. Enter the Virtual Lab and follow your instructor's directions to select one of the Respiratory options from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study carefully, and use the information provided to answer the following questions (consult your textbook for additional information). Identify the organism using VirtualUnknown™ Microbiology to provide information necessary to the complete understanding of the disease in the Case Study.

2. Which unknown did you select?  
   Respiratory 1  Respiratory 2  (circle one)  
   Case Study # ____________.  What is the identity of the microbe?

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

3. Draw the respiratory system below. Label the illustration to note what is considered “upper respiratory” and what is considered “lower respiratory”. Indicate where the organism was causing the problems associated with the disease described in the Case Study.

4. Locate a list of bacteria that are commonly recovered from the upper or lower respiratory systems. Would this organism be considered part of the normal flora for either?

5. What normal nonspecific defenses prevent more common infections in (1) the upper and (2) the lower parts of the respiratory system?
6. Would this patient be considered immunocompromised? Based on the information from the Case Study and your identification of the causative organism, was the patient's general health a factor in the initiation and progression of this disease? Explain.

7. What traits of this organism can equip it to cause a variety of infections?

8. Write out a narrative to describe a scenario by which the growth of this microbe could have led to this infection.

9. Follow your instructor's directions for submitting this MDM exercise and your Virtual Lab Report.
Activity 27  
Infections of the Digestive System

1. Enter the Virtual Lab and follow your instructor’s directions to select one of the Digestive options from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study carefully, and use the information provided to answer the following questions (consult your textbook for additional information). Identify the organism using VirtualUnknown™ Microbiology to provide information necessary to the complete understanding of the disease in the Case Study.

2. Which Digestive Unknown did you select? Digestive 1 Digestive 2 Digestive 3 (circle one)

   Case Study #___________. What is its identity?

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

3. Draw and label the digestive system, pointing out the location of the “upper GI” and “lower GI” portions. Indicate where the infection was located.

4. What normal nonspecific defenses prevent more frequent infections in (1) the upper and (2) the lower regions of the digestive system?

5. Is this species commonly encountered in epidemics in the United States? What is its ecological niche?
6. Was this organism more likely to be introduced into the foodstuff by improper food processing or improper food handling? Explain how these differ.

7. How would the types of organisms introduced into foods by improper food processing differ from those introduced by improper food handling? Give examples of contaminating organisms likely encountered by each means.

8. Up your game! The following questions require you to be a super sleuth and will take you to websites around the world. **Instructors: Let your class know what parts you want answered!** Conduct an Internet search to find out more about this microbe and disease. Resources might include the websites for the World Health Organization and the Centers for Disease Control.
   - How many cases of this disease were reported in the United States last year?
   - How many cases of this disease are estimated to have occurred world-wide last year?
   - What is the mortality rate for infections caused by this microbe?
   - What course of treatment is usually prescribed for this disease when caused by this organism?

9. Follow your instructor’s directions for submitting this MDM exercises and your Virtual Lab Report.
Activity 28  Infections of the Reproductive & Urinary Systems

1. Enter the Virtual Lab and follow your instructor’s directions to select one of the UrinaryTract options from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study carefully, and use the information provided to answer the following questions (consult your textbook for additional information). Identify the organism using VirtualUnknown™ Microbiology to provide information necessary to the complete understanding of the disease in the Case Study. Case Study # ____________.

NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

2. The unknown selected was          Urinary Tract 1      Urinary Tract 2      Urinary Tract 3       (circle one)

3. Complete the following table of diseases of the reproductive and urinary systems, and make a list of the symptoms of each.

<table>
<thead>
<tr>
<th>Description</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystitis</td>
<td></td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td></td>
</tr>
<tr>
<td>Urethritis</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection (UTI)</td>
<td></td>
</tr>
</tbody>
</table>

4. Which of the above diseases is most likely described by the Case Study above? Explain your rationale.
5. Complete the identification of this pathogen using VirtualUnknown™ Microbiology. What is its identity?

6. Is this one of the more common causative agents of this disease? What other microbes are recovered from cases of this disease?

7. Did this organism more likely to infect the diseased organ through the urinary tract or through the bloodstream? Explain your reasoning.

8. In collecting urine samples for testing, it is preferable to obtain a “mid-stream catch” or a “clean catch”; that is, to only collect urine after the flow has started. Why is this necessary?

9. What is the normal habitat for this organism? Provide an explanation for its recovery from urine.

10. Follow your instructor’s directions for submitting this MDM exercise and your Virtual Lab Report.
Activity 29  Infections of the Nervous System

1. Enter the Virtual Lab and select Nervous Infection from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the Case Study carefully, and use the information provided to answer the following questions (consult your textbook for additional information). Identify the organism using VirtualUnknown™ Microbiology to provide information necessary to the complete understanding of the disease in the Case Study. Case Study # __________.

   NOTE: If your instructor is creating your unknowns with the Admin Console, you may be assigned Case Studies chosen specifically for you. If not, your Case Studies are assigned randomly by the software based on those that are appropriate to the varied environmental and medical conditions linked to the microbe you were assigned. If the case study you receive is inappropriate to the particular Exercise you are completing, your instructor may suggest you continue creating additional new unknowns until you receive a case study that fits the Exercise assigned.

2. What is the identity of this microbe?

3. What is the normal ecology of this organism?

4. What are some common diseases caused by this organism?

5. Based on the symptoms described in the case study, what would be the logical diagnosis?

6. The organism responsible for this infection was not one routinely encountered. Which organisms are more likely to be recovered from patients with this disease? How might age of the patient help one predict the causative organism for this disease?
7. Based on the facts presented in the Case Study, provide a scenario to explain why this unlikely organism was able to cause this infection.

8. What challenges are encountered in treating victims with this disease through administration of antibiotics?

9. Follow your instructor’s directions for submitting this MDM exercise and your Virtual Lab Report.
Creating Scientific Posters

Science is an enterprise that relies upon the collaborative efforts of workers. Each study reported to the scientific community builds on the work of other studies completed before and communicates new findings to the next generation of scientists to broaden and deepen our understanding of how life works.

The process of scientific communication is unlike the writing done for most other classes. **Its purpose is to accurately, clearly, and succinctly communicate a research study to others so the work can be reproduced and verified as true and accurate.** Good scientific writing is not measured by how many words are used. It is measured by its completeness and clarity in expressing the thought behind a project, the methods used, the results obtained, and the meaning derived from the work.

One format for scientific communication is **the scientific poster.** This is a less formal approach to conveying information than a podium talk or journal publication and provides a more visible and lasting artifact. **Scientific posters can also be used as a replacement for traditional lab reports,** and in doing so the student fulfills their obligation for reporting lab results in a format that also provides practice in this valuable writing technique.

The parts of a scientific poster vary, but below is one approach that would work well:

- **Title and Credits.** Name of the project, who did the work, and where they are affiliated.
- **Introduction.** The background information that explains the importance of this project and why the work was done.
- **Materials and Methods.** You must clearly and completely explain what you used and how you did your work so that others could read your poster and repeat the work with the same results.
- **Results and Discussion** In a poster, the Results are often simply presented in data form – tables, figures (photos, drawings, graphs) and legends for each that explain the “what and how” for each experiment depicted. The Discussion is the researcher’s analysis of the meaning each piece of data and explanation of how the data contributes to finding the answer to the problem at the heart of the study.
- **Conclusions.** Here the author generalizes the analysis and explanation to provide the “take-home message” and to relate this study to its significance in relation to previous work done on the problem.
- **Literature Cited.** It is not expected that everything on a poster is original thought or work. Every study builds on knowledge contributed by others, and the citations would give credit to those previous studies that are quoted or have their methods or findings referred to or used in this poster.
- **Acknowledgements.** Often there are individuals who contributed to the work but who were not directly involved in doing the work going into this poster. Their contributions do not warrant being included in the list of authors in the Title and Credits, but they should be mentioned here.
- **For More Information.** One individual from the research team will serve as the communications liaison for any incoming questions related to the study. This person, the “Corresponding Author”, provides contact information here.
Posters are traditionally several feet tall and several feet across. You may download a template at http://www.virtualunknown.com/student-resources.html or create your own using Microsoft PowerPoint™ and setting page dimensions to 28”x42”. If resources for printing posters are not available on your campus, try saving the poster as a photo image for display on flatscreen monitors or digital projectors. Or, this format for information can be recreated in typical lab report format for submission to your instructor.

Some hints on poster creation and scientific writing…

- **Tense is important.** Introduction: this is what we know (present), this is what has been done (past), this is what we intend to do (future). Materials and Methods: this is what we used and did (past). Results: this is what we found (past). Discussion: this is what the results mean (present).

- **Style is important.** Do not write as you speak. It is not a conversation, but a more stylized professional presentation format. Do not (unless told otherwise you by instructor) use bulleted lists, but instead write out sentences with nouns and verbs and capitalization and punctuation.

- **Get your scientific names right!** The easiest way to undermine a really nice poster is to be careless in the spelling and conventions used for scientific names. Capitalize genus but not species, italicize both. Don’t let spell-check ruin correct scientific spellings!

- **Fonts are important.** Use a font size sufficient to be read from a distance of four (4) feet. Try 20-pt or larger to begin with and see if that can be read from a distance of four feet and yet allows enough content to tell your story. Your narrative should use a different font from the one used for legends to tables and figures so that a reader can quickly tell what is a part of the body of the writing and what is instead explaining the tables and figures (suggestion of a serif font like Times New Roman for the narrative and sans serif font like Arial for the legends).

- **Remember scale.** Photos put into a poster are blown up significantly when the 28”x42” poster is printed out. Be sure to review the poster at 100% size before printing to be sure all graphics maintain clarity.

- **Use color and contrast effectively.** Light colored text on a black background is very effective. However, it also eats up black toner, which adds expense to the poster. Be practical. Likewise, graphic-intensive backgrounds can play havoc with text when the contrast between portions of the photo or graphic and the color of the text is constantly changing. Think about using color fill in text boxes as a way to distinguish text from legends, titles, and other sections. But always remember that simple is better.

- **Test it out before you print it out.** A dark red font against a black fill in the title of a poster may sound like a nice aesthetic touch, but if the lack of contrast results in the title being illegible it is not a good move. Make it easy for the reader to enjoy your work.

Creating Posters for *Micro Digital Media™* Exercises

1. You may work in research teams or individually, as decided by your instructor. If you work in teams, your instructor will need to create an unknown for everyone in your group to share. Even then, remember that Case Studies are randomly assigned based on the assigned microbe. You will have to determine which case study all in your group will use to serve as the basis for your poster-building activity. If you work individually, you may choose the “Poster Exercise” subgroup (at the bottom of the Subgroup list) when creating a new unknown and base your poster on the Unknown Microbe and Case Study randomly assigned to you.

2. Complete the identification of an organism as outlined in the *Micro Digital Media™ 3/e* exercises for microbes of that Gram reaction, shape, and grouping (see exercises 8 and 14 for guidance).
3. Use the content of the Case Study and your Gram stain as your starting point. You might want to look at the questions for appropriate exercises in *MDM3e* to help guide some of your analysis. For instance, if the Case Study seems to suggest a public health issue with an epidemic of food poisoning, you might want to look at Exercise 21 for some tips on how to view and interpret the information provided. And you have abundant resources in the software Help files available to provide additional information.

4. Use these as starting points for building the sections of the poster as described below:

- **Title and Credits.** Come up with an appropriate title for the project. List all members of your group (first name, middle initial, last name), your department affiliation (the department where your major is delivered), and the college where the department is located (along with its city/state/zip code).

- **Introduction.** Use the Case Study for introducing the problem being addressed. Do some Internet research and expand upon this as requested by your instructor. End with a statement about the goal being to identify the cause of the problem stated in the Case Study and how such information can help provide guidance for avoiding future problems of this nature.

- **Materials and Methods.** Begin your M&M with information about the patient from which the isolate was identified (Case Study). If given, state the source of the isolate (blood, cerebrospinal fluid, exudates, etc.) Then, state that it was Gram stained (take a screen capture of the Gram stain in the software and put into the report as “Fig. 1” to represent the “results” of your Gram stain.). **Do not describe the Gram reaction, shape, and grouping of the organism here – that is a “result” and so should be saved for that section.** Your narrative should continue with a statement like this: “Identification of a pure culture of the bacterium was accomplished by using conventional biochemical tests, as listed in Table 1.” Then list the tests used (and results obtained) for the identification in a Table and label it Table 1. Continue: “Test methods used and interpretation were accomplished following guidelines found in VirtualUnknown™ Microbiology Help files.” [Note: you would then include reference to this in your Literature Cited section]. Your own version of this will be tailored to the exercise and Case Study used.

- **Results and Discussion.** You might provide an image from the Gram stain, a table of tests and results, a decision tree, and other bits of data from identifying the unknown organism in VirtualUnknown™ Microbiology as your data for the R&D section. Here you state and explain your results – Gram stain, biochemical testing, etc. Guidance on ID strategies is found in previous MDM exercises, and these can help guide the approach taken. Relate the results of the identification to the Case Study and other pertinent information about the identified microbe to begin to tie the loose ends of the identity of the microbe and the Case Study together. For instance, the “B” button in the software takes you to the list of Bacteria and a click on the microbe in question brings up additional information that could be useful. Any of the questions posed for Exercises 21-29 in this lab manual that seem to fit the scope of this project can serve as effective starting points for building your discussion. **However, skip Epidemiology 2 as an option – it will be used in the sample poster below on p. 99!**

- **Conclusions.** This is the summary of the project – identity of the organism, how it relates to the Case Study (knowledge about the microbe confirms this is a logical organism to find in these circumstances), external confirmation that this is a logical identification (from Literature on this disease), a scenario for how the events of the Case Study were brought about by this microbe, and advice on how future incidents of this nature can be avoided. All of these would be nice inclusions, though not all are essential.

- **Literature Cited.** If you go beyond the Case Study to build your Introduction, you need to cite those sources here. Methods used in the Virtual Lab are presented in the software Help files, so those files need to be cited. If you use any outside information on the microbe, the Case Study, or in your analysis of the identification or its meaning or relation to the Case Study it also needs to be cited. Any methods or processes from others, any ideas of others that you rely on to build your case – they need to be cited.

- **Acknowledgements.** Your instructor will guide you in making decisions on how to approach this section. Thank them for their supervision and input toward completion of the project.

- **For More Information.** One individual from the research team will serve as the communications liaison for any incoming questions related to the study. This person, the “Corresponding Author”, provides contact information here.
To see how this might look, a sample poster has been provided on page 99 that shows what this might look like for the case study below and the microbe associated with it:

Organizers were alarmed when 174 convention-goers experienced severe diarrhea within 24 hours of their awards banquet. Fifty-seven had to be admitted to hospitals after blood appeared in their stools and their diarrhea continued unabated. Fifteen others were also admitted and treated for dehydration and electrolyte imbalance. City health officials questioning the caterer and patrons found that those who had skipped the salad were unaffected by the outbreak. An inspection of the kitchen found the area where the salad was prepared to be adjacent to the area where the chicken had been prepared. Cooks insisted they had not shared utensils. This organism was recovered from both food preparation surfaces.

Look over the poster that follows on page 99 of this manual to see how that information and the guidance provided above helped create a very attractive and informative research poster for this fictitious event and organism.

Want more examples? Need some pointers? Microsoft™ Help Online provides this helpful link:

This site also has useful information:

And this one provides templates free of charge:  http://www.postersession.com/poster-templates.php

For a video tutorial on the process, look here:  http://www.youtube.com/watch?v=MqgjgwIXadA

The content and style may vary slightly among these resources, but the end result is always the same – effective presentation of scientific information. With these resources, the process of creating outstanding posters for this class and others should be easy. You can actually print an 8.5”x14” version that is legible to allow you to preview the product before its submission.

Final word from VirtualUnknown™ Microbiology: We hope that through these activities you have grown in your knowledge and sharpened your skills in basic microbiology, have experienced the use of your new-found knowledge and skills to identify bacteria and research problems, and have learned new and interesting ways to report your work to others. If we have helped make microbiology relevant, engaging, and fun, then our job is complete. Please let us know of your experience with this software (and your recommendations for its improvement in the future) by dropping us a line at micro@intuitiveinc.com.
Outbreak of Salmonellosis due to contaminated food preparation surfaces

Smar T. Pants*, Ima Genius**, Clever Feller***

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Introduction

At a recent convention, 174 participants experienced gastrointestinal symptoms within 24 hours of a banquet. Seventy-two fell below the normal hospital capacity, and all 53 were admitted to three hospitals. An inspection of the kitchen found that one area appeared to be the source of the outbreak. An analysis of the food samples confirmed that the cause of the outbreak was Salmonella enterica serotype enterica.

Table 1. Preliminary tests indicated the contamination of food to be Salmonella enterica serotype enterica

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-F fermentation</td>
<td>Positive</td>
</tr>
<tr>
<td>Growth on TSI</td>
<td>Acidic/Indole-positive</td>
</tr>
<tr>
<td>H2S production</td>
<td>Positive</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Figure 1. Gram stain of bacteria isolated from food preparation surfaces. Bacteria were gram-negative rods.

Materials and methods

Sampling Methods

Food samples were collected from the convention center and telephone interviews with attendees. Samples were taken from food preparation surfaces, including countertops, oven racks, and cutting boards. These samples were sent to the microbiology lab for analysis.

Table 2. Possible indicators of Health Department’s role in following standards of food safety operations

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provision of kitchen equipment</td>
<td>Yes</td>
</tr>
<tr>
<td>Preparation of food in a clean environment</td>
<td>Yes</td>
</tr>
<tr>
<td>Cleaning and sanitizing of kitchen surfaces</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Results & Discussion

Initial analysis of the suspected source of the outbreak was accomplished on standard biochemical tests. Upon examination of the Gram-stained smear, the organisms were determined to be Gram-negative rods. The cells were arranged as long, slender, and occasionally in clusters. Based on the information provided, the organism was identified to be Salmonella enterica serotype enterica.

Table 3. Identification scheme for the identification of Salmonella enterica serotype enterica

<table>
<thead>
<tr>
<th>Strain</th>
<th>Identification Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterica serotype enterica</td>
<td>Positive fermentation of lactose</td>
</tr>
</tbody>
</table>

Conclusion

Based on the results of the study, the outbreak of gastrointestinal illness caused by Salmonella enterica serotype enterica was identified. The illness was transmitted from the prepared food to the attendees through the food preparation surfaces. Since the vegetables served were not cooked, live enteric bacteria were transmitted to patients through this route in the normal food supply procedures.

Literature Cited


Acknowledgements

We would like to acknowledge the help of our lab instructor for her patience and support throughout this project.

For further information

Please contact Smar T. Pants, pants@enormous.edu, for further information.
Now that you have developed mad Ninja skills in Microbiology, it’s time to put them to good use and have some fun. The growth of “fantasy sports” has exploded in recent years, and now we are ready to bring the fun to science in a new game called…. Fantasy Microbiology™!

How does it work?

- Your instructor is the Commissioner of your Fantasy Microbiology League™ (FML for short).
- Students sign up to be “players” in the game. This may be a required or optional activity for your class. For even more fun, convince your instructor to be a player in the league!
- Each week, players will participate in a “game” consisting of identifying a VUMIE™ 2012 unknown organism. The Commissioner will assign the “game microbe” of the week, either by allowing random assignment of unknowns by a pre-defined subgroup or by creating specific unknowns from scratch for students using the Admin Console.
- The season is five weeks, so players identify five unknowns during that time period.
- Points earned in each game are assigned for various elements of identifying an unknown:
  - minimizing the number of virtual days used,
  - minimizing the number of tests used,
  - minimizing the number of mistakes and warnings recorded in the lab report, and
  - whether the ID was correct.
- The official report used for calculating points is the Virtual Lab Report. The player accumulating the most points in a week wins that week’s game. Your Commissioner will decide whether to have head-to-head competition each week, or simply look at total accumulated points for the season to determine the league champion.
- At the conclusion of the regular season, your Commissioner may call for seeded single-elimination playoffs between top finishers, conducted during your lab session.
- If your institution has more than one section of lab using VUMIE™ 2012, perhaps winners from each lab section (or each instructor) are pitted against each other in the Fantasy Microbiology Campus Championship! Winner receives the Robert Koch Trophy!

How are points earned and lost in Fantasy Microbiology League™?

- Whether the ID was correct
  - Correct ID earns 25 points
  - No points for Incorrect ID
- Number of virtual days used
  - 3 days or fewer used earns 25 points
  - 4 days earns 20 points
  - 5 days earns 15 points
  - 6 days earns 10 points
  - 7 days earns 5 points
  - No points for more than 7 days
- Number of tests performed
  - Points earned = 25 – number of tests used
- Number of mistakes and warnings recorded in the lab report
  - 0 mistakes earns 25 points
  - 4 points are deducted for each mistake or warning in the Virtual Lab Report

Look for Fantasy Microbiology League™ to be an automatically-scored and managed option in future versions of VUMIE™. Who knows? Maybe campus champs competing in FML World Championships won’t be far behind!