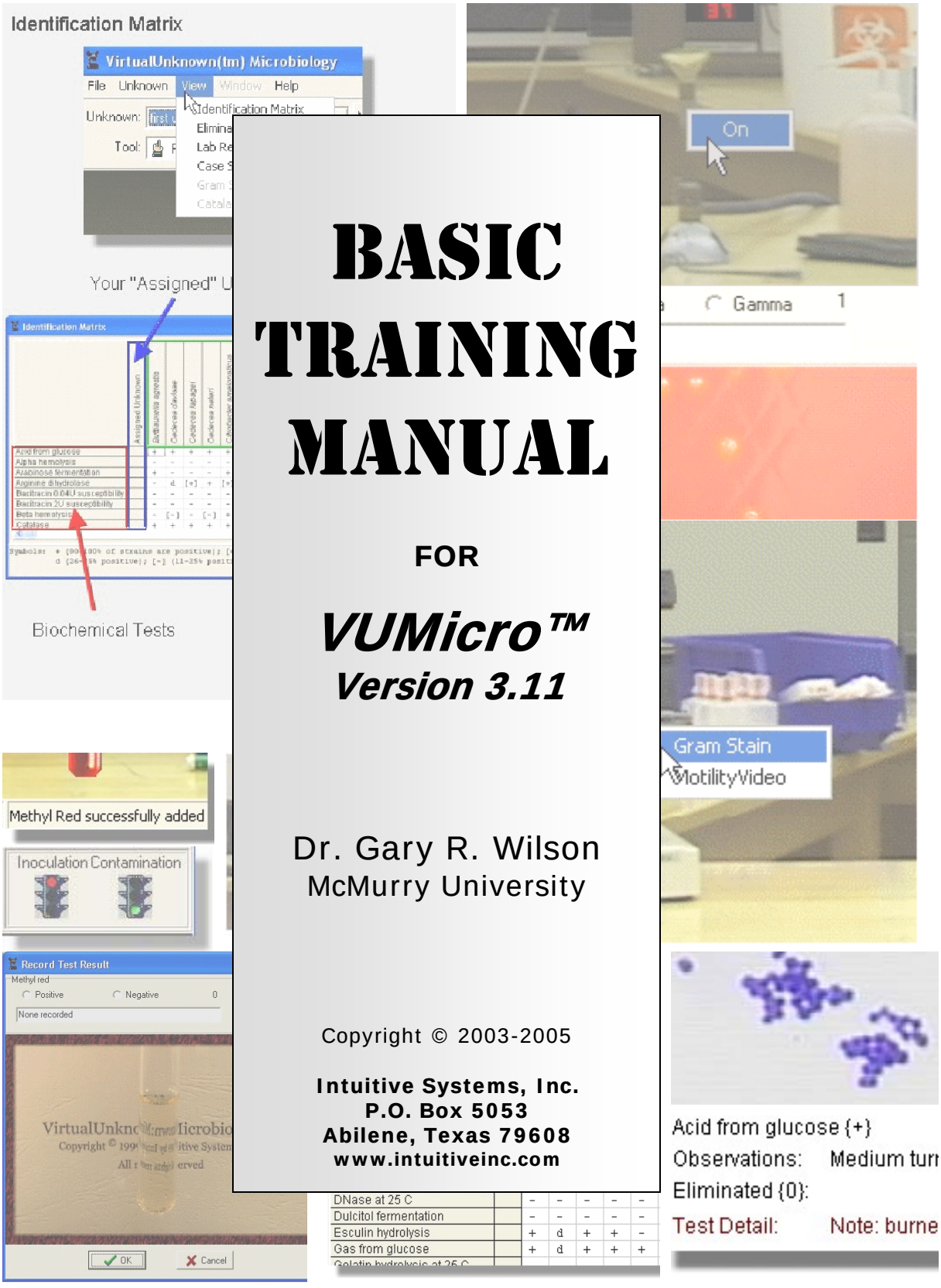


# BASIC TRAINING MANUAL

**FOR**

## *VUMicro™*

### *Version 3.11*


Dr. Gary R. Wilson
McMurry University





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**www.intuitiveinc.com**

# FOREWORD

***Basic Training Manual for VUMicro™ 3.11*** is a supplementary package available to users of ***VirtualUnknown™ Microbiology*** software. It contains 51 exercises designed to help enhance and deepen the students' understanding of basic microbiology through the use of this exciting simulation software. Great effort has gone into demonstrating the connections between the theory and concepts of lecture with the practical experience of the lab.

You will note that the exercises are organized in six sections: ***Getting Started***, ***General Microbiology***, ***Biochemical Tests and Microbial Metabolism, Identifying Bacteria, Medical Microbiology***, and ***Other Microbiology Topics***. The intent is to provide an easy way of integrating ***VUMicro™*** into both the lab and the lecture portions of any general microbiology course. You now have a simulation that will "bring to life" topics in your courses, from first week to last.

***Basic Training Manual for VUMicro™ 3.11*** is the successor to (1) the ***Virtual Laboratory Guide™*** written to provide support to users of earlier versions of ***VirtualUnknown™ Microbiology***, and (2) the exercises supporting version ***2.03BC***, which was bundled with the ***Fundamentals of Microbiology*** textbook by Tortora, Funke, and Case published by Benjamin Cummings. It combines the best of both supplements – excellent guidance on using the software to provide instruction and practice for the lab, AND excellent exercises for lecture classes to bring added relevancy to topics being covered in the lab. New innovations include pictorial guides to many of the key "first steps" described in the ***Getting Started*** section, and a greater focus on the use of the multitude of resources provided in the ***Help*** section of the software.

If you are a microbiology instructor, you probably have encountered the same problems I did in teaching microbiology labs – lack of time for students to practice their technique, lack of opportunity for students to work in the lab "after hours", the expense of supplies and cultures, limitations on what media and microbes could be provided for students, and no effective way to provide meaningful "make-up" work for students with legitimate absences. ***VUMicro™*** was developed to provide a solution. It soon became clear that its utility could be extended to enhance instruction on topics all semester long. That provided the motivation for writing the ***Virtual Laboratory Guide™***. In this latest metamorphosis, it becomes even more integral to the total microbiology experience. We hope you will agree that ***Basic Training Manual for VUMicro™ 3.11*** gives ***VirtualUnknown™ Microbiology*** unparalleled value as a supplemental package for teaching microbiology to today's students.

We invite your comments and suggestions for improvements. Visit the website at www.intuitiveinc.com and let us know what you think. Feedback from instructors around the nation has helped shape and improve the software time and again. Work is already underway for a new concept that will be a "must-have" for the new generation of microbiology students. Tell us what you wish to see in that product!

Gary R. Wilson, PhD
Professor of Biology
McMurry University

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## V. Medical Microbiology



## VI. Other Microbiology Topics

Name:_____

Section:_____

# SPECIFICATIONS AND SOFTWARE INSTALLATION INSTRUCTIONS

***VirtualUnknown™ Microbiology*** is a lab simulation that assigns you an unknown microbe at the beginning of each session. It is then up to you to perform biochemical tests to identify your mystery microbe! Over 50 familiar and common tests and more than 120 bacterial species are included.

## *MINIMUM REQUIREMENTS*

Operating Systems (any of the following):

- Windows® 98™ OSR2
- Windows® ME™
- Windows® 2000
- Windows® XP®.

Minimum hardware requirements:

- 64MB RAM
- 50 MB hard disk space
- 800x600 resolution monitor (SVGA)
- 2MB or more video graphics card (65K color depth palette)
- A 500MHz or faster processor is recommended
- If the university lab server expansion package is used by your school, an internet connection is required (broadband is recommended, but dialup will suffice).

## *INSTALLATION INSTRUCTIONS*

To use ***VirtualUnknown™ Microbiology*** you must complete a two-part process involving (1) acquiring and installing the software on your computer and (2) registering your copy so that it is activated. The software may be obtained by free download from our website OR by installing the software from a ***VirtualUnknown™ Microbiology CD-ROM*** made available by your school, a friend, or by purchase. Once it is installed, you will be asked to input a registration number to activate the software. You will not be able to use the software until you have done so by purchasing a license registration number for a nominal charge from your school/bookstore or from our website (www.virtualunknown.com). Your instructor will inform you of the preferred method to use at your school to get your copy of the software and license registration number.

Once you have the software, follow the installation instructions you see on the screen:

1. Read the Welcome screen, then click NEXT to continue.

2. To accept the End User License Agreement (EULA), click YES. The software will not install if you do not accept the terms of the EULA.

3. Read the user information that appears, then click NEXT.

4. You will be asked to select an installation directory. It is recommended that you use the default directory specified where it says "Destination Folder." Click NEXT to continue.

5. You will be prompted for a program folder into which shortcuts will be placed to launch the software. The program folder refers to the entry in the Start-->Programs bar where the software will be found. It is recommended that you use the default program folder specified by the installer. Click NEXT to continue. Click YES to create a shortcut to the program on your desktop.

Name:_____

Section:_____

6. Review the installation settings, then click NEXT to begin copying program files to your computer. Use the BACK button to change/correct any previously entered information.

7. The install will they copy files to your hard drive. This should only take a few seconds. You may be prompted to re-start your computer. If so, be sure to close all applications you have running on the computer and then click FINISH to re-start your computer.

### *ACTIVATING VIRTUALUNKNOWN™ MICROBIOLOGY*

1. Once you are done with installation, the program should automatically launch you into the registration and activation process. You will not be able to use the software until the registration process is complete.

2. If you had to re-start your computer, then click Start-->Programs-->VirtualUnknown-->Microbiology Student Laboratory 3.11 to launch the virtual laboratory software.

3. Activation will require purchase of a license registration number from Intuitive Systems, as explained previously. Be sure your computer is connected to the Internet, and then input the license registration number into the fields in the registration wizard. If the number is valid, a message will appear indicating that your copy of ***VUMicro™*** has been activated.

### *FINDING HELP, RESOURCES, AND INFORMATION*

There is an abundance of information available under the “Help” menu when you enter the virtual lab. Click on the “Contents” option to be taken to a list of features. In addition to the extensive reference books provided, there is an alphabetical listing of topics under the “Tell Me More About…” option. Each entry includes screen shots and explanations to provide information of value. There are pictorial “how to” descriptions of aseptic technique and other essential elements of the software to bring fast and complete success in using the virtual lab. In addition, a “Search” option is also available, as well as an “Index” of all topics included in the software, each entry linked to a page containing further information. Use these features to maximize your benefit from the software.

### *TECHNICAL SUPPORT*

For technical support, please visit the support page at www.virtualunknown.com, or contact Intuitive Systems via email at: support@intuitiveinc.com

### *INSTRUCTIONAL SUPPLEMENTS*

For information on instructional supplements and distance learning using the internet, visit www.virtualunknown.com

Name:_____

Section:_____

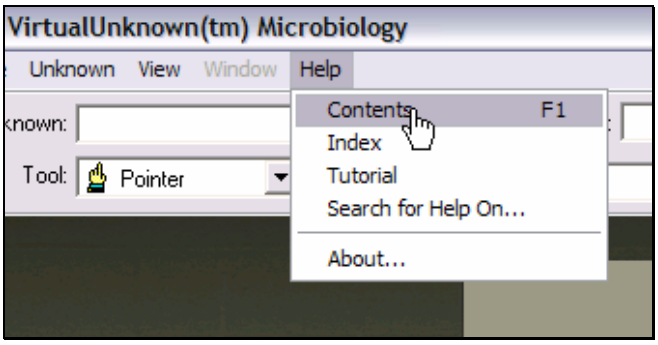
# 1… TOURING THE VIRTUAL LAB

**The goals for this exercise are**:

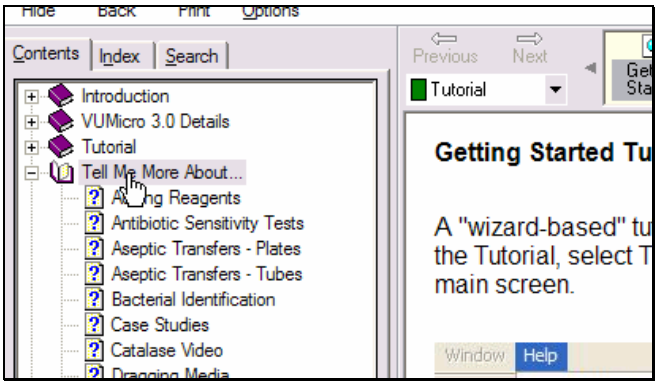
- to become familiar with the parts and features of the Virtual Lab
- to become familiar with the Tutorial provided in ***VirtualUnknown™ Microbiology***
- to learn how the Virtual Lab operates

***HINTS: For additional help in completing this exercise***:

*More detail on the content and format of the software is available from the "Help" provided. When you click on "Help" you will be to access the "Help" contents, an index of all help topics, the tutorial, and even a search engine that will enable you to find out more information on topics that are otherwise elusive.*

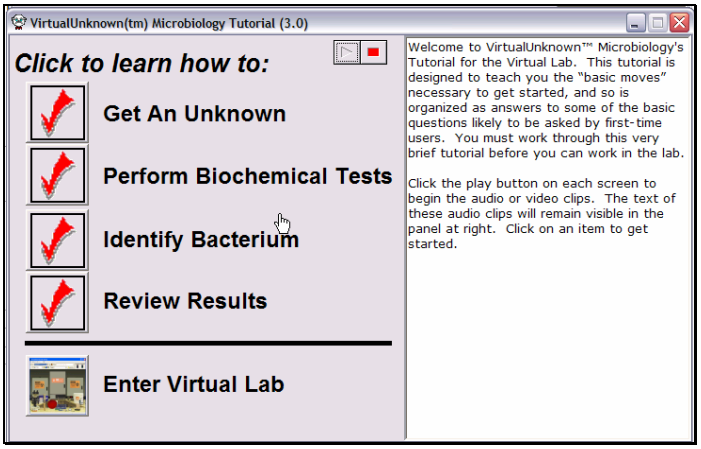



*Besides being able to review the tutorial from "Help", you will also find **illustrated** descriptions of the lab features and activities that you will study through the use of this software. If you have trouble finding the information you need following the instructions below, use the "Help" as another approach to completing these assignments.*




**Instructions**:

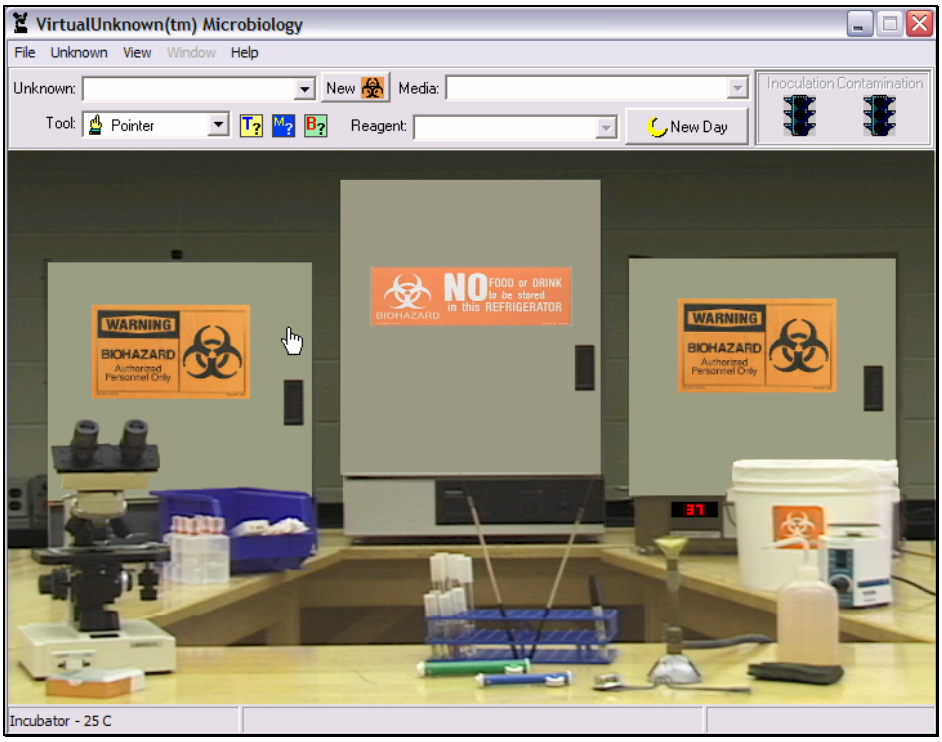
1. Open the software. **Work through the Tutorial to become acclimated to the means by which the Virtual Lab functions**. Once you feel comfortable with the workings of the lab, exit the Tutorial and enter the Virtual Lab.



Name:_____

Section:_____

2. Investigate the many features of the Virtual Lab by moving the cursor over the various pieces of lab equipment. They are identified in the field at the lower left below the lab. Use this information to answer the following questions:




a. **Which incubator is set at 25° C**? one at left one in center one at right

b. **What is the volume of the pipettes in the blue bin**?

c. **As you move your cursor across the different pieces of equipment, how many are described in the left field found in the bar below the lab**?

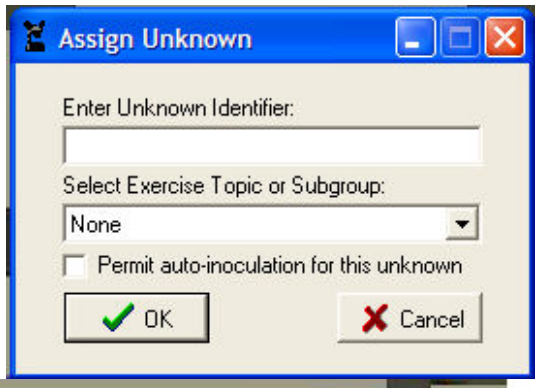
3. **Fill in the Table below to reflect the features available at this point**.

| | Is this feature active? |
|---|---|
| "Tests", "Media", "Bacteria" reference book buttons | |
| "Help" features | |
| "View" features | |
| Media dropdown box list of media | |
| Tools dropdown box list of lab tools | |
| Reagent dropdown box list of test reagents | |

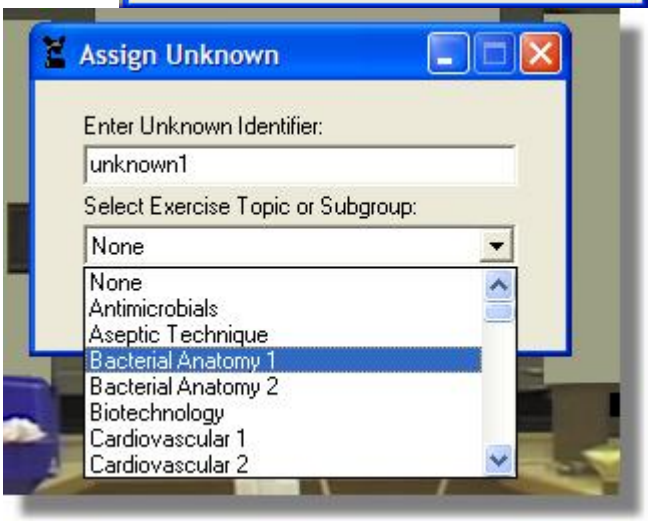
Name:_____

Section:_____

4. Click on the "New" button (with the orange biohazard symbol) to have an unknown organism assigned for you to work with.

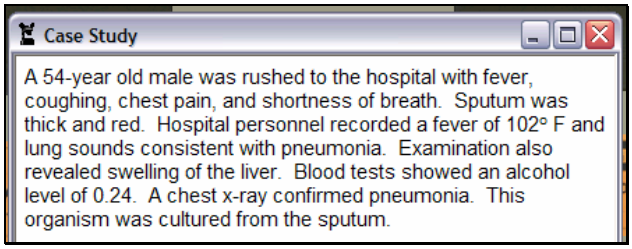

a. **Provide an identifying name for the unknown**. An example might be the date, or "ex 1" for the unknown used with Exercise 1. Inputting this identifier adds this unknown to the list of defined unknowns in the dropdown list of topics or subgroups. When you re-enter the Virtual Lab you can resume work on the same organism by selecting the name from the list in the dropdown box.




b. **Select the lab exercise or type of microbe from the dropdown list**. The selection you make will limit the types and number of bacteria possible. Specially-assigned unknowns and case studies are activated for use in the various exercises in this manual when they are selected from this list. For this exercise, please select "**None**", indicating you are not choosing a specific pre-defined unknown from the list and wish to have one randomly assigned. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box to permit auto-inoculation for this unknown.

c. **Click on "okay" to have an unknown assigned**. This will set the parameters for your unknown.

d. **Read the Case Study**. **When you are done, exit the Case Study**. It can be revisited by selecting "Case Study" from the options found under the "View" dropdown list.




e. **Review the Gram stain**. In the process of receiving your unknown, you will complete your first test when you review the Gram stain to determine the Gram reaction, morphology, and grouping of the unknown microbe. Try to exit the Gram stain image without making any selections. **What message do you receive**?

Name:_____

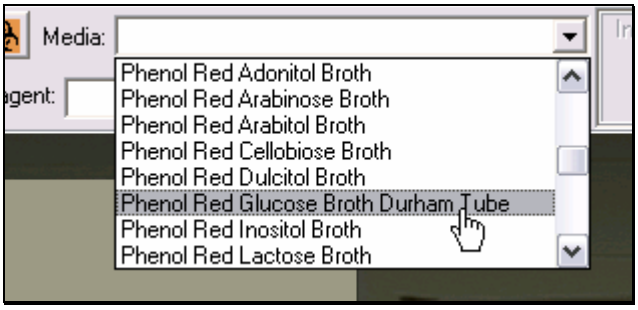
Section:_____

f. Next, provide an incorrect interpretation of the Gram stain results (say, "Spiral and Curved Bacteria"). **What message do you receive**?

5. **Fill in the Table below to reflect the features available at this point**.

| | Is this feature active? |
|---|---|
| "Tests", "Media", "Bacteria" reference book buttons | |
| "Help" features | |
| "View" features | |
| Media dropdown box list of media | |
| Tools dropdown box list of lab tools | |
| Reagent dropdown box list of test reagents | |

6. 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" and click "okay". Two tubes appear in the foreground on the lab bench. These are the inoculum at left and the sterile tube of phenol red glucose broth with Durham tube at the right.




7. **Fill in the Table below to reflect the features available at this point**.

| | Is this feature active? |
|---|---|
| "Tests", "Media", "Bacteria" reference book buttons | |
| "Help" features | |
| "View" features | |
| Media dropdown box list of media | |
| Tools dropdown box list of lab tools | |
| Reagent dropdown box list of test reagents | |

Name:_____

Section:_____

8. **Based on your experience in this exercise and your understanding of the Tutorial, place the letters in the blanks for the following events in their proper order to demonstrate the sequence in which features of the software are activated in the Virtual Lab**. If two events happen simultaneously, either may be listed first.

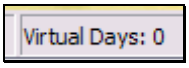
| | | |
|---|---|---|
| First | _____ | A. Media appear in Media dropdown box |
| | _____ | B. Case Study appears |
| | _____ | C. "Tests", "Media", and "Bacteria" reference book buttons function |
| | _____ | D. Tubes appear on screen |
| | _____ | E. "View" features become activated |
| | _____ | F. Gram stain image appears for your interpretation |
| | _____ | G. Reagents appear in Reagent dropdown box |
| Last | _____ | H. Tools appear in the Tools dropdown box |

9. With the selection of a medium for use in testing, the two traffic signals appear in the upper right above the lab change. **What is the initial color of each**?


Inoculation:_____ Contamination:_____

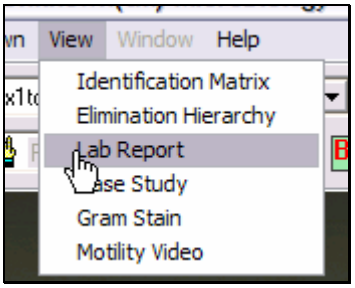
**What might be the meaning of the color in each instance**?

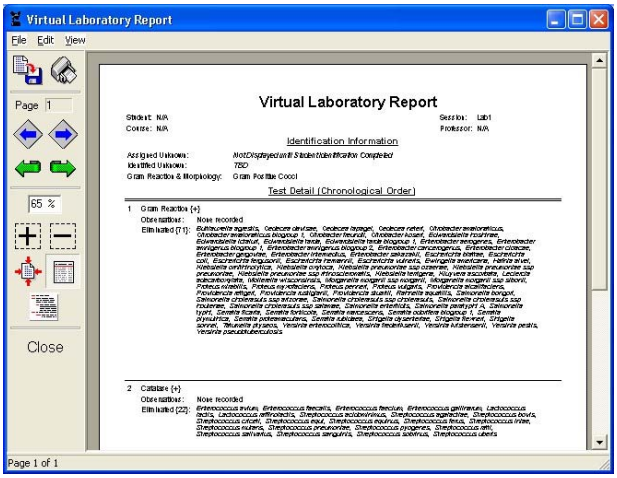
10. At the bottom right is a field indicating the number of "Virtual Days" that have transpired in the lab. Click on the "New Day" button above the lab scene and note that time has advanced 24 hours. **Why would this be a necessary feature for working in the Virtual Lab**?





11. 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 lab report**:




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

Name:_____

Section:_____

b. **What is the first test recorded**?

**c. How many organisms were eliminated by this result**?

d. **What detail is provided in the Test Detail (shown in red) which explains your errors and mistakes at this point?**

12. **Print out the Virtual Lab Report and attach it to this Exercise**. Exit the lab report.

13. Right-click on the various pieces of lab equipment in the Virtual Lab. **Which objects contain popup menus revealed by a right click of the mouse**? **List each and the options available for each**.

14. Try to exit the Virtual Lab. **What message do you receive**?

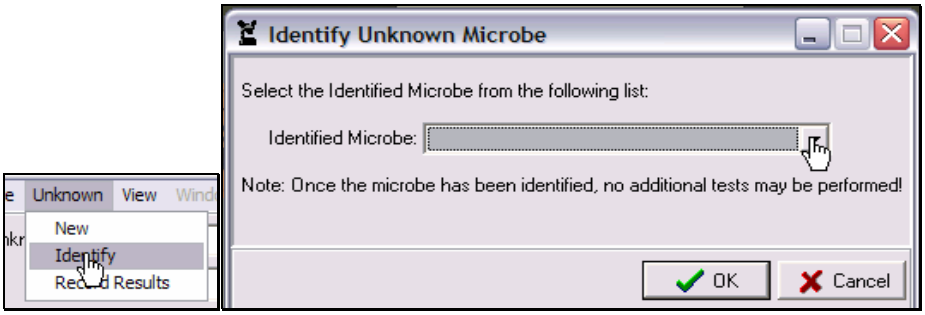
15. Use the skills learned in the Tutorial to attempt to dispose of the media. **What message do you receive**?

Name:_____

Section:_____

16. **Click 'Yes" and input results (make guesses)**. Then repeat the disposal of the media.

17. 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. Make a guess.




**What feedback is given**?

18. When prompted about whether to return to 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**?

19. Exit the Virtual Lab Report and continue your tour of the Virtual Lab. When you feel comfortable with the lab and believe you can navigate through the features of the Virtual Lab sufficiently, you may exit. Turn this exercise and your Virtual Lab Report in to your instructor.

Name:_____

Section:_____

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Name:_____

Section:_____

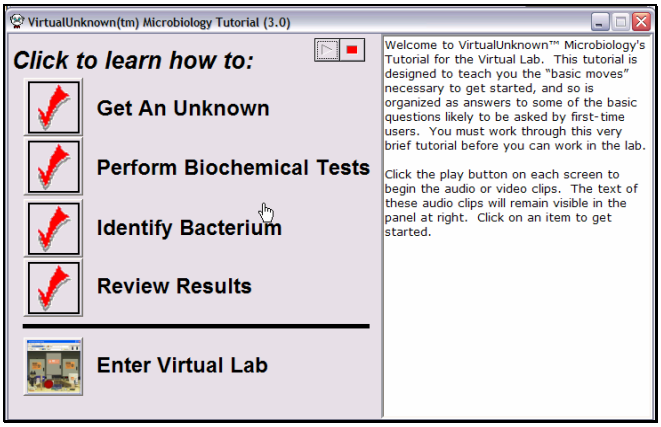
# 2… THE *VUMICRO™ 3.11* REFERENCE LIBRARY

**The goals for this exercise are**:

- to become familiar with the parts and features of the Virtual Lab
- to become familiar with the Reference books on media, tests, bacteria, reagents, and antibiotics provided in ***VirtualUnknown™ Microbiology 3.11***
- to learn how the Virtual Lab operates

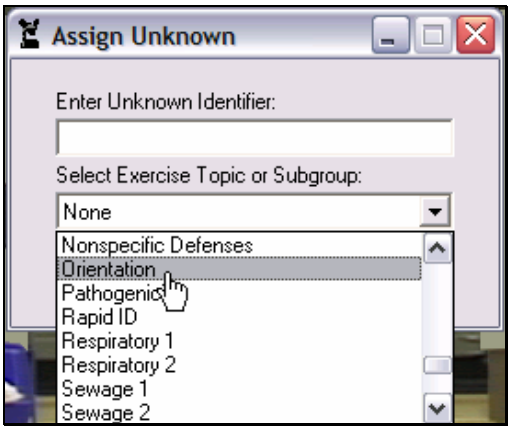
**Instructions**:

1. Open the software. **Work through the Tutorial to become acclimated to the means by which the Virtual Lab functions**. Once you feel comfortable with the lab, exit the Tutorial and enter the Virtual Lab.

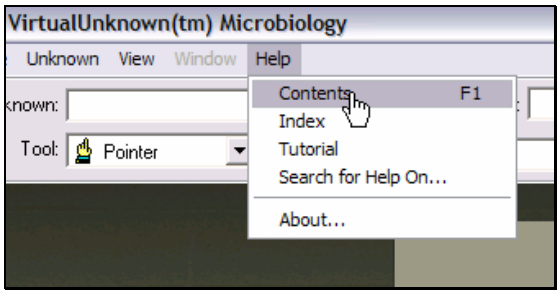



**2. Click on the "New" button to create a new unknown.**


**A box appears requesting you to provide an identifier for the unknown so that you can locate this work at a later time. Identify the unknown as "Orientation" and select "Orientation" from the options available in the "Exercise Topic or Subgroup" list.** *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. **DO NOT check the box to permit autoinoculation.**




**3.** Click on "Help" and review the options at your disposal



Name:_____

Section:_____

4. Below are listed the options available. **Briefly explain the contents of each**:

   a. Contents

   b. Index

   c. Tutorial

   d. Search for Help On…

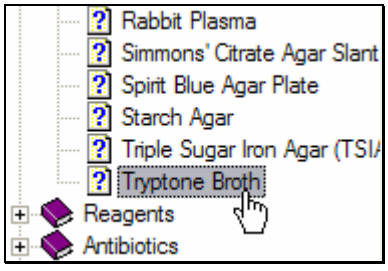
5. Above the Virtual Lab is a yellow button with the letter "T", which explains the biochemical tests in the software. Click on this button. **What is the first test in the alphabetical list?**

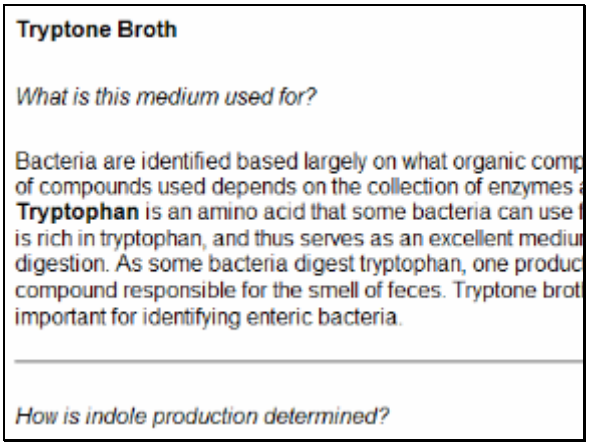
6. Exit this reference book and repeat the process for the other two. **What are the first items found in each of the alphabetized lists**?

   a. Blue button with letter "M" (media):

   b. Green button with letter 'B" (bacteria):

7. Open the Media reference book. Scroll down the list at left to find "tryptone broth". Clicking on the item in this list brings up detailed information about its content and use in the panel at right.

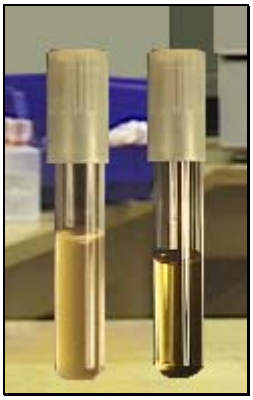


Name:_____

Section:_____

8. Select tryptone broth from the media dropdown list. You will be asked to provide an identifying name for medium. Type "trypone" in the appropriate field and click "OK". The medium will appear on the benchtop along with a tube of inoculum to its left.

9. Select the magnifier from the list of tools and use it to view close-ups of both tubes. The tube at left is the culture of unknown that will be used to inoculate the sterile tryptone broth at right. View the close-up of the tryptone broth tube to answer the following questions.


**What are the following characteristics of tryptone broth medium**?

a. Color:_____

b. Opacity:_____

c. Presence of Durham tube?_____

d. Presence of agar?_____

11. Answer the following questions using the reference books and links (words or phrases underlined and in green) between them:

a. **What test is this medium (tryptone broth) used for in *VUMicro™ 3.11***?

**b. How is indole production determined**?

c. **What is the content of this medium**?

d. **What reagent is used to complete the test, and what is its chemical composition**?

Name:_____

Section:_____

e. **How is the test performed**?

**f. What is the appearance of positive and negative test results?**

**12. Now, use the reference files to answer the following questions about other tests, media, and microbes**:

a. **What is the concentration of adonitol in phenol red adonitol broth**?

b. **At what p*H* does bromo-thymol blue indicator turn blue**?

c. **What is the chemical content of Barritt's reagents A & B**?

d. **What is the appearance of a positive DNase test**?

e. **What are the incubation conditions needed for performing a gelatinase test**?

Name:_____

Section:_____

f. **Why are the incubation conditions for the arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase tests different from all other tests described**?

g. **Where is the bacterium *Escherichia blattae* normally encountered**?

h. **Explain why most bacteria growing on mannitol salt agar would be expected to also grow in 6.5% NaCl broth**.

i. **How does gamma-hemolysis on blood agar differ from alpha-hemolysis and beta-hemolysis**?

Name:_____

Section:_____

j. **What is the antimicrobial mode of action for novobiocin**?

k. **What is the purpose for 0.1N hydrochloric acid in *VUMicro™ 3.11***?

l. **How is hydrogen sulfide production detected**?

m. **Which of the antibiotics listed is/are effective in preventing peptidoglycan synthesis**?

n. **How is it possible for a microbe to be positive for nitrate reductase whether or not the medium turns red after the addition of nitrate reagents A & B**?

Name:_____

Section:_____

o. **Where is the bacterium *Serratia ficaria* normally encountered**?

p. **Which *Staphylococcus* is used in sausage production**?

q. **Name one of the Gram positive cocci "found on the skin, udders, and lips of cattle, and in raw milk"**.

r. **How many of each are included in *VUMicro™ 3.11***?

- **Gram + bacteria**:
- **Gram – bacteria**:
- **identification tests**:
- **media**:

13. When you have completed your work, exit the Virtual Lab and turn in your work to your instructor.

Name:_____

Section:_____

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Name:_____

Section:_____

# 3… ASEPTIC TECHNIQUE IN *VUMICRO™ 3.11*

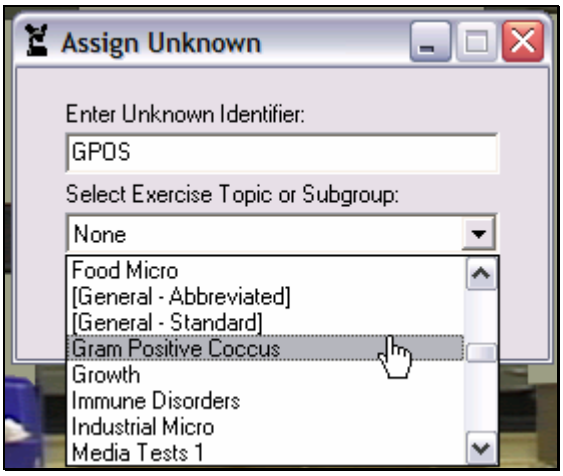
**The goals for this exercise are**:

- to become familiar with the parts and features of the Virtual Lab
- to become familiar with the means by which asepsis is recreated in the Virtual Lab and by which aseptic technique is simulated in ***VirtualUnknown™ Microbiology***
- to become familiar with means by which proficiency in aseptic technique can be communicated to the instructor through the Virtual Lab Report.
- to allow students opportunity to practice "wetlab" techniques beyond the limitations of time and space imposed by access to the wetlab.

**Instructions**:

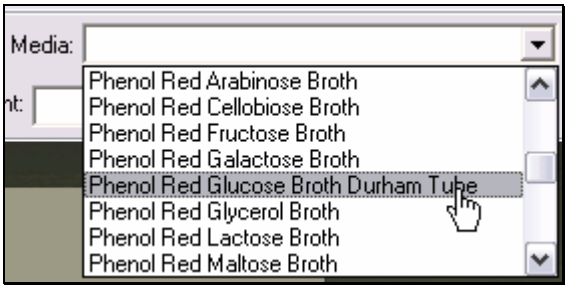
1. Open the software. **Work through the Tutorial to become acclimated to the means by which the Virtual Lab functions**. Once you feel comfortable with the workings of the lab, exit the Tutorial and enter the Virtual Lab.

   **Create a new unknown named "GPOS", using "Gram Positive Coccus" as the "exercise topic or subgroup"** *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box permitting auto-inoculation.




2. Read through the Case Study and interpret the Gram stain. Obtain a tube of phenol red glucose broth with Durham tube and provide an identifying label – prgb.

   Appearing will be two tubes – one at left which is your culture of unknown organism and the one at right which is sterile phenol red glucose broth.



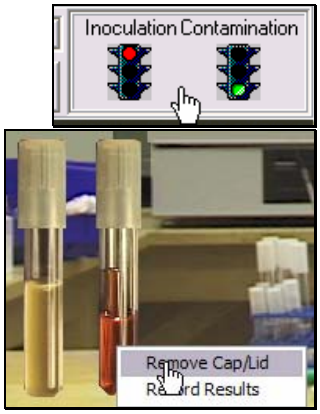

3. The purpose of aseptic technique is to control the conditions of the work environment so that sterile conditions and media remain that way until inoculated deliberately by the lab worker. The two tubes represent the "before" and "after" results when this is accomplished – a sterile medium and a pure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

Name:_____

Section:_____

4. 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 *VUMicro™ 3.11* before the Contamination light turns red**?

***NOTE: The steps needed to complete an aseptic transfer with VUMicro™ mirror those used in the wetlab. To review how to accomplish these steps using the software, review the "Tutorial" or "Tell Me More About Aseptic Transfers" in "Help".***




5. Replace the caps on those tubes, then drag the phenol red glucose broth tube and drop it in the 37° C incubator. Immediately retrieve the medium from the incubator by right-clicking on it and selecting the medium. **Explain why a contaminated culture has not demonstrated any observable change at this point**?


6. Replace the contaminated tube into the incubator. Click "New Day". Retrieve the medium from the incubator. **What does the turbidity and color change of the medium indicate**?


7. **Did you intentionally inoculate the medium**? **Interpret the meaning of these results and predict the source of the inoculum**.

8. **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**?

Name:_____

Section:_____

9. Dispose of the media by dragging the tube and dropping it in the biohazard waste container. You do not need to record any results.

10. Obtain another tube of phenol red glucose broth with Durham tube. **This time, watch the traffic signals and keep track of time as you complete the following steps**:

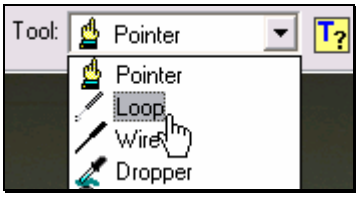
    a. Select the inoculating loop or wire from the Tool dropdown list.

    b. Remove the caps from the tubes.

    c. Obtain an inoculum from the culture at left and transfer it to the sterile medium at right.

    d. Replace the caps on the tubes.

***NOTE: The steps needed to complete an aseptic transfer with VUMicro™ mirror those used in the wetlab. To review how to accomplish these steps using the software, review the "Tutorial" or "Tell Me More About Aseptic Transfers" in "Help".***




11. **Did you complete the transfer within the allotted time period**? Yes No
**If not, repeat until you can do so**.

12. **Explain why the traffic signal indicating "Contamination" turned red even when the tubes were not open longer than allowed**.

13. Dispose of the media in the biohazard waste container. You do not need to record any results.

14. **Place the following steps in their proper order to indicate the sequence of events necessary to successfully complete an aseptic transfer in the wet lab**. NOTE: Some of the steps are performed twice.

| | | |
|---|---|---|
| First | _____ | A. dip loop into inoculum |
| | _____ | B. replace caps |
| | _____ | C. dip loop into sterile broth |
| | _____ | D. flame loop |
| | _____ | E. remove caps |
| | _____ | F. flame tube mouths |
| | _____ | G. ignite burner |
| | _____ | |
| Last | _____ | |

15. Sometimes an aseptic transfer is described as entering and working in a closet – you must go out the same way you came in. **Why is this description of the steps of an aseptic transfer appropriate**?

Name:_____

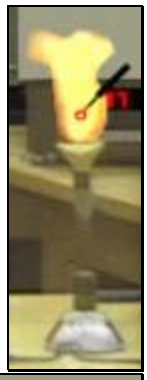
Section:_____

16. Obtain a third tube of phenol red glucose broth with Durham tube. **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.

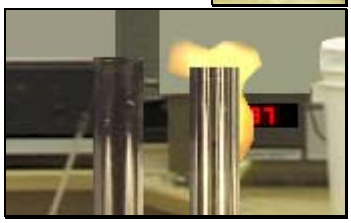
    b. Ignite the burner by right-clicking on the burner.

    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 text fields below the lab 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 "Tell Me More About…" feature.]***



    e. Perform the aseptic transfer by dipping the 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. **What change in the traffic signals occurs as this step is completed**? **Does the color change make sense from your knowledge of aseptic technique in a wetlab**? **Explain**.

    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**.

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

    h. Place the inoculated culture in the 37$^{o}$ C incubator. Click "New Day". Retrieve the medium and record the results. For information in the interpretation of the results, refer to the "Acid from Glucose" 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. Dispose of the media in the biohazard waste container.

Name:_____

Section:_____

17. Obtain yet another tube of phenol red glucose broth with Durham tube. Select the cotton swab as the tool for the aseptic transfer. **What is the warning message that you receive**?

18. Select the inoculating loop as your tool for inoculating the phenol red glucose broth that is present. Inoculate the sterile medium using your best aseptic technique. However, after sterilizing the loop, removing the caps, and flaming the tubes, move the sterile loop slowly away from the central part of the lab scene toward the four corners of the virtual lab. Do so briefly before completing the aseptic transfer without any other mistakes, making sure to complete the task within the allocated period of time.

    a. **At what point during the transfer did the contamination light turn red**? **Why does contamination during unnecessary movement away from the central work area make perfect sense**?

    b. Dispose of the media in the biohazard waste container.

19. **Perform without contamination the three tests 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 a table below.

| Medium Used | Appearance Following Incubation | Interpretation of Results |
|---|---|---|
| DNase agar with methyl green | | |
| Simmons citrate agar slant | | |
| Christiansen's urea broth | | |

20. Print out your Virtual Lab Report and attach it to this Exercise for submission to your instructor. Take a moment to review all of the comments on your work in the Virtual Lab. **What warnings or messages appear most frequently**?

Name:_____

Section:_____

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Name:_____

Section:_____

# 4… REPORTS AND DOCUMENTS IN *VUMICRO™ 3.11*

**The goals for this exercise are**:

- to become familiar with the parts and features of the Virtual Lab
- to become familiar with the reports and documents internally generated for monitoring progress, accuracy, and proficiency in ***VirtualUnknown™ Microbiology***
- to become familiar with means by which labwork can be communicated to the instructor.
- to learn how the Virtual Lab operates

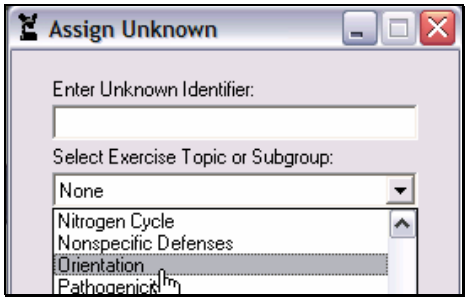
**Instructions**:

1. ***VUMicro™ 3.11*** uses a variety of reporting features and allows printing of helpful documents. The reporting features consist of
   - ***a. Things you report.*** In your work you will be interpreting tests and reporting results. These are recorded in the Virtual Lab Report and may result in immediate reporting back to you by the software.
   - ***b. Things reported to you by the software.*** When you make a critical mistake in some of the tests and procedures, you will receive immediate feedback reporting your error before you are allowed to proceed.
   - ***c. Things recorded on the Virtual Lab Report and in the Elimination Hierarchy.*** The software monitors your accuracy in performing and interpreting tests and procedures. Though some errors are reported back to you immediately, most are merely recorded on the Virtual Lab Report. This includes incorrect interpretation of test results and inaccurate identifications, which are only revealed upon completion of the work on the unknown organism. The Elimination Hierarchy simply provides a visual means for following the elimination of microbes as you work your way through the battery of tests.

   To demonstrate how the software responds to data provided by you, follow these directions:

   Open the software. **Work through the Tutorial to become acclimated to the means by which the Virtual Lab functions**. Once you feel comfortable with the workings of the lab, exit the Tutorial and enter the Virtual Lab. **Select the unknown labeled "Orientation" to work with in this exercise**. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box allowing autoinoculation.

   ***NOTE: The steps needed to complete an aseptic transfer with VUMicro™ mirror those used in the wetlab. To review how to accomplish these steps using the software, review the "Tutorial" or "Tell Me More About Aseptic Transfers" in "Help".***


2. Read the Case Study and proceed to the Gram stain image. Interpret the Gram stain ***incorrectly***.

   **Correct Gram stain interpretation:**_____

   **Incorrect Gram stain interpretation entered:**_____

   **What notice appears on the screen**?

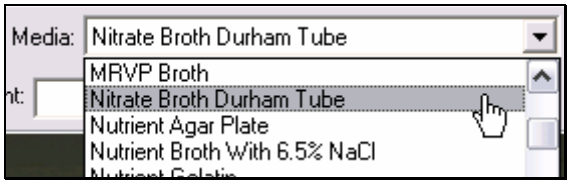
Name:_____

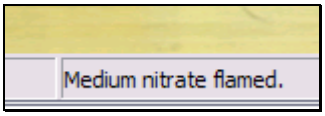
Section:_____

3. This is an example of immediate feedback from the software when data is input incorrectly. The software must correct your interpretation of the Gram stain because the selection of possible unknowns and test media available must be set before proceeding.

4. Perform the **nitrate reduction test**. If you are unfamiliar with this test, you may read up on in by locating the discussion in the Test Reference book. Below are the steps for completing this test:

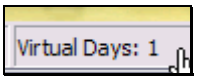
   a. Select nitrate broth from the Media list. Inoculate the broth using proper aseptic technique for a tube-to-tube transfer. Be sure to observe the status comments in the field below the Virtual Lab, as they represent a form of immediate feedback from the software on the status of events in the lab. **List three messages appearing there and the conditions under which they appeared**:

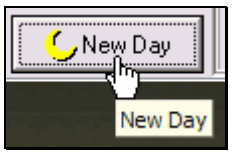



   b. The inoculated culture for a nitrate test is supposed to be incubated in the 37° C incubator (the one at right). Attempt to place it in the 25° C incubator (the one at left). **What message is given**?

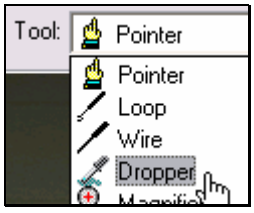
   c. Place the culture in the correct incubator. Note the field below the Virtual Lab where the number of Virtual Days is indicated, and then click "New Day" to advance and incubate your culture. This is another form of reporting provided by the software – immediate feedback on the status of events in the lab. **Does the number of Virtual Days increase**?

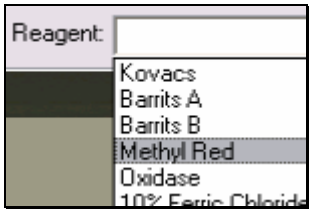



   d. **Was your burner still lit when the "New Day" button was clicked**?

   e. Remove the culture from the incubator. This is a test requiring addition of reagents to complete the chemical reactions necessary for interpreting results. If you are unfamiliar with the chemistry of the test and the reagents used, consult the Test Reference book's description of the nitrate reduction test. Select the dropper from the list of tools and an ***incorrect*** reagent from the list of reagents. Remove the cap lid from the culture and add the incorrect reagent to the tube. **What happens**?

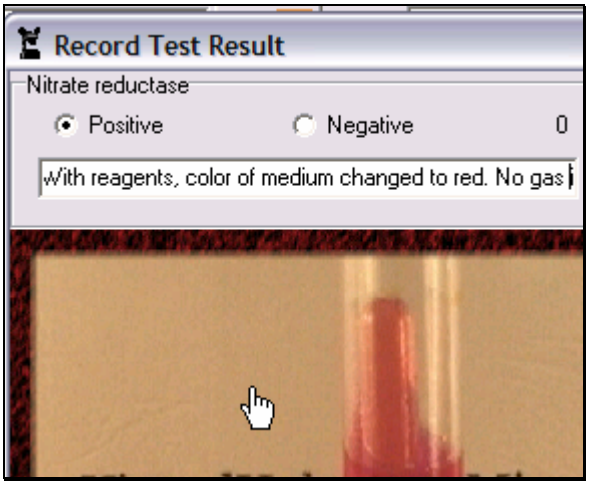


Name:_____

Section:_____

f. Next, add the correct reagents. Interpret the results and refer back to the Test Reference book in Help if necessary. Replace the cap, and select the option to record results. Be sure to record some notes on the appearance of the culture and Durham tube in the field provided for that purpose.




**Reproduce the information entered in the software in the field below**:

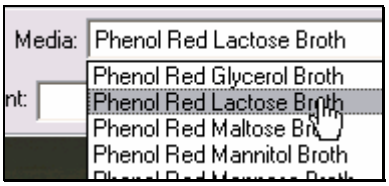
Positive or Negative (circle one)

Notes:

g. Dispose of the medium.

5. In the nitrate reduction test you were prevented from making critical mistakes by feedback from the software. However, you were not prevented from making mistakes in interpreting the results recorded for the test. Next perform a test where an incorrect result is recorded in the Virtual Lab Report.

a. Select phenol red lactose broth from the media list. If you are unfamiliar with the **lactose fermentation test** you may read up on it in the Test Reference book in Help. Inoculate the medium using proper aseptic technique, incubate it properly, and retrieve it from the incubator.




b. The lactose fermentation test is positive when the original red medium is turned yellow by the acids produced during lactose fermentation. If the tube remains red or turns hot pink, the result is negative. **What color is your culture, and what is the result observed**?

c. Select the option to record results for the test, and record the ***incorrect*** result. **What feedback is given by the software when this happens**?

d. Dispose of the medium.

Name:_____

Section:_____

6. What feedback is provided when contamination occurs? To find out, let's purposely contaminate a plate of medium.

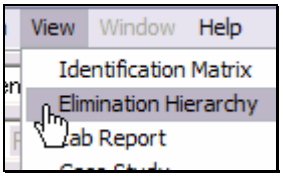
   a. Select the DNase plate with methyl green from the media list. **What are the colors of the "Inoculation" and "Contamination" traffic signals in the upper right corner above the lab**?

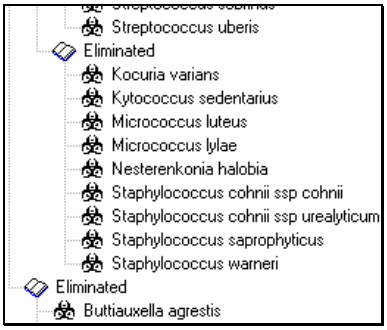
   b. Select the option to remove the tube cap and plate lid. **What change occurs immediately in the traffic signals, and what does this indicate about the cultures**?

   c. Wait for at least two minutes. **What final change occurs in the traffic signals in the upper right corner**?

   d. This is another form of reporting by the software to help you follow your success in accomplishing your work in the lab successfully. **Right-click on the tube and select the option to record a result for this test (either positive or negative is fine) and dispose of the medium**.

7. Open the Elimination Hierarchy and study its contents. This report on your work differs in many ways from the other reporting methods in ***VUMicro™ 3.11***. Compare the Elimination Hierarchy with the Virtual Lab Report. **What effect does your report of results have on the makeup of the Elimination Hierarchy**?



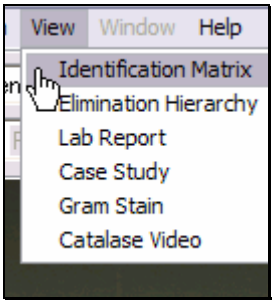




8. **How might the Elimination Hierarchy be useful in conducting work to identify an unknown organism**?

Name:_____

Section:_____

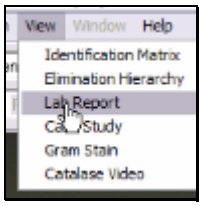
9. Open the Identification Matrix and note the results recorded in the column at left for "Assigned Microbe". **Are these the results you submitted for tests performed or the correct results in every case**?

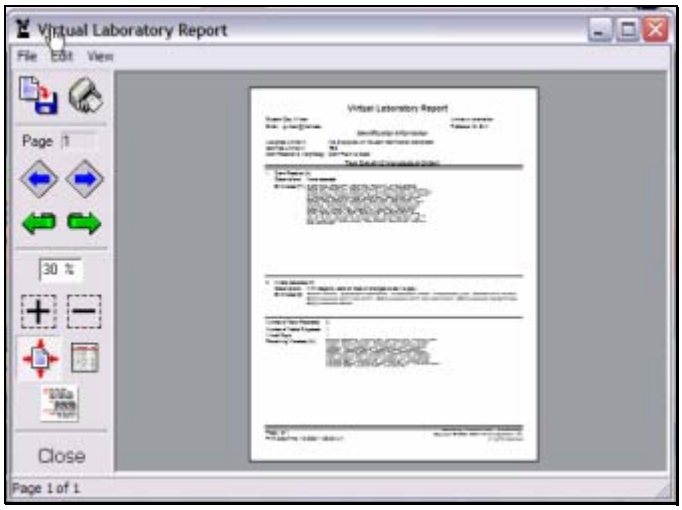



| | Assigned Unknown | *Dermacoccus nishinomiyaensis* | *Enterococcus avium* | *Enterococcus faecalis* | *Enterococcus faecium* | *Enterococcus gallinar* |
|---|---|---|---|---|---|---|
| Acid from glucose | | [-] | + | + | + | + |
| Alpha hemolysis | | - | + | d | + | d |
| Arabinose fermentation | | [-] | + | - | d | + |
| Arginine dihydrolase | | - | - | + | + | + |
| Bacitracin 0.04U susceptibility | | + | - | - | - | - |
| Bacitracin 2U susceptibility | | + | - | - | - | - |
| Beta hemolysis | | - | - | + | - | + |
| Catalase | | + | - | - | - | - |
| Cellobiose fermentation | | + | d | [-] | [+] | [+] |
| Citrate Utilization (Simmons) | | - | - | - | - | + |
| Coagulase | | | | | | |

10. You know that you have recorded one test result correctly (nitrate reduction), one incorrectly (lactose fermentation) and had one test contaminated (DNase). Now it is time to see how these results were recorded.

    a. Open the Virtual Lab Report. **Record below all Test Details presented for each of the tests performed**:




Gram Stain:

Nitrate Reductase:

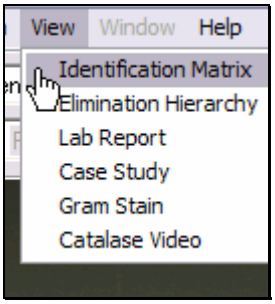
Lactose Fermentation:

DNase:

Name:_____

Section:_____

11. Open the Identification Matrix and note the results recorded in the column at left for "Assigned Microbe". **Are these the results you submitted for tests performed or the correct results in every case**?




| | Assigned Unknown | *Dermacoccus nishinomiyaensis* | *Enterococcus avium* | *Enterococcus faecalis* | *Enterococcus faecium* | *Enterococcus gallinar* |
|---|---|---|---|---|---|---|
| Acid from glucose | | [-] | + | + | + | + |
| Alpha hemolysis | | - | + | d | + | d |
| Arabinose fermentation | | [-] | + | - | d | + |
| Arginine dihydrolase | | - | - | + | + | + |
| Bacitracin 0.04U susceptibility | | + | - | - | - | - |
| Bacitracin 2U susceptibility | | + | - | - | - | - |
| Beta hemolysis | | - | - | + | - | + |
| Catalase | | + | - | - | - | - |
| Cellobiose fermentation | | + | d | [-] | [+] | [+] |
| Citrate Utilization (S mons) | | - | - | - | - | + |
| Coagulase | | | | | | |

12. **Indicate whether the following statements about the Virtual Lab Report are True or False. Explain any observations you have about these statements.**

    a. The Virtual Lab Report let me know when I made mistakes in ***technique***: True False

    b. The Virtual Lab Report let me know when I made mistakes in ***interpreting tests***: True False

    c. Feedback appearing immediately in the Virtual Lab does not necessarily also appear in the Virtual Lab Report, and vice versa. True False

    d. Possible microbes are eliminated based on the correct test results: True False
    Observations:

13. Identification tables similar to the VUMicro™ Identification Matrix are often included in laboratory manuals and reference books. They share in common certain terminology for results: +, (+), d, (-), and -, among other terms and symbols. What do each of these results indicate?

    +

    (+)

    d

    (-)

    -

14. **Print the Virtual Lab Report for submission to your instructor along with this Exercise**. Close the Virtual Lab Report.

Name:_____

Section:_____

# 5… UBIQUITY OF MICROBES

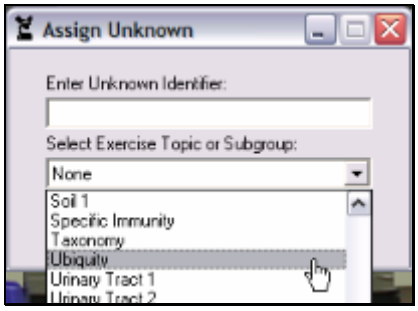
**The goals of this exercise are**:

- to gain an understanding of the presence of microbes in our surroundings
- to gain an understanding of the effects microbes have on the appearance of culture media
- to gain an understanding of the means by which microbial contamination of media can occur
- to connect the landmark experiments of Pasteur in the study of spontaneous generation to the containers now used in working with microbial cultures.

**Instructions**:

1. Familiarize yourself with ***VirtualUnknown™ Microbiology*** by working through the Tutorial as you enter the software. Once you are comfortable with the mechanics of the software, enter the Virtual Lab.

   Select **Ubiquity** from the list of predefined unknowns to activate the lab. No work can be attempted until this step is completed. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box allowing autoinoculation.




2. Select **phenol red glucose broth with Durham tube broth** from the media list. Label the tube by typing “glucose” in the field provided in the “Medium Label” box that appears. This allows you to locate and retrieve the culture tube from the incubator later.

3. Think back to Exercise 3 and your investigation of the way ***VUMicro™*** recreates the hazards to asepsis found in the wetlab. **What was the source for contamination of open test tubes and plates?**

**4.** Think about the culture tubes and the petri dishes you may have used in your microbiology wetlab. Neither of these types of containers is air-tight. **Illustrate the route air must take to enter (1) a tube of culture medium and (2) a petri dish of agar medium.**

Culture tube Petri dish

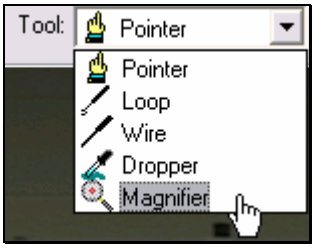
**5. Using your textbook or other resources, draw below the path that air would take to enter one of Pasteur’s swan-neck flasks.**

Name:_____

Section:_____

**6.** Compare your drawing for the swan-neck flask with the ones drawn for the airflow route or air entering culture tubes and Petri dishes. **Why are these designs effective in preventing microbial contamination?**

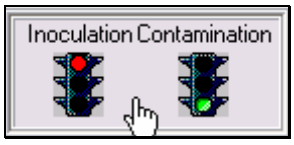
**7.** Back to the Virtual Lab… Visible will be the tube of your unknown organism at left and a tube at phenol red glucose broth at right. Use the magnifying glass tool to observe close-ups of both. **Can the text be read through the broth?**




| | | |
|---|---|---|
| **Unknown culture:** | **Yes** | **No** |
| **Phenol red glucose broth:** | **Yes** | **No** |

**8. What is responsible for the turbidity in the tube at left?**

9. Observe the two "traffic signals" above the right corner of the virtual lab. The red light in the signal at left indicates the inoculation of the sterile medium has not been accomplished. The green light in the signal at right indicates no contamination has occurred.




10. As described in the Tutorial, remove the caps from the two tubes and leave them open for 15 seconds before replacing them in the same manner. **Why do the two signals turn yellow when the caps are off?**

11. **Compare the color of the signals after replacing caps with their color before removing caps.**

Name:_____

Section:_____

12. Repeat steps 11 & 12, this time leaving the tubes open until there is a change in the colors displayed by the signals. **How would Louis Pasteur explain the change in the traffic signals, in comparison with the results observed in steps 11 & 12?**

13. **Why does the phenol red glucose broth with Durham tube still appear clear even when the signals indicate contamination has occurred**?

14. Dispose of the medium in the biohazard container. **When prompted to record results for the test being performed, do so by guessing**. **What was your guess?**

15. Obtain another tube of phenol red glucose broth with Durham tube. This time, change your cursor into an inoculating loop by selecting that option from the Tool menu.

16. Remove the tube caps, and without flaming the loop or tubes, run the loop down into the phenol red glucose broth. **What does this action do to the color of the right stoplight above the lab**?

17. **Why is this indication of contamination completely understandable and expected, based on the action you just took**?

18. Replace the cap on the contaminated medium and place it in the $37^{o}$ C incubator. **Predict the appearance of the culture medium after 24 hours of incubation**.

Name:_____

Section:_____

19. Click the "New Day" button to advance virtual time 24 hours. Remove the medium from the incubator. **Was your prediction accurate**?

20. **Right-click on the tube and record the results you observe for the test**. Then, dispose of the medium in the biohazard waste container.

21. Open the Virtual Lab Report and review the results recorded for the "acid with glucose" and "gas with glucose" tests you have just reported using the phenol red glucose broth with Durham tube. Although you are unable to determine whether your "guess" was accurate, other error messages and warnings appear in red under the Test Details. **What were the messages recorded**?

22. **What do these error messages and warnings tell you about how closely *VUMicro*™ mimics nature in demonstrating the ubiquity of microbes**?

23. Exit the Virtual Lab.

Name:_____

Section:_____

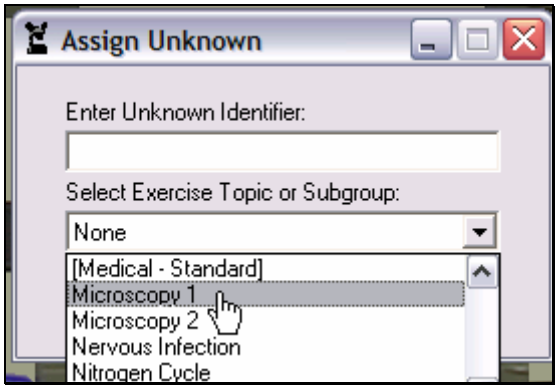
# 6… MICROSCOPY

**The goals of this exercise are**:

- to gain the ability to identify the type of microscope used by the appearance of the image produced
- to gain practice in connecting the type of slide preparation used to the appearance of the image observed.
- to gain an appreciation for the differences in size of various microbes when observed under similar magnification.
- to gain the ability to distinguish between Brownian movement and true motility in bacteria.

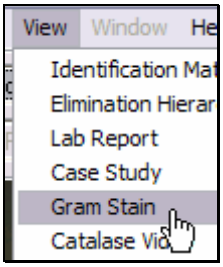
**Instructions**:

1. Enter the Virtual Lab and select **Microscopy 1** from the predefined unknowns available from the Unknown dropdown box. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box allowing autoinoculation.




2. After reading the case study you will be taken to a Gram stain image of the unknown organism. You cannot work in the Virtual Lab until the Gram stain results are recorded. Do so and enter the lab.

3. You may review the **Gram stain image** at any time by selecting that option from the View list found in the Main Menu bar above the Virtual Lab, or by right-clicking on the microscope.





   a. **Which of the following dyes is responsible for the color of the cells**?

   A. Malachite green  B. Iodine  C. Safranine  D. Methylene blue  E. Crystal violet

   b. **Which of the following types of microscopy was most likely used to get this image**?

   A. Fluorescence Microscopy
   B. Transmission Electron Microscopy
   C. DIC Microscopy
   D. Scanning Electron Microscopy
   E. Bright Field Microscopy
   F. Confocal Microscopy

   c. **Would yeast cells appear smaller, larger, or the same size as the bacteria observed here**? **Explain**.

Name:_____

Section:_____

d. Next, change bacteria by selecting **Microscopy 2** from the predefined unknown list.

e. Bring up the **Gram stain image** by selecting that option from the View list found in the Main Menu bar above the Virtual Lab, or by right-clicking on the microscope. **How does the appearance of these bacteria differ from those observed in the Gram stain image for Microscopy 1**?

f. **Which of the following dyes is responsible for the color of the cells in Microscopy 2**?

A. Malachite green B. Iodine C. Safranine D. Methylene blue E. Crystal violet

g. **Which of the following types of microscopy was most likely used to get this image**?

A. Fluorescence Microscopy

B. Transmission Electron Microscopy

C. DIC Microscopy

D. Scanning Electron Microscopy

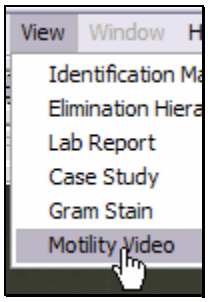
E. Bright Field Microscopy

F. Confocal Microscopy

h. **Which of the following ranges of magnification most likely is depicted in the software images**?

A.40-100X B.100-400X C.400-1000X D. Over 1000X

i. Exit the Gram stain image and bring up the **motility video clip** by selecting that option from the View list found in the Main Menu bar above the Virtual Lab, or by right-clicking on the microscope. Watch the video clip. **Are these bacteria motile**? **What key observations lead you to this conclusion**?





j. **What type of slide preparation was used to observe the bacteria for motility**?

k. **Which of the following types of microscopy was most likely used to obtain this video clip**?

A. Fluorescence Microscopy

B. Scanning Electron Microscopy

C. Transmission Electron Microscopy

D. Bright Field Microscopy

E. DIC Microscopy

F. STEM

l. Practice laboratory tests on this organism, if desired. When you are done in the Virtual Lab, exit the software.

Name:_____

Section:_____

# 7… BACTERIAL ANATOMY, MORPHOLOGY, AND ARRANGEMENT

**The goals of this exercise are**:

- to further understanding of bacterial structure
- to further understanding of how structure is related to function in bacteria
- to further understanding of how tests and methods for identification often are reliant on microbial structure
- to further understanding of the factors responsible for cell integrity, shape, grouping, and motility.

**Instructions**:

1. Enter the Virtual Lab and select **Bacterial Anatomy 1** from the predefined unknown list found in the Unknown dropdown box above the Virtual Lab.

2. Display the Gram stain for this unknown organism by selecting **Gram stain** from the View options in the Main Menu bar, or by right-clicking on the microscope. 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**.

3. **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 |

4. **Which dye from the Gram stain procedure is responsible for the observed color of the cells**?

5. **What cell structure(s) is(are) stained by the dye to give this organism the color observed**?

Name:_____

Section:_____

6. **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. periplasmic space present
   E. flagella with only two rings in basal body
   F. endotoxin present
   G. resistance to salts and drying
   H. resistance to penicillins

7. **What is the terminology used for the cell anatomy and arrangement observed for this organism**?

8. Next, observe a different organism by selecting **Bacterial Anatomy 2** from the predefined unknowns listed.

9. Display the Gram stain for Bacterial Anatomy 2 by selecting **Gram stain** from the View options in the Main Menu bar, or by right-clicking on the microscope. 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**.

10. **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 |

11. **Which dye from the Gram stain procedure is responsible for the color of the cells**?

12. **What cell structure(s) is(are) stained to give this organism the color observed**?

Name:_____

Section:_____

13. **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. periplasmic space present
E. flagella with only two rings in basal body
F. endotoxin present
G. resistance to salts and drying
H. resistance to penicillins

14. **What is the terminology used for the cell anatomy and arrangement observed for this organism**?

15. 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**?

16. **Does the lack of motility among bacteria prove the lack of flagella**? **Explain**.

17. **Does the lack of flagella among bacteria prove the lack of motility**? **Explain**.

18. **Draw and label below how flagella are anchored in Gram + and Gram – cell walls**.

Gram +

Gram -

Name:_____

Section:_____

19. The number and arrangement of flagella observed is consistent for the members of a species. **Draw below and label three arrangements of flagella possible in bacteria**.

20. Bacteria possess two sets of enzymes involved in peptidoglycan synthesis. One set is responsible for cell lengthening and the other set is responsible for building the crosswall to separate daughter cells. **Explain how the relative rates of activity of these two systems can influence the cell shape**.

21. 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. **Would the unknown bacteria observed in the Gram stain and motility video 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?**

Name:_____

Section:_____

# 8… GROWTH MEDIA AND PATTERNS OF GROWTH

**The goals for this exercise are**:

- to demonstrate the differences in content and uses for culture media
- to demonstrate the ways in which media can supply the substances required for growth
- to connect the discussion of growth patterns and media in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Enter ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Growth** from the list of predefined unknowns. Use your textbook and the Reference Resources provided in the ***VUMicro™ 3.11*** Help files to complete the following questions. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box allowing autoinoculation.

2. Complete the table below to provide descriptions for the following media:

| | Complex or Synthetic? | Selective, Differential, or Both? |
|---|---|---|
| Malonate broth | | |
| Tryptone broth | | |
| KCN broth | | |
| Nutrient gelatin | | |
| OF glucose broth | | |
| DNase agar | | |
| Ornithine decarboxylase broth | | |

3. Complete the table below to provide descriptions for the following media:

| | Carbon Source | Nitrogen Source |
|---|---|---|
| Phenol red adonitol broth | | |
| KCN broth | | |
| Phenylalanine deaminase agar | | |
| Simmons' citrate agar | | |
| Spirit blue agar | | |
| Nutrient gelatin | | |

4. 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.
   a. **Based on these results, circle the classification below that best fits your unknown microbe**:

      Strict aerobe  Strict anaerobe  Facultative anaerobe  Microaerophile

   b. **What is responsible for the color change observed in a positive test**?

Name:_____

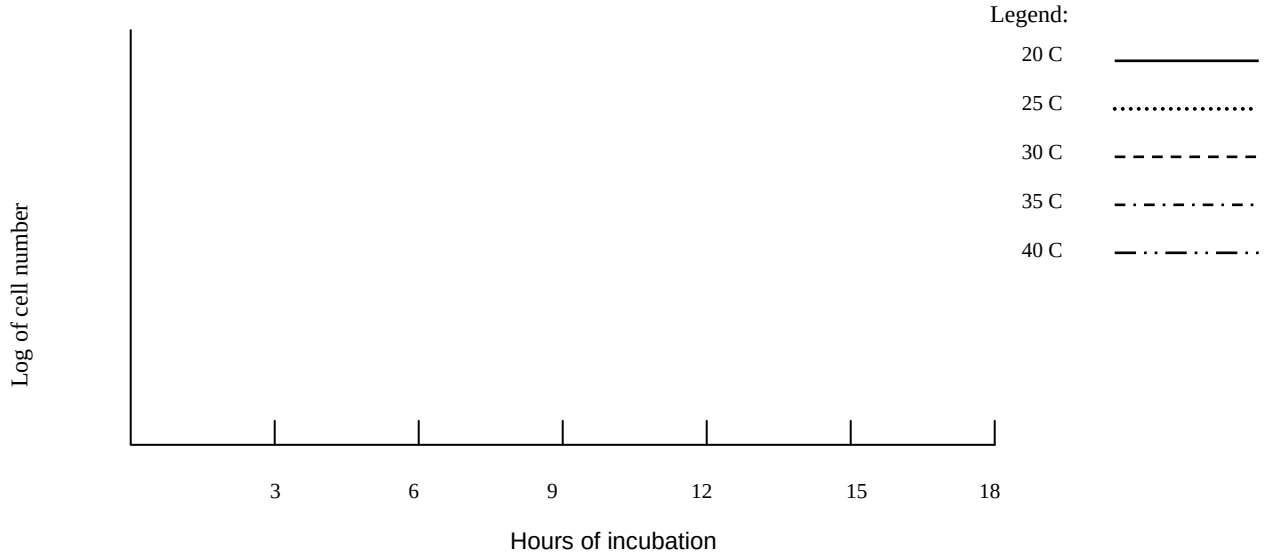
Section:_____

c. **Predict (and draw) the expected appearance of fluid thioglycollate medium containing a culture of your unknown**.

5. Select tryptone broth from the media list. **Explain why the inoculum at left and the sterile medium initially provided at right have very different appearances** (NOTE: You may view closeups of the tubes by using the magnifying tool in the tool bar above the Virtual Lab.).

6. Next, inoculate the tryptone broth and incubate it for 24 hours. Use the magnifier and compare its appearance after incubation to its appearance before. Repeat this exercise with malonate broth.
   a. **Explain the cause of the different appearances for the tryptone broth and the malonate broth following incubation**.

   b. **Graph below the expected results after 18 hours for six sterile tubes of an identical medium inoculated simultaneously with an enteric microbe and incubated at 20 C, 25 C, 30 C, 35 C, 40 C and 45 C**.



Name:_____

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# 9… CONTROLLING MICROBIAL GROWTH

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to illustrate the ways in which chemicals can alter the growth of microbes.
- to connect the steps in aseptic technique to their role in preventing contaminants from entering and thriving in an otherwise sterile medium.
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Enter the Virtual Lab and select **Controlling Growth** from the list of predefined unknowns. Use your textbook and the Reference Resource books in the software to help answer the following questions. *(refer to Exercise 1, step 4 for instructions on how to create an unknown)*. For this exercise, DO NOT check the box allowing autoinoculation.

2. One of the oldest food preservatives is salt. **How does salt control microbial growth and reduce spoilage?**

3. Click on the Media references icon (designated by the letter "M") to display the media ***VUMicro™ 3.11*** includes for identifying bacteria. **What two media include elevated concentrations of sodium chloride for preventing growth of most bacteria**?

4. **Would bacteria growing on these media be more likely than those that cannot to cause food spoilage**?

5. Bile salts are natural products of the digestive system that prevent the growth of many bacteria. **How do bile salts exert antimicrobial activity**?

6. **What is the concentration of bile salts in bile esculin agar**?

Name:_____

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7. **What quality do the bacteria in *VUMicro™ 3.11* that will grow on bile esculin agar have in common**?

8. Nitrite is often used as a preservative in cured meats to prevent anaerobic growth of spoilage bacteria. **Based on your understanding of the nitrate reductase and nitrite reductase tests, why would the inclusion of nitrite inhibit anaerobic growth**?

9. One would think the potent poison potassium cyanide (KCN) would be toxic to bacteria also. Instead, potassium cyanide can actually serve as a source of nutrition to support the growth of certain bacteria. **Provide an explanation for its toxicity to humans and its nutritional qualities for some bacteria**.

10. **Draw below the appearances of strict aerobes, strict anaerobes, facultative anaerobes, and microaerophiles in thioglycollate broth**. **Explain the metabolic abilities resulting in each pattern of growth**.

Name:_____

Section:_____

11. **How would the growth patterns in thioglycollate relate to problems with food spoilage in canned vegetables**?

12. Some bacteria are able to shift their metabolism from growth in air to anaerobic growth. **How would such a shift in abilities make many common means for controlling microbial growth ineffective**?

13. **List below two culture medium employing an inhibitor to prevent the growth of unwanted microbes**. **Explain how the inhibitors accomplish their task**.

14. Microbes sometimes also can be inhibited by introducing antibiotics to their growth environment. Read the discussion of how antibiotic sensitivity tests found in the Test reference book (accessed by clicking the "T" icon) of ***VUMicro™ 3.11***. Antibiotic tests supported in the software include **bacitracin** and **novobiocin** sensitivity tests.
    a. **Why does the zone of inhibition form around the sensitivity disks when a microbe is susceptible to the chemical**? **How does the pattern of growth relate to the minimum inhibitory concentration**?

    b. **Why must the antibiotic be water soluble**?

Name:_____

Section:_____

15. Design an experiment that can be performed using ***VirtualUnknown™ Microbiology*** to investigate the effect of heat sterilization methods on the successful completion of an aseptic transfer. Your experiment should include such common steps as keeping tubes capped, sterilizing the transfer tools, and flaming the mouths of tubes.

    EXPERIMENTAL DESIGN:

16. Using your experimental design, test your hypothesis using ***VirtualUnknown™ Microbiology*** and present your results in a table. Discuss your observations.

    RESULTS:

| | | |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

OTHER OBSERVATIONS:

DISCUSSION:

Name:_____

Section:_____

# 10… MICROBIAL GENETICS AND BIOTECHNOLOGY

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using the reference materials found in ***VirtualUnknown™ Microbiology***
- to connect the discussion of molecular manipulations and biotechnology in lecture with the organisms in ***VirtualUnknown™ Microbiology,*** their habitats, and their traits
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Enter the Virtual Lab and select **Biotechnology** from the list of predefined unknowns. For this exercise check the box to enable auto-inoculation to speed up the work on this exercise. Use this predefined unknown for your work on the following problem:

   *You have been hired by a biotech firm to find new insecticides that are more environmentally-friendly. You believe there are safe and effective ways to genetically-engineer natural flora of pest insects to produce a recently discovered insecticidal protein. Always looking for potential bacterial hosts for the toxic gene, you have called your old classmates. One, an environmental microbiologist working for NASA, sent this as a candidate. Use the media and tests at your disposal in* ***VirtualUnknown™ Microbiology*** *to identify this unknown organism.* ***What is its identity****?*

2. **Would you suggest that this microbe has economic potential for genetic engineering to control pest insects**? **Explain**.

3. **If you found the organism to have promise as a vector to introduce a toxin gene into target insects, what would be its potential strengths and its potential weaknesses**?

Name:_____

Section:_____

4. Whether or not this organism shows promise, you know introduction of the toxin gene into its genome is possible. **Provide an experimental plan to introduce the toxin gene, now found in a Gram negative bacillus, expressed by the bacterium just identified**.

   GOAL OF THE PROJECT:

   METHODS AND SEQUENCE TO BE USED:

   HOW GENE EXPRESSION WOULD BE MONITORED:

   HOW EFFECTIVENESS WOULD BE DETERMINED:

Name:_____

Section:_____

# 11… CARBOHYDRATE TESTS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Be sure to familiarize yourself with the techniques used to perform biochemical tests in the laboratory and in ***VirtualUnknown™ Microbiology***. Use the Tutorial available upon entering the software and refer to Exercises 1-4 in this ***Basic Training Manual for VUMicro™ 3.11*** manual to learn the basics of doing aseptic transfers and interpreting test results. The reference library (explained in Exercise 2), your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation.

3. **Complete the tests listed in the table below and record the results**. They are automatically recorded in the Virtual Laboratory Report as well. 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. Answer these questions as you proceed:
   **a. What medium is used for determining production of acid and gas from glucose?**

   **b. What is the medium's initial color? What color change indicates a positive test result? Why does the medium change color in a positive test?**

   **c. What is the initial p*H* of the medium?**

   **d. Estimate the final p*H* of the medium in a positive test.**

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**e. Provide an explanation for the change in color and p*H* in a positive test.**

**f. What is the purpose of the Durham tube?**

4. Repeat the above assignment for the different microbes assigned for each of the following predefined unknowns:
   **Media Tests 2**: *Enterobacter aerogenes;*
   **Media Tests 3**: *Salmonella typhi; and*
   **Media Tests 4**: *Proteus vulgaris.*

   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:**

| Test | ***E. coli*** Media Tests 1 | ***Enterobacter aerogenes*** Media Tests 2 | ***Salmonella typhi*** Media Tests 3 | ***Proteus vulgaris*** Media Tests 4 |
|---|---|---|---|---|
| Gram stain result: | | | | |
| Acid from glucose: | | | | |
| Gas from glucose: | | | | |
| Lactose fermentation test: | | | | |
| Sucrose fermentation test: | | | | |

**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 the next few exercises.**

Name:_____

Section:_____

5. Use this table, the 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. Consult your textbook to provide an explanation for the inability of some bacteria to produce gas from glucose. **Predict the metabolic products expected for the four bacteria.**

      *E. coli*

      *Enterobacter aerogenes*

      *Salmonella typhi*

      *Proteus vulgaris*

   b. **If all four bacteria were able to metabolize glucose, explain why all four were not able to metabolize lactose and sucrose**.

   c. Sugar fermentation tests are positive when the p*H* turns acidic. **How are media for these tests modified to allow production of acids to be detected?**

   d. How many carbohydrate fermentation tests are supported in ***VirtualUnknown™ Microbiology***?

   e. 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?**

Name:_____

Section:_____

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Name:_____

Section:_____

# 12… AMINO ACID METABOLISM

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software and refer to Exercises 1-4 in this ***Basic Training Manual for VUMicro™ 3.11*** manual to learn the basics of performing biochemical tests. The reference library (explained in Exercise 2), your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation

3. **Complete the tests listed in the table below, recording the results for *E. coli*.** They are automatically recorded in the Virtual Laboratory Report as well.
   a. Arginine dihydrolase test, lysine decarboxylase test, and ornithine decarboxylase test use similar media and are performed in the same manner.
   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**: *Enterobacter aerogenes;*
   **Media Tests 3**: *Salmonella typhi; and*
   **Media Tests 4**: *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:

| Test | ***E. coli*** Media Tests 1 | ***Enterobacter aerogenes*** Media Tests 2 | ***Salmonella typhi*** Media Tests 3 | ***Proteus vulgaris*** Media Tests 4 |
|---|---|---|---|---|
| Arginine dihydrolase test: | | | | |
| Lysine decarboxylase test: | | | | |
| Ornithine decarboxylase test: | | | | |
| Phenylalanine deaminase test: | | | | |

Name:_____

Section:_____

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**?

7. **Why must this test be observed at 24 and 48 hours**?

8. **Why must there be a color change at 24 hours in order for results at 48 hours to be valid**?

9. **Indicate your observations for lysine decarboxylase broth during the lysine decarboxylase test**:

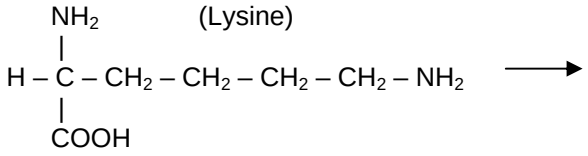
| | Color of medium | p*H* of medium |
|---|---|---|
| Uninoculated broth | | |
| Observation at 24 hours | | |
| Observation at 48 hours | | |

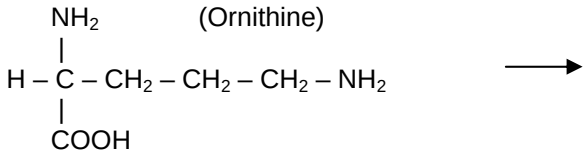
Name:_____

Section:_____

10. **Based on the results in the table above, what sequence of events must take place before the amino acid in each medium is metabolized**?

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




12. 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**?

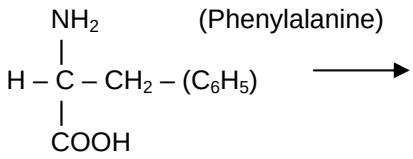
13. **Why must reagents be added to the phenylalanine agar slant *after*, rather than *before*, incubation**?

Name:_____

Section:_____

14. **How do the two reagents added to complete the phenylalanine deaminase test each contribute to the determination of phenylalanine deamination**?

15. Decarboxylases remove carboxyl groups. This enzyme is a deaminase. **With this in mind, complete the reaction below to show the structure of the product of phenylalanine deaminase**.



Name:_____

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# 13… NITRATE METABOLISM

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software to learn the basics of doing aseptic transfers and interpreting test results in ***VirtualUnknown™ Microbiology***. The reference library in the software, your textbook, and your lab manual will provide additional information on the tests, media, and reagents required to complete this assignment.

2. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Media Tests 5** from the list of predefined unknowns. This organism is *Staphylococcus aureus*. DO NOT check the box permitting autoinoculation.

3. Complete the nitrate test. Details on the medium used, the steps for completing the test, and the determination of the result are all found in the software's reference books. Click on the "T" icon to access information on tests and on the "M" icon to gain information on media. Answer the following questions:

4. **What is the source of nitrate in the medium**?

5. **When the 24-hour culture in nitrate broth is retrieved from the incubator, what is the appearance of (1) the broth and (2) the Durham tube**?

6. In order to complete this test, reagents must be added. **Indicate in the space below the chemical content of the reagents**:

   - Nitrate A

   - Nitrate B

7. **What chemical is indicated in the broth culture when it turns red after the addition of Nitrate A and Nitrate B reagents**?

8. **What enzyme(s) is(are) indicated when the culture turns red after Nitrate A and B are added**?

Name:_____

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9. **What chemical is collected in the Durham tube when it fills with gas**?

10. **What are the possible results indicated when the broth culture fails to turn red when Nitrate A and Nitrate B reagents are added**?

**How can one determine which possible result is, in fact, the outcome for this microbe?**

11. **Below, draw out the chemical reactions involved in the metabolism of nitrate when nitrate reductase and nitrite reductase are produced by a culture**.

12. Repeat the nitrate reduction test using the microbe assigned when **Media Tests 6** is selected from the Unknown list. This organism is *Staphylococcus saprophyticus.* **Fill in the table with the results for both microbes. Print out and attach the Virtual Laboratory Reports for both microbes:**

| Test | ***Staphylococcus aureus*** Media Tests 5 | ***Staphylococcus saprophyticus*** Media Tests 6 |
|---|---|---|
| Gram stain result: | | |
| Presence of gas in Durham tube? | | |
| Culture medium turned red when Nitrate A and Nitrate B added? | | |
| Results indicate presence of nitrite reductase? | | |
| Results indicate presence of nitrate reductase? | | |

Name:_____

Section:_____

# 14… THE IMViC SERIES

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software to learn the basics of doing aseptic transfers and interpreting test results in ***VirtualUnknown™ Microbiology***. The reference library in the software, your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation.

3. **Complete the tests listed in the table below, recording the results below and in the Virtual Laboratory Report**.

4. Repeat the above assignment for the different microbes assigned for each of the following unknowns:
   **Media Tests 2**: *Enterobacter aerogenes;*
   **Media Tests 3**: *Salmonella typhi; and*
   **Media Tests 4**: *Proteus vulgaris.*

   These can be selected from the list of predefined unknowns. Fill in the table with the results for all four microbes. Print out the Virtual Laboratory Reports for all four microbes:

| Test | ***E. coli*** Media Tests 1 | ***Enterobacter aerogenes*** Media Tests 2 | ***Salmonella typhi*** Media Tests 3 | ***Proteus vulgaris*** Media Tests 4 |
|---|---|---|---|---|
| Indole test: | | | | |
| Methyl red test: | | | | |
| Voges-Proskauer test: | | | | |
| Citrate utilization: | | | | |

Use the table, the Help files in ***VirtualUnknown™ Microbiology***, and the information in your textbook and laboratory manual to answer the following questions:

Name:_____

Section:_____

5. **Which medium is used for performing the INDOLE TEST**?

6. **What is the product being detected in a positive indole test**? **Where does it come from**?

7. **Why must a reagent be added to the culture to complete this test**? **What reagent is used**?

8. **Why does the reagent float atop the broth culture**?

9. **Fill in the following table for the Indole Test**:

| | Appearance of medium | |
|---|---|---|
| | In a positive test | In a negative test |
| Uninoculated | | |
| After incubation | | |
| After adding reagent | | |

10. **Which medium is used for performing the METHYL RED TEST**? **What is the content of this medium**?

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

Name:_____

Section:_____

12. **Contrast the means by which the specific metabolic products are detected by the reagents being added in the methyl red and indole tests**.

13. **Predict the expected results for these organisms**:
    - **the result for the methyl red test of an organism that cannot ferment glucose. Explain**.

    - **the result for the glucose fermentation test for an organism that is methyl red positive. Explain.**

14. **Fill in the following table for the Methyl Red Test**:

| | Appearance of medium | |
|---|---|---|
| | In a positive test | In a negative test |
| Uninoculated | | |
| After incubation | | |
| After adding reagent | | |

15. **What medium is used to perform the VOGES-PROSKAUER TEST**?

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

17. **Why must a reagent be used for completion of this test**? **What reagent is used**?

Name:_____

Section:_____

18. **Predict the possible results for these organisms**:
    **- possible results for the Voges-Proskauer test of an organism that cannot ferment glucose. Explain.**

    **- possible glucose fermentation test results for an organism that is Voges-Proskauer positive. Explain.**

19. **Fill in the following table for the Voges-Proskauer Test**:

| | Appearance of medium | |
|---|---|---|
| | In a positive test | In a negative test |
| Uninoculated | | |
| After incubation | | |
| After adding reagent | | |

20. **Which medium is used for performing the CITRATE UTILIZATION TEST**?

21. **What is the metabolic ability being detected in a positive citrate test**?

    **How is this activity accomplished?**

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

Name:_____

Section:_____

23. **Fill in the following table for the Citrate Utilization Test**:

| | Appearance of medium | |
|---|---|---|
| | In a positive test | In a negative test |
| Uninoculated | | |
| After incubation | | |

24. 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**:

| | |
|---|---|
| *E. coli* | + + - - |
| *Enterobacter aerogenes* | |
| *Proteus vulgaris* | |
| *Salmonella typhi* | |

25. The results for the four tests in the IMViC series are automatically entered into the Virtual Laboratory Report. Select **Media Tests 1** from the list of predefined unknowns and view the **Identification Matrix**, an option under "View" in the main menu bar. This unknown also contains results from any other tests you have performed while using the Media Tests 1 unknown, such as those from previous **Basic Training Manual** exercises. All bacteria with results directly contradicting those you have recorded have been eliminated as possible matches for your unknown. Those listed are the ones that are in agreement with your results. **List below those bacteria yielding a pattern of results agreeing with yours**.

26. To see what bacteria have been eliminated by the results you have recorded, select the **Elimination Hierarchy** from the "View" option in the main menu bar. Select "Expand All" to reveal all bacteria that have been eliminated. **How many bacteria have been eliminated by the tests performed so far?**

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Name:_____

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# 15… TSIA AGAR

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software to learn the basics of doing aseptic transfers and interpreting test results using ***VirtualUnknown™ Microbiology***. The reference library in the software, your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation.

3. Complete the inoculation and incubation of a tube of the medium. Use the information you learn to answer the following questions:

4. **TSIA allows detection of what gases produced as byproducts of growth**? **What is the chemical origin of each**?

5. **What p*H* indicator is included in this medium? At what p*H* does it turn yellow? At what p*H* does it turn magenta?**

6. **How is hydrogen sulfide production detected in TSIA**?

7. **Without Durham tubes, how is gas production detected in TSIA**?

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8. **What does a color change in the slant of the TSIA slant indicate, vs. a color change in the butt of the tube**?

9. **Fill in this table to indicate the possible results for TSIA cultures and what they mean**.

| | Which sugar(s) are fermented? | Gas produced? Yes/No | $H_2S$ produced? Yes/No |
|---|---|---|---|
| Yellow slant, yellow butt, cracks in agar | | | |
| Magenta slant, yellow butt, cracks in agar | | | |
| Magenta slant, magenta butt, cracks in agar | | | |
| Yellow slant, yellow butt, no cracks in agar | | | |
| Magenta slant, yellow butt, no cracks in agar | | | |
| Magenta slant, magenta butt, no cracks in agar | | | |
| Yellow slant, yellow butt, cracks in agar, black discoloration in agar | | | |
| Magenta slant, yellow butt, cracks in agar, black discoloration in agar | | | |
| Magenta slant, magenta butt, cracks in agar, black discoloration in agar | | | |
| Yellow slant, yellow butt, no cracks in agar, black discoloration in agar | | | |
| Magenta slant, yellow butt, no cracks in agar, black discoloration in agar | | | |
| Magenta slant, magenta butt, no cracks in agar, black discoloration in agar | | | |
| Any other result | | | |

Name:_____

Section:_____

10. Note that the results for the TSIA slant are reported in this order: slant/butt, gas production, $H_2S$ production. A yellow slant, yellow butt, cracks in the agar, and black precipitate would be reported as A/A +gas +$H_2S$. **Fill in the table with the results obtained in Exercise 11: Carbohydrate Tests. Then, complete the table by adding the results for the TSIA slant**.

    Complete the table by repeating the inoculation of TSIA slants for the microbes assigned by the following predefined unknowns:

    **Media Tests 2**: *Enterobacter aerogenes;*
    **Media Tests 3**: *Salmonella typhi; and*
    **Media Tests 4**: *Proteus vulgaris.*

| | Glucose fermentation | Lactose fermentation | Sucrose fermentation | TSIA result |
|---|---|---|---|---|
| *E. coli* | | | | |
| *Enterobacter aerogenes* | | | | |
| *Proteus vulgaris* | | | | |
| *Salmonella typhi* | | | | |

11. **Predict which of the following statements are TRUE, based on the results in the table above**:

    - Bacteria that ferment glucose yield yellow butts in TSIA. TRUE FALSE
    - One cannot tell if the yellow slant in TSIA is due to fermentation of lactose or sucrose. TRUE FALSE
    - Bacteria that cannot ferment glucose will produce magenta butts in TSIA. TRUE FALSE

12. **Why do TSIA slants turn magenta when sugars are not fermented**?

13. **How does the p*H* indicator in the TSIA slant compare with that found in the media used for carbohydrate fermentation tests, such as phenol red glucose broth**?

Name:_____

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# 16… BLOOD HEMOLYSIS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software to learn the basics of doing aseptic transfers and interpreting test results using ***VUMicro™ 3.11***. Use the reference library, your textbook, and your lab manual will provide additional information on the tests, media, and reagents required to complete this assignment.

2. **Indicate below what the expected appearances would be for blood agar in the vicinity of colonies exhibiting each type of hemolysis**:

   Alpha-hemolysis

   Beta-hemolysis

   Gamma-hemolysis

3. **What is actually happening to the erythrocytes in the media when these types of hemolysis are taking place**?

   Alpha-hemolysis

   Beta-hemolysis

   Gamma-hemolysis

Name:_____

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

   *Staphylococcus aureus*

   *Streptococcus pyogenes*

   *Streptococcus pneumoniae*

   *Streptococcus mutans*

   *Enterococcus faecalis*

5. Answer the following questions about the medium used:

   a. **What is the source and concentration of blood used in the medium for detecting hemolysis**?

   b. **Would results differ if other forms of blood were substituted**? **Explain**.

   c. **Which of the following terms describe blood agar**? (circle all that apply)

      Selective  Differential  Chemically-defined  Enriched

   d. **How is blood agar related to chocolate agar**?

6. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Blood Hemolysis 1** from the list of predefined unknowns. Follow these steps to perform the blood hemolysis test within ***VUMicro™***.

   a. Obtain a plate of 5% sheep blood agar. Select the inoculating loop from the Tools dropdown box. Ignite the burner and sterilize your loop.

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b. Remove the cap and lid from the tube and plate. Flame the mouth of the tube of culture, then obtain an inoculum with the sterile loop. Be sure to enter and exit the tube through the mouth.

c. Run the loop of inoculum over the blood agar plate until streak lines appear. Flame the mouth of the tube by dragging it through the flame of the burner until the indicator field below the Virtual Lab indicates this has been accomplished. **What color are the traffic signals**?

**Inoculation:**_____ **Contamination:**_____

d. Replace the cap and lid. Deposit the inoculated plate in the $37^{o}$ C incubator. Sterilize your loop to complete the aseptic transfer.

e. Click on the "New Day" button to advance time by one virtual day. Retrieve the blood agar plate from the incubator and compare its appearance to the descriptions just listed. **What is type of hemolysis is exhibited by this microbe**?

f. Before disposing of this test medium, record the results as directed.

7. **Repeat this test for each of the situations listed below, in which the course of events was altered**. **For each, explain what the effect was on the process**:

a. After streaking the plate, replace the cap before flaming the mouth of the tube of inoculum.

b. Before placing the streaked plate in the incubator, attempt to dispose of the culture used for inoculation.

c. **What do these events say about the importance of following proper aseptic technique in *VUMicro™ 3.11***?

8. Click the "New" button to obtain a new unknown. Select **Blood Hemolysis 2** from the list of predefined unknowns. Complete the blood hemolysis test for this microbe. **What is the observed result**?

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Name:_____

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# 17… ANTIBIOTIC SENSITIVITY TESTING

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the Tutorial available upon entering the software to learn the basics of doing aseptic transfers and interpreting test results in ***VirtualUnknown™ Microbiology***. The reference library in the software, your textbook, and your lab manual will provide additional information on the tests, media, and reagents required to complete this assignment.

2. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and click the "New" button to obtain an unknown. Select **Cardiovascular 1** from the list of predefined unknowns. This organism is a *Streptococcus*. DO NOT check the box permitting autoinoculation.

3. Details on the medium used for antibiotic sensitivity tests, the steps for completing the tests, and the determination of the results are all found in the software's reference books. Click on the "T" icon to access information on tests and on the "M" icon to gain information on media. Answer the following questions:

   a. **Which of the following describe(s) the medium used**:

      Selective  Differential  Complex  Chemically-defined

   b. **What is the mode of action by which each of these chemicals kills bacteria or inhibits their growth**?

      Bacitracin:

      Novobiocin:

   c. **Explain why positive tests of antibiotic sensitivity contain areas of confluent bacterial growth on the plate except in the area immediately surrounding the impregnated disk**. **What is happening here**?

Name:_____

Section:_____

d. **How would the size of the molecule and its solubility in water affect the results observed in a positive antibiotic sensitivity test**?

e. **What is the minimum inhibitory concentration in an antibiotic sensitivity test**? **Where is it found on the agar plate**?

4. Complete the four sensitivity tests supported in ***VUMicro™ 3.11*** for this organism: bacitracin (0.04 Units), bacitracin (2.0 Units), novobiocin, and optochin. **Fill in the appropriate column in the table below with the results observed**. Then, repeat the tests using the *Staphylococcus* organism assigned when you select **Digestive 2** from the list of predefined unknowns.

| Antibiotic | Sensitivity in this *Streptococcus*? | Sensitivity in this *Staphylococcus*? |
|---|---|---|
| Bacitracin (0.04 U) | | |
| Bacitracin (2.0 U) | | |
| Novobiocin | | |
| Optochin | | |

5. Open the Identification Matrix. Compare your results to those for all Streptococci. **List below the species of Streptococci that follow the pattern of results observed for your Streptococcus**.

Name:_____

Section:_____

# 18… EXOENZYMES

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the reference library in the software, your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation. **Complete the tests listed in the table below, recording the results below and in the Virtual Laboratory Report. If the test is not available for bacteria of that Gram reaction, write N/A in the field**.

3. **Repeat the above assignment for the different microbes assigned for each of the following unknowns**:
   **Media Tests 2**: *Enterobacter aerogenes;*
   **Media Tests 3**: *Salmonella typhi;*
   **Media Tests 4**: *Proteus vulgaris;*
   **Media Tests 5**: *Staphylococcus aureus*
   **Media Tests 6**: *Staphylococcus saprophyticus*

| Exoenzyme | ***E. coli*** Media Tests 1 | ***Enterobacter aerogenes*** Media Tests 2 | ***Salmonella typhi*** Media Tests 3 | ***Proteus vulgaris*** Media Tests 4 | ***Staphylococcus aureus*** Media Tests 5 | ***Staphylococcus saprophyticus*** Media Tests 6 |
|---|---|---|---|---|---|---|
| Alpha-amylase: | | | | | | |
| Catalase: | | | | | | |
| Coagulase: | | | | | | |
| DNase: | | | | | | |
| Gelatinase: | | | | | | |
| Alpha or Beta Hemolysins (see exercise 16): | | | | | | |
| Lipase: | | | | | | |
| Oxidase: | | | | | | |
| Urease: | | | | | | |

Name:_____

Section:_____

Use the table, the Help files in ***VirtualUnknown™ Microbiology***, and the information in your textbook and laboratory manual to answer the following questions:

4. **Which medium is used to detect alpha-amylase**?

5. **What reagent is used to detect the presence of starch**?

6. **Are the clear halos around colonies the result of a chemical reaction between the reagent and the products of alpha-amylase? Explain.**

7. **What function does catalase serve**?

8. **What reagent is used to detect the presence of catalase**?

9. **What gas(es) is(are) found in the bubbles generated by catalase? Show the chemical reaction.**

Name:_____

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

11. **What substance is used to detect coagulase activity**?

12. **What organism(s) is(are) most commonly associated with coagulase activity**?

13. **What pathogenic advantage would coagulase-producing bacteria have over those lacking the enzyme**?

14. **Compare and contrast the appearance of a positive coagulase test with that of a positive gelatinase test**.

15. **What is the basis for (1) uninoculated DNase agar with methyl green having a green color, and (2) the clearing of the green color from DNase agar with methyl green only by colonies of bacteria possessing DNase**?

Name:_____

Section:_____

16. **What is the chemical nature of gelatin? What general class of enzymes does gelatinase belong to? What is the solidifying agent in nutrient gelatin?**

17. **Why are 24-hour nutrient gelatin cultures placed in the refrigerator prior to their interpretation?**

18. **Why is it necessary to use a tween-like detergent in the preparation of Spirit Blue agar plates?**

19. **When performing lipase tests, what type of lipid is commonly used?**

20. **What are the typical breakdown products of lipids generated by lipases?**

21. **What role does the oxidase enzyme play in the metabolism of cells?**

Name:_____

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22. **How does the oxidase enzyme cause the color change in the oxidase reagent**?

23. **Would oxidase be more likely in strict aerobes or in strict anaerobes? Explain.**

**24. Write a chemical formula that describes the formation of urea by cells from the waste products of metabolism**. **State the metabolic source for each of the reactants**.

**25. Which of the six microbes tested possessed urease? What niche might one to expect these microbes to thrive in that would be less hospitable for those not possessing the enzyme?**

**26.** Refer to discussions of the normal habitats for these six microbes, and their involvement in infections of urea-rich regions. **Does this evidence support your prediction in question #25**? **Explain**.

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**27. Which of the six microbes tested possessed the greatest number of exoenzymes? How might its niche be a reflection of the activity of these enzymes**?

**28. Which of the six microbes tested possessed the fewest exoenzymes? How might its niche be altered by its sudden acquisition of the entire complement of exoenzymes listed?**

Name:_____

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# 19… OTHER BIOCHEMICAL TESTS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to demonstrate the differences in test results that are observed for different species of bacteria of a similar Gram reaction, morphology, and arrangement
- to connect the discussion of metabolism in lecture with the tests and results observed in lab
- to provide practice in using test results for the purpose of identifying bacteria

**Instructions**:

1. Use the reference library in the software, your textbook, and your lab manual will provide additional 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** from the list of predefined unknowns. This organism is *Escherichia coli*. DO NOT check the box permitting autoinoculation. **Complete the tests listed in the table below, recording the results below and in the Virtual Laboratory Report. If the test is not available for bacteria of that Gram reaction, write N/A in the field**.

3. **Repeat the above assignment for the different microbes assigned for each of the following unknowns**:
   **Media Tests 2**: *Enterobacter aerogenes;*
   **Media Tests 3**: *Salmonella typhi;*
   **Media Tests 4**: *Proteus vulgaris;*
   **Media Tests 5**: *Staphylococcus aureus*
   **Media Tests 6**: *Staphylococcus saprophyticus*

| Biochemical Test | ***E. coli*** Media Tests 1 | ***Enterobacter aerogenes*** Media Tests 2 | ***Salmonella typhi*** Media Tests 3 | ***Proteus vulgaris*** Media Tests 4 | ***Staphylococcus aureus*** Media Tests 5 | ***Staphylococcus saprophyticus*** Media Tests 6 |
|---|---|---|---|---|---|---|
| Esculin Hydrolysis: | | | | | | |
| Growth on Bile: | | | | | | |
| Growth in KCN: | | | | | | |
| Growth in Malonate: | | | | | | |
| Growth in 6.5% NaCl: | | | | | | |

Use the table, the Help files in ***VirtualUnknown™ Microbiology***, and the information in your textbook and laboratory manual to answer the following questions:

Name:_____

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4. **Where are bile salts likely to be encountered by bacteria? Would it be expected that the six microbes tested in this exercise would be found there? Explain.**

5. **What do bile salts do to sensitive bacteria? What makes bile salt-tolerant bacteria able to thrive in their presence?**

6. **What compound is responsible for the brown discoloration that develops in bile esculin agar when esculin is metabolized**?

7. 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.**

8. **How does potassium cyanide exert toxicity toward cells?**

9. **What adaptations to metabolism allow some bacteria to grow in the presence of potassium cyanide?**

Name:_____

Section:_____

10. **What is responsible for the initial color of malonate broth, and the color change observed in a positive malonate test?**

11. **Which other common test for identifying Gram negative facultatively anaerobic bacilli (the "enterics") shares many similarities in design and interpretation with the malonate test? List the similarities.**

12. **Indicate which of the following describe the medium used for detecting ability to grow in 6.5% NaCl**.

Selective Differential Chemically-defined Complex

13. **What is the means by which elevated salt content in the environment inhibits growth of some bacteria**?

14. **What adaptations are present in salt-tolerant bacteria to allow their survival**?

15. **What result would be expected in the "growth in 6.5% NaCl test" for bacteria capable of growth on mannitol salt agar**? **What result would be expected in the "growth on mannitol salt agar test" for bacteria capable of growth in 6.5% NaCl**? **Explain**.

Name:_____

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Name:_____

Section:_____

# 20… CLASSIFICATION SCHEMES AND BACTERIAL TAXONOMY

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to demonstrate the importance of good reference materials toward providing an identification for an unknown organism
- to connect the discussion of laboratory tests in lab with the characteristics used to classify microbes discussed in lecture

**Instructions**:

1. Enter the Virtual Lab and select **Taxonomy** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation.

2. **Identify the unknown organism using the tests and media at your disposal in *VirtualUnknown™ Microbiology***. Print out your Virtual Laboratory Report when you are done.

3. **Fill in the table below for the results observed**. You will need to use your textbook and other resources to find the full information requested.

| Test, procedure, or other data | Result |
|---|---|
| Gram reaction, morphology, arrangement | |
| Source of specimen | |
| Identity of pathogen | |
| Kingdom | |
| Domain | |
| Phylum or Division | |
| Class | |
| Order | |
| Family | |

4. **List below three important bacteria found in the same**…
   - **Family (but not the same genus)**

   - **Order (but not the same family)**

5. **Give a brief description of the ecology of this organism**.

Name:_____

Section:_____

6. **Use references at your disposal to create a dichotomous tree listing the tests and results necessary to discriminate among the six bacteria listed in the previous question and the unknown you identified for this exercise**.

7. **Create below a dichotomous tree that would place any living bacterium into its correct Class**.

Name:_____

Section:_____

# 21… IDENTIFYING GRAM POSITIVE COCCI

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to demonstrate the importance of good reference materials toward providing an identification for an unknown organism
- to connect the discussion of laboratory tests in lab with the characteristics used to classify microbes discussed in lecture

**Instructions**:

1. Consult a reference table on bacterial classification, such as a medical microbiology textbook or *Bergey's Manual of Systematic Bacteriology,* to find listed the traits used for placement of unknown bacteria in the appropriate order, class, and phyla. Determine what laboratory tests you might perform and key features to look for to provide initial help in placing an unknown bacterium in one of the phyla and orders**. List those initial key tests and special features below**:

2. Enter the Virtual Lab and select **Gram Positive Coccus** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation.

3. Read the case study carefully. Perform the initial key tests from your list above using ***VirtualUnknown™ Microbiology***. **Based on the information provided in your reference table, in which phylum and order would you predict the unknown microbe should be placed**?

Name:_____

Section:_____

4. Using information you provided in step 1 as your guide, complete some tests in the Virtual Lab that would allow you to give a more informed placement of the organism in the taxonomic scheme. **Do these results confirm your original prediction based solely on the Case Study? If not, what is your modified prediction for its taxonomic placement**?

5. After completing additional key tests, compare the survivors in the Identification Matrix in ***VirtualUnknown™ Microbiology*** with genera listed in your reference table. **Did your predicted taxonomic placement match the final placement**? **Explain your reasoning for both the prediction and final placement**.

6. If you have not done so, complete your identification of the unknown organism. Consult the Bacteria Reference resource book in ***VirtualUnknown™ Microbiology*** for additional information concerning the identified bacterium. **Using the information at your disposal (including the Case Study), provide an explanation for its presence in this habitat**.

7. Print out your Virtual Laboratory Report and attach it to this exercise before submitting this to your instructor.

Name:_____

Section:_____

# 22… IDENTIFYING GRAM NEGATIVE ENTERIC BACILLI

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to demonstrate the importance of good reference materials toward providing an identification for an unknown organism
- to connect the discussion of laboratory tests in lab with the characteristics used to classify microbes discussed in lecture

**Instructions**:

1. Consult a reference table on bacterial classification, such as *Bergey's Manual of Systematic Bacteriology, 2nd Edition* to find listed the traits used for placement of bacterial species in the appropriate order, class, and phyla. Determine what laboratory tests you might perform and key features to look for to provide initial help in placing an unknown bacterium in one of the phyla and orders. **List those initial key tests and special features below**:

2. Enter the Virtual Lab and select **Enteric Bacillus** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation.

3. Read the case study carefully. Perform the initial key tests from your list using ***VirtualUnknown™ Microbiology***. **Based on the information provided in your reference table, in which phylum and order would you predict the unknown microbe should be placed**?

Name:_____

Section:_____

4. Complete some key tests from your list in step 1 in the Virtual Lab that would allow you to give a more informed placement of the organism in the taxonomic scheme. **Do these results confirm your original prediction**? **If not, what is your modified prediction for its taxonomic placement**?

5. Complete additional tests. Compare the survivors in the Identification Matrix in ***VirtualUnknown™ Microbiology*** with genera listed in your reference table. **Did your predicted taxonomic placement match the final placement**? **Explain your reasoning for both the prediction and final placement**.

6. If you have not done so, complete your identification of the unknown organism. Consult the Bacteria Reference resource book in ***VirtualUnknown™ Microbiology*** for additional information concerning the identified bacterium. **Using the information at your disposal (including the Case Study), provide an explanation for its presence in this habitat**.

7. Print out your Virtual Laboratory Report and attach it to this report before submitting to your instructor.

Name:_____

Section:_____

# 23… CLASSIFICATION USING RAPID ID SYSTEMS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to demonstrate the connection between the tube and plate tests performed in the lab and combined rapid identification systems used in clinical labs

**Instructions**:

1. Obtain product information listing the biochemical tests included in a rapid identification system tube, card, or strip. Commonly used systems include Enterotube™ II from Becton Dickinson and API Rapid ID™ strips from bioMerieux. You may use these rapid identification systems, or any other system that uses biochemical test results as the basis for its identification.

2. Enter the Virtual Lab and select **Rapid ID** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. **Determine which tests *VirtualUnknown™ Microbiology* has in common with your rapid ID system**. **List those tests below**:

3. Perform the tests listed above and determine the identity of the unknown organism. **What is its identity**?

Name:_____

Section:_____

4. **Based on your results with the software, predict the appearance of the rapid ID system for this organism**. **Do the corresponding tests in VUMicro™. Draw the results below and determine its numerical identity based on the rapid ID system (to the degree that the software tests will allow)**.

5. Compare the numerical identity determined in the above step to the reference book for the rapid ID system. **What identity would the rapid ID system give to the unknown organism assigned by the computer in this exercise**?

6. **How does this ID match up with the identification resulting from completing tests within the software**?

Name:_____

Section:_____

# 24… INFECTIOUS DISEASE AND EPIDEMIOLOGY – PART 1

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Epidemiology 1** 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 each Case Study.

2. **What the identity of this microbe**?

3. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. local b. systemic c. subclinical d. focal e. none of these apply

   **What evidence supports your selection(s) here**?

4. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. primary infection
   b. secondary infection
   c. involved a fomite
   d. a nosocomial infection
   e. none of these apply

Name:_____

Section:_____

5. **Which of these was the reservoir for the microbe**?

   a. animal reservoir
   b. human reservoir
   c. non-living reservoir
   d. none of these apply

6. **Which of the following categories would this disease fall into**?

   a. communicable disease  b. contagious disease  c. noncommunicable disease

   **What evidence supports your selection here**?

7. **Which of the following categories would this disease fall into**?

   a. acute disease  b. chronic disease  c. subacute disease  d. latent disease

8. **Were predisposing factors involved in this disease**? **Was the host compromised**? **Explain**.

9. **How was the infection spread**? **Is this a notifiable disease**?

Name:_____

Section:_____

# 25… INFECTIOUS DISEASE AND EPIDEMIOLOGY – PART 2

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Epidemiology 2** 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 each Case Study.

2. **What the identity of this microbe**?

3. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. local b. systemic c. focal d. subclinical e. none of these apply

   **What evidence supports your selection(s) here**?

4. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. primary infection
   b. secondary infection
   c. involved a fomite
   d. a nosocomial infection
   e. none of these apply

5. **Which of these was the reservoir for the microbe**?

   a. animal reservoir
   b. human reservoir
   c. non-living reservoir
   d. none of these apply

Name:_____

Section:_____

6. **Which of the following categories would this disease fall into**?

   a. communicable disease  b. contagious disease  c. noncommunicable disease

   **What evidence supports your selection here**?

7. **Which of the following categories would this disease fall into**?

   a. acute disease  c. subacute disease

   b. chronic disease  d. latent disease

8. **Were predisposing factors involved in this disease**? **Was the host compromised**? **Explain**.

9. **How was the infection spread**? **Is this a notifiable disease**?

Name:_____

Section:_____

# 26… INFECTIOUS DISEASE AND EPIDEMIOLOGY – PART 3

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Epidemiology 3** 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 each Case Study.

2. **What the identity of this microbe**?

3. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. local b. systemic c. focal d. subclinical e. none of these apply

   **What evidence supports your selection(s) here**?

4. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. primary infection
   b. secondary infection
   c. involved a fomite
   d. a nosocomial infection
   e. none of these apply

5. **Which of these was the reservoir for the microbe**?

   a. animal reservoir
   c. non-living reservoir

Name:_____

Section:_____

b. human reservoir d. none of these apply

6. **Which of the following categories would this disease fall into**?

a. communicable disease b. contagious disease c. noncommunicable disease

**What evidence supports your selection here**?

7. **Which of the following categories would this disease fall into**?

a. acute disease b. chronic disease c. subacute disease d. latent disease

8. **Were predisposing factors involved in this disease**? **Was the host compromised**? **Explain**.

9. **How was the infection spread**? **Is this a notifiable disease**?

Name:_____

Section:_____

# 27… INFECTIOUS DISEASE AND EPIDEMIOLOGY – PART 4

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Epidemiology 4** 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 each Case Study.

2. **What the identity of this microbe**?

3. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. local b. systemic c. focal d. subclinical e. none of these apply

   **What evidence supports your selection(s) here**?

4. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. primary infection
   b. secondary infection
   c. involved a fomite
   d. a nosocomial infection
   e. none of these apply

5. **Which of these was the reservoir for the microbe**?

   a. animal reservoir
   b. human reservoir
   c. non-living reservoir
   d. none of these apply

Name:_____

Section:_____

6. **Which of the following categories would this disease fall into**?

   a. communicable disease  b. contagious disease  c. noncommunicable disease

   **What evidence supports your selection here**?

7. **Which of the following categories would this disease fall into**?

   a. acute disease  c. subacute disease

   b. chronic disease  d. latent disease

8. **Were predisposing factors involved in this disease**? **Was the host compromised**? **Explain**.

9. **How was the infection spread**? **Is this a notifiable disease**?

Name:_____

Section:_____

# 28… INFECTIOUS DISEASE AND EPIDEMIOLOGY – PART 5

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests
- to provide practice in using test results for the purpose of identifying bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Epidemiology 5** from the list or predefined unknowns. 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 each Case Study.

2. **What the identity of this microbe**?

3. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. local b. systemic c. focal d. subclinical e. none of these apply

   **What evidence supports your selection(s) here**?

4. **Which of the following descriptions apply to the infection described**? (circle all that apply)

   a. primary infection
   b. secondary infection
   c. involved a fomite
   d. a nosocomial infection
   e. none of these apply

5. **Which of these was the reservoir for the microbe**?

   a. animal reservoir
   b. human reservoir
   c. non-living reservoir
   d. none of these apply

Name:_____

Section:_____

6. **Which of the following categories would this disease fall into**?

   a. communicable disease  b. contagious disease  c. noncommunicable disease

   **What evidence supports your selection here**?

7. **Which of the following categories would this disease fall into**?

   a. acute disease  c. subacute disease

   b. chronic disease  d. latent disease

8. **Were predisposing factors involved in this disease**? **Was the host compromised**? **Explain**.

9. **How was the infection spread**? **Is this a notifiable disease**?

Name:_____

Section:_____

# 29… PATHOGENICITY AND VIRULENCE FACTORS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious disease and epidemiology concepts covered in lecture with the case studies and data provided in the software
- to demonstrate the connection between bacterial structure and products with their role in pathogenicity and virulence

**Instructions**:

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 the resources within ***VirtualUnknown™ Microbiology*** obtained during identification to answer the following questions.

2. **What is the identity of this microbe**?

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

   a. Enterotoxin b. Cytotoxin c. Neurotoxin d. Endotoxin

   **What evidence supports your selection**?

4. **Which of the following would be likely to be found in the microbe's cell wall**? (circle all that apply)

   a. M protein
   b. Teichoic acids
   c. Lipid A
   d. Mycolic acid
   e. Waxes
   f. Peptidoglycan

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

Name:_____

Section:_____

6. **Which of the following would best predict symptomology observed in an infection caused by this organism**? (circle your answer)

   a. red skin rash, sore throat, and fever
   b. blurred vision, vomiting, and watery diarrhea
   c. uncontrolled muscle contraction, fever, and convulsions
   d. fever, cramps, and diarrhea
   e. 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)

   a. heat stable
   b. low $LD_{50}$
   c. impact frequently lessened by immunization
   d. can lead to septic shock
   e. not released until cell death
   f. prevents function of neurotransmitter chemicals

   **What evidence leads you to this conclusion**?

Name:_____

Section:_____

# 30… NONSPECIFIC DEFENSES TO DISEASE

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the contributing processes of nonspecific defense to disease and the case studies and data provided in the software
- to demonstrate the connection between bacterial structure and products with their role in pathogenicity and virulence

**Instructions**:

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

2. **What is the identity of this unknown bacterium**?

3. **Explain what differences define nonspecific immunity, as opposed to specific immunity**.

4. Use your textbook and other references to provide a list of the nonspecific means by which humans fight off infections.
   a. **What are the barriers that contribute to the nonspecific immune response**?

   b. **What are the physiological reflexes that contribute to the nonspecific immune response**?

Name:_____

Section:_____

c. **What are the nonspecific responses observed in the inflammatory response when the barriers are breached due to injury or infection**?

d. **What role does phagocytosis play in the process of nonspecific immunity**?

e. **Which of these would be involved in fighting the infection described in this case study**?

5. 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**.

Name:_____

Section:_____

# 31… SPECIFIC IMMUNE RESPONSE

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the elements of specific immunity covered in lecture and the case studies and data provided in the software
- to demonstrate the connection between specific immunity and the route it takes in fighting bacterial infections.

**Instructions**:

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 carefully, and identify the bacterium if instructed to do so. Use this information and that from other sources to answer the following questions.

2. **If you were asked to identify the microbe, what was its identity**?

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

4. **Which branch of the immune system (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**.

Name:_____

Section:_____

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 T-cells and B-cells in the process,

   c. the classes of immunoglobulins active at each step in the immunoactivation process, and

   d. the way immunological memory is generated.

Name:_____

Section:_____

# 32… IMMUNE SYSTEM DISORDERS

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between the infectious agents and immune system disorders covered in lecture with the case studies and data provided in the software
- to demonstrate the connection between specific immunity and the path taken by our bodies to defeat invading pathogens

**Instructions**:

1. Enter the Virtual Lab and select **Immune Disorders** 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 this organism. Use the information obtained in the identification and from other sources to answer the following questions.

2. **What was the identity of this organism**?

3. The microbe responsible for this illness is seldom a pathogen in healthy individuals.
    a. **What is the normal ecology of this organism**?

    b. **What is the normal role of this organism in causing diseases**?

    c. **Explain how it is normally kept in check by a healthy immune system**.

Name:_____

Section:_____

4. **What route did this organism most likely take to get from its normal ecological niche to the point of causing this disease?**

5. **Which of these nonspecific defenses had to be breached for this organism to cause this disease**? (circle all that apply)

   a. barriers  b. flushing mechanisms  c. inflammatory response  d. phagocytosis

6. **Which branch of specific immunity (cellular immunity or humoral immunity) normally protects individuals from diseases caused by this organism**?

7. **Which of the following elements of blood would most likely be in short supply in an individual with an immunodeficiency allowing this disease**? (circle all that apply)

   a. T-lymphocytes
   b. B-lymphocytes
   c. platelets
   d. clotting factors
   e. neutrophils
   f. erythrocytes

8. **Explain the impact of each of these partial immune deficiencies on a body's ability to fight off infections by this organism**. **Be sure to reflect the types of infections and locations where they would be likely to occur for each**:
   a. **Inability to produce secretory IgA**:

   b. **Inability to produce IgG**:

Name:_____

Section:_____

# 33… ANTIMICROBIAL AND CHEMOTHERAPEUTIC AGENTS

**The goals for this exercise are**:

- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between bacterial structure and the modes of action of antimicrobial agents and chemotherapeutic agents covered in lecture with the case studies and data provided in the software
- to demonstrate the connection between bacterial structure and products with their role in pathogenicity and virulence

**Instructions**:

1. Enter the Virtual Lab and select **Antimicrobials** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Proceed to identify the unknown organism. Print out the Virtual Laboratory Report to submit to your instructor if requested. Use the information in ***VirtualUnknown™ Microbiology*** and other resources to answer these questions.

2. **What is the identity of this microbe**?

3. If you did not perform antibiotic and chemical sensitivity tests during the identification process, do so now. **Fill in the table below with the results you recorded in your lab report for this organism**.

| Antibiotic or Chemical | Zone of inhibition observed? | Resistant or Sensitive? |
|---|---|---|
| Novobiocin | | |
| Bacitracin – 2 units | | |
| Bacitracin – 10 units | | |
| Optochin | | |

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

5. **How do semisynthetic antibiotics compare with antibiotics and with chemotherapeutic agents**?

Name:_____

Section:_____

6. 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**.

| Class | Mode of Action | Example | Effective? |
|---|---|---|---|
| Penicillins | | | |
| Cephalosporins | | | |
| Vancomycin | | | |
| Isoniazid | | | |
| Aminoglycosides | | | |
| Tetracyclines | | | |
| Chloramphenicol | | | |
| Macrolides | | | |
| Streptogramins | | | |
| Polymyxins | | | |
| Rifamycins | | | |
| Quinolones | | | |
| Sulfonamides | | | |

7. **Which of the antimicrobials listed in the table above are antibiotics and which are synthetic chemotherapeutic agents**?
   - **Antibiotics**:

   - **Chemotherapeutic agents**:

8. **Why would antibiotics interfering with peptidoglycan biosynthesis frequently be more effective against Gram positive bacteria than against Gram negative bacteria**?

9. Eukaryotic cells also have ribosomes. **Why do antibiotics that interfere with ribosome function (and thus protein synthesis) kill bacteria but not the patient**?

Name:_____

Section:_____

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

11. Some antimicrobial agents imbed in membranes, thus leading to microbial death. **How could the presence of a chemical like this cause the death of the bacterial cell**?

12. **Explain why bacteriostatic antibiotics can be as effective as bacteriocidal antibiotics**.

13. **What conditions would lead a physician to prescribe bacteriostatic antibiotics preferentially**? **What conditions would lead a physician to prescribe bacteriocidal antibiotics preferentially**?

14. **Explain what special problems are encountered when attempting to fight an infection of the central nervous system**.

Name:_____

Section:_____

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Name:_____

Section:_____

# 34… INFECTIONS OF THE NERVOUS SYSTEM

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between nervous system anatomy, the invading microbes that can cause disease there, and the symptoms of nervous system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

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 proceed to identify the organism if so instructed. Use the information obtained from this and other sources to answer the following questions.

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**? **Under what circumstances is each one most likely to be found**?

Name:_____

Section:_____

7. **How might age of the patient help one predict the causative organism for this disease**?

8. **Based on the facts presented in the Case Study, provide a scenario to explain why this unlikely organism was able to cause this infection**.

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

Name:_____

Section:_____

# 35… INFECTIONS OF THE CARDIOVASCULAR SYSTEM – PART 1

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between cardiovascular anatomy, the invading microbes that can cause disease there, and the symptoms of cardiovascular system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Cardiovascular 1** 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 unknown organism. Use information from this and other sources to answer the following questions.

2. **What was the identity of this microbe**?

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

4. **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

Name:_____

Section:_____

5. **Provide a likely scenario by which this victim would have been exposed to this organism**.

6. **What is the mode of transmission for this organism in the case of this disease**?

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

Name:_____

Section:_____

# 36… INFECTIONS OF THE CARDIOVASCULAR SYSTEM – PART 2

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between cardiovascular anatomy, the invading microbes that can cause disease there, and the symptoms of cardiovascular system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Cardiovascular 2** 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 unknown organism. Use information from this and other sources to answer the following questions.

2. **What was the identity of this microbe**?

3. The diagnosis for this patient is subacute endocarditis. **Explain what this means**.

4. **Explain why a microbe responsible for disease might not be detectable in blood while still causing damage and disease**.

5. **How does the growth of this organism in the host lead to the signs and symptoms associated with this disease**? **What was the source of the deposits revealed by echocardiography**?

Name:_____

Section:_____

6. **Which would have had to precede the final diagnosis of this disease**? (circle all that apply)

   a. Skin infection
   b. Sore throat
   c. Diarrhea
   d. Pneumonia
   e. Sepsis
   f. Seizure

7. **Provide an explanation for the inability of the nonspecific immune processes to prevent this infection**.

8. **Provide a likely scenario by which this victim acquired this disease.**

Name:_____

Section:_____

# 37… INFECTIONS OF THE RESPIRATORY SYSTEM – PART 1

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between respiratory anatomy, the invading microbes that can cause disease there, and the symptoms of respiratory system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Respiratory 1** 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 unknown organism. Use information from this and other sources to answer the following questions.

2. **What is the identity of the microbe**?

3. **Diagram 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 infections of the upper or lower respiratory systems. **Would this organism be considered a common causative agent for respiratory diseases**? **What other organisms would commonly be associated with this disease**?

Name:_____

Section:_____

5. **What normal nonspecific defenses prevent more common infections in (1) the upper and (2) the lower parts of the respiratory system**?

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

7. **What predisposing factors could have led to this patient's disease**?

8. **Provide a scenario by which the growth of this microbe could have led to this infection**.

Name:_____

Section:_____

# 38… INFECTIONS OF THE RESPIRATORY SYSTEM – PART 2

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between respiratory anatomy, the invading microbes that can cause disease there, and the symptoms of respiratory system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Respiratory 2** 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 unknown organism. Use information from this and other sources to answer the following questions.

2. **What is the identity of the microbe**?

3. **Is this organism a frequent cause of lung abscesses**? **What other microbes are known causes**?

4. **Draw and label below a cross section of the respiratory membrane and pleural cavity**. **Why would a buildup of fluid in the pleural space result in such overwhelming chest pain**?

Name:_____

Section:_____

5. **Was the infection resulting from the patient's ruptured esophagus more likely caused by esophageal matter being aspirated into the respiratory system, or by contamination during the surgery that followed**? **Explain your rationale**.

6. **Would this patient be considered immunocompromised**? **Based on the information from the Case Study and your identification of the causative organism, is it likely this factor was important in the progress of the disease**?

7. **Provide a scenario by which an infection might result in a "collapsed lung"**.

Name:_____

Section:_____

# 39… INFECTIONS OF THE DIGESTIVE SYSTEM – PART 1

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between digestive system anatomy, the invading microbes that can cause disease there, and the symptoms of digestive system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Digestive 1** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **Locate a table of bacterial diseases of the digestive system and causative agents, and make a list of the possible causes of this infection**.

| Disease | Causative Agents | Possible Cause of This Infection? |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

2. **From the information provided, does it appear that this disease is due to infection or to intoxication**? **Explain the difference, and how the Case Study points to one rather than the other**.

3. Complete the identification of this pathogen using ***VirtualUnknown™ Microbiology***. **What is its identity**?

Name:_____

Section:_____

4. **Draw and label the digestive system, pointing out the location of the "upper GI" and "lower GI" portions**. **Indicate where this infection was located**.

5. **Explain how this can be a gastroenteric infection when there was no diarrhea or vomiting**.

6. **Provide three possible means by which this man could have acquired this infection, based on the information provided**.

7. **Based on the identification and the appropriate information provided by the textbook, predict the source of his infection**.

Name:_____

Section:_____

# 40… INFECTIONS OF THE DIGESTIVE SYSTEM – PART 2

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between digestive system anatomy, the invading microbes that can cause disease there, and the symptoms of digestive system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Digestive 2** from the list of predefined unknown organisms. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **Locate a table of bacterial diseases of the digestive system and causative agents, and make a list of the possible causes of this infection and routes of entry for the pathogen**.

2. Complete the identification of this pathogen using ***VirtualUnknown™ Microbiology***. **What is its identity**?

3. **Was this organism more likely to be introduced into the foodstuff by improper food processing or improper food handling**? **Explain how these differ**.

Name:_____

Section:_____

4. **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**.

5. **Provide a likely scenario by which this organism got into the pizza**.

6. **What steps could have been taken to reduce the risk of food poisoning**?

7. **What capability of this organism makes its emergence in pizza in abundant numbers possible**?

Name:_____

Section:_____

# 41… INFECTIONS OF THE DIGESTIVE SYSTEM – PART 3

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between digestive system anatomy, the invading microbes that can cause disease there, and the symptoms of digestive system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Digestive 3** from the list of predefined unknown organisms. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. Locate a table of bacterial diseases of the digestive system and causative agents, and make a list of the possible causes of this infection and routes of entry for the pathogen.

2. Complete the identification of this pathogen using ***VirtualUnknown™ Microbiology***. **What is its identity**?

3. **Is this species commonly encountered in the epidemics in the United States**? **What is its ecological niche**?

4. **What would the symptoms provided in the Case Study and the identity of the microbe point to as the diagnosis for this disease? Do the symptoms observed match those expected? Explain.**

5. **What other microbes might be expected as alternate causes of this disease**?

Name:_____

Section:_____

6. **Based on the information provided and the typically pattern of transmission for this microbe, provide a plausible scenario to explain the transmission of this organism among the affected group**.

7. **What course of treatment would be appropriate for someone with an infection of this type**?

8. 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**?
   - **Are there any groups that are more susceptible to infections by this organism**?
   - **What course of treatment is usually prescribed for this disease when caused by this organism**?

9. **How do the results you recorded in questions 1-7 compare with the data supplied from these health organizations**?

Name:_____

Section:_____

# 42… INFECTIONS OF REPRODUCTIVE & URINARY SYSTEMS – PART 1

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between reproductive and urinary system anatomy, the invading microbes that can cause disease there, and the symptoms of reproductive and urinary system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Urinary Tract 1** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **Locate a table of bacterial diseases of the reproductive and urinary systems and causative agents, and make a list of the possible causes of this infection and routes of entry for the pathogen**.

2. **Is this infection most likely cystitis, pyelonephritis, urethritis, or ureteritis**? **Explain your reasoning**.

3. Complete the identification of this pathogen using ***VirtualUnknown™ Microbiology***. **What is its identity**?

Name:_____

Section:_____

4. **Is this one of the more common causative agents of this disease**?

5. **What other microbes cause this disease**?

6. **Does the occupation of the victim complicate the investigation of this disease**? **What role could it play in the development of this disease**?

7. **Based on the identification and the appropriate information provided by the textbook, predict the events leading to this infection**.

Name:_____

Section:_____

# 43… INFECTIONS OF REPRODUCTIVE & URINARY SYSTEMS – PART 2

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between reproductive and urinary system anatomy, the invading microbes that can cause disease there, and the symptoms of reproductive and urinary system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Urinary Tract 2** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **Locate a table of bacterial diseases of the reproductive and urinary systems and causative agents, and make a list of the possible causes of this infection and routes of entry for the pathogen**.

2. Complete the identification of this pathogen using ***VirtualUnknown™ Microbiology***. **What is its identity**?

3. **Is this one of the more common causative agents of this disease**?

4. **Was this organism more likely to infect the diseased organ through the urinary tract or through the bloodstream**? **Explain your reasoning**.

5. **How does the history of low-grade fever factor into the course of this infection**?

Name:_____

Section:_____

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Name:_____

Section:_____

# 44… INFECTIONS OF REPRODUCTIVE & URINARY SYSTEMS – PART 3

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between reproductive and urinary system anatomy, the invading microbes that can cause disease there, and the symptoms of reproductive and urinary system infections covered in lecture with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Urinary Tract 3** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **Locate a table of bacterial diseases of the reproductive and urinary systems and causative agents, and make a list of the possible causes of this infection and routes of entry for the pathogen**.

2. Perform the urea hydrolysis test on your unknown. **What is the result observed**?

3. **Why does urea hydrolysis result in a basic p*H*?**

4. **Is the urea hydrolysis result consistent with what would be expected for an organism found in urine**? **Explain**.

Name:_____

Section:_____

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**?

7. **What are some of the complications that are associated with this organism causing urinary tract infections**?

8. **Based on the information provided, provide a plausible scenario to explain the transmission of this organism into the diseased tissue**.

9. **What course of treatment would be expected for an individual with such an infection**?

Name:_____

Section:_____

# 45… SOIL MICROBIOLOGY

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between discussion in lecture of bacterial physiology and nutrient cycling with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Soil** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **What is the identity of the unknown organism**?

2. **Based on your biochemical test results, does this organism fit the definition for a coliform**?

3. **Would this organism be a satisfactory indicator organism for indicating fecal contamination of the soil**? **Explain**.

4. **Would this microbe be more likely to be classified as a nitrifying bacterium, a denitrifying bacterium, a nitrogen-fixing bacterium, or an ammonifying bacterium**? **Explain your answer**.

5. **Explain how these two physical factors might determine how long an organism can persist in the soil**.
   i. soil p*H*

   ii. sunlight

Name:_____

Section:_____

6. Soils are usually fairly well aerated. However, when they are saturated with water, conditions become anaerobic. **Under which of the following conditions would this microbe most likely thrive in the soil**? (circle one)

   a. very dry soil  b. saturated soil

   **Explain your reasoning**.

7. **What is the factor most likely to limit the rate of growth of bacteria in the soil**? **Explain why**.

8. **Perform some Internet research to determine how many bacteria would be expected in a gram of soil of the following types**.
   a. sandy soil

   b. soil rich in clay

   c. organically-rich soil
   d. Provide here the URL address for the reference used to provide this information.

Name:_____

Section:_____

# 46… THE NITROGEN CYCLE

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to investigate how biochemical abilities of bacteria correspond with their role in natural environmental processes
- to demonstrate the connection between discussion in lecture of bacterial physiology and nutrient cycling with the case studies and data provided in the software

**Instructions**:

1. Use the resources within the ***VUMicro™ 3.11*** Help Reference books, your textbook, your laboratory manual, and the Internet to find a discussion of the nitrogen cycle.
   a. In the space provided, diagram the nitrogen cycle, demonstrating the relationships between the following compounds and processes: Nitrification; Denitrification; Nitrogen Fixation; Nitrogen Assimilation; Ammonification; Nitrate; Nitrite; Dinitrogen; Nitrous oxide; Ammonia; Amino functional group.
   b. Include in your diagram the chemical formulas for the compounds and write out the chemical reactions taking place.
   c. List at least one genus of bacteria capable of accomplishing each process in the description of the process. Circle those genera that are included in the bacteria found in ***VUMicro™ 3.11***.

Name:_____

Section:_____

2. Click on "Help" in ***VUMicro™ 3.11*** and select the "Index" option. Do a search for the compounds and processes found in the nitrogen cycle. Read up on these topics and their insight into the nitrogen cycle. **What biochemical tests included in the software would be useful in an investigation of the role of its bacteria in the nitrogen cycle**?

3. Listed at right are the processes of the nitrogen cycle. At left are some tests featured in ***VUMicro™ 3.11***. **Place the letter in the blank if the test measures chemical reactions central to the nitrogen cycle process**.

| | |
|---|---|
| _____nitrate reductase test | A. nitrification |
| _____urease test | B. denitrification |
| _____arginine dihydrolase test | C. assimilation |
| _____lysine decarboxylase test | D. ammonification |
| _____citrate utilization test | E. nitrogen fixation |
| _____oxidase test | F. none of these nitrogen cycle processes |

4. Enter the Virtual Lab and select **Nitrogen Cycle** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. Complete tests needed to identify this organism. **What is the identity of the unknown organism**?

5. **Investigate whether the organism is able to perform each of the following nitrogen cycle processes** (Hint: This may require you to look at the organism's results for some of the tests listed in question #3):

| | | |
|---|---|---|
| Nitrification | Yes | No |
| Denitrification | Yes | No |
| Assimilation | Yes | No |
| Ammonification | Yes | No |
| Nitrogen Fixation | Yes | No |

6. Microbes that perform denitrification do so because of enzymes accomplishing a process similar to that studied in the oxidase test. **Use this information to explain why water-saturated soils lose plant-accessible nitrogen**.

Name:_____

Section:_____

# 47… WATER MICROBIOLOGY

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between discussion in lecture of bacterial ecology and water microbiology with the case studies and data provided in the software

**Instructions**:

1. Enter the Virtual Lab and select **Water** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **What is the identity of the unknown organism**?

2. **Based on your test results, does this organism fit the definition for a coliform**? **Explain**.

3. **Would this microbe be a satisfactory indicator organism for indicating fecal contamination of the water**? **Explain your answer**.

4. **Does the presence of this microbe in the spring water indicate that it is unhealthy for drinking**? **Explain**.

Name:_____

Section:_____

5. **Based on evidence and information at your disposal, would this organism more likely be found in the *limnetic zone*, the *profundal zone*, or the *benthic zone* of a mountain lake**? **Explain your answer**.

6. **How does the water depth impact the numbers and types of bacteria present**?

7. **How do microbial populations in streams differ from those in lakes**? **How do freshwater populations differ from those in marine environments**?

Name:_____

Section:_____

# 48… SEWAGE TREATMENT

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to investigate how biochemical abilities of bacteria correspond with their role in natural and commercially-important processes
- to demonstrate the connection between discussion in lecture of bacterial physiology and sewage treatment with the case studies and data provided in the software

**Instructions**:

1. Use the resources within the ***VUMicro™ 3.11*** Help Reference books, your textbook, your laboratory manual, and the Internet to find a discussion of sewage and wastewater treatment.

2. Define the following terms as they would pertain to a discussion of sewage treatment:

   - Effluent

   - Sludge

   - Activated sludge system

   - Trickling filter system

   - floc

   - Biofilm

3. Describe the importance of BOD (1) on the health of the fish and plant population in a body of water, and (2) as an indicator of water quality.

Name:_____

Section:_____

4. In the space below create a schematic for a sewage treatment plant using the "activated sludge" method. Be sure to include the following features. For each, explain their role in the process. Be sure to trace the flow of solid- and liquid wastes through the process.
   - sewage input line
   - primary treatment tank
   - activated sludge tank
   - anaerobic sludge digestor

5. How do the parts in the above system correspond to parts of alternate sewage treatment systems?

| Activated Sludge Method | Equivalent in Septic Tank | Equivalent in Industrial Stabilization Ponds/Lagoons |
|---|---|---|
| Primary sedimentation tank | | |
| Activated Sludge System | | |
| Anaerobic sludge digester | | |
| Settling tank | | |

Name:_____

Section:_____

6. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Sewage 1** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Perform the tests listed in the table below for this organism. Repeat these tests using a different organism, **Sewage 2**.

| Test | Result for Sewage 1 | Result for Sewage 2 |
|---|---|---|
| Nitrate and nitrite reductase | | |
| Oxidation and Fermentation of glucose | | |
| Urease | | |
| Gas from glucose | | |

7. Based on these results, which microbe would be more likely found in effluent from each of the following. Justify each answer.

   - primary sedimentation tank:_____ Why?

   - activated sludge or trickling filter tank:_____ Why?

   - anaerobic sludge digester:_____ Why?

8. Continue testing until you have identified both Sewage 1 and Sewage 2 organisms.

   Sewage 1: _____

   Sewage 2: _____

9. Which of these organisms is more likely to be found in raw sewage?_____

10. Which of these organisms is more likely to be found in untreated fresh water?_____

11. Which of these organisms is more closely related to bacteria of importance to sewage treatment like *Zooglea, Sphaerotilus,* and *Caulobacter*?

12. What is the importance of the organism assigned with Sewage 2 toward monitoring of water quality?

Name:_____

Section:_____

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Name:_____

Section:_____

# 49… FOOD MICROBIOLOGY

**The goals for this exercise are**:

- to investigate the ways microbes are involved in the production and spoilage of food
- to learn of the ways in which microbes can be introduced into foods during processing and preparation
- to provide practice in using facts to determine the cause of microbially-based problems

**Instructions**:

1. Use information from your textbook and other reference sources to answer the following questions. Be sure to provide your reference for each item.
   a. Provide real-life examples to verify the following statement: "Bacteria are important in the production and in the spoilage of food".

   b. List five common foods and two common beverages that are produced by of the metabolic activity of microbes.

   c. List five food-borne diseases and the agents responsible for their symptoms.

Name:_____

Section:_____

2. List two bacteria included in ***VUMicro™ 3.11*** that are used in the production of foods.

3. Provide below a chemical formula for a metabolic change accomplished by microbes that results in the production of a food product.

4. Explain why early man might have purposely encouraged microbial activity resulting in metabolic changes to his foods.

5. List three Gram negative bacteria and three Gram positive bacteria included in ***VUMicro™ 3.11*** that are commonly associated with food-borne illnesses.

6. Two different means for introducing pathogens into foods are through improper food processing and improper food preparation. Contrast these two different routes. Then, provide an example (complete with the pathogen responsible) to illustrate how disease could be caused by each.

Name:_____

Section:_____

7. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Food Micro** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read through the Case Study. Explain why this scenario, which has little to do with the quality of the food product produced by the plant, poses an environmental problem for its owners.

8. What dangerous microbes might be expected in the effluent being generated by a poultry processing plant?

   Which of these microbes are included in ***VUMicro™ 3.11***?

9. Identify the unknown assigned in this exercise.
   a. What is its identity?

   b. Where is this microbe typically found?

   c. Which of the following would be the most likely reason for this microbe to be found in the effluent?
      - It was released into the processing plant environment through the processing of raw chickens.
      - It was introduced into the processing plant environment by the improper handling of the raw chickens by workers.
      - It was naturally in the environment and its presence is not directly related to the processing of chickens.

      Select one of the reasons above and write your rationale for that decision below:

   d. Presence of which ***VUMicro™ 3.11*** bacteria in this scenario would have led investigators to worry about improper food processing taking place?

Name:_____

Section:_____

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Name:_____

Section:_____

# 50… DAIRY MICROBIOLOGY

**The goals for this exercise are**:

- to investigate the ways microbes are involved in the dairy industry
- to learn how microbes can impact the grading and quality of milk
- to learn how microbial loads in milk can be reduced by the various forms of pasteurization
- to provide practice in using facts to determine the cause of microbially-based problems

**Instructions**:

1. Use information from your textbook and other reference sources to answer the following questions. Be sure to provide your reference for each item.

2. **What is mastitis, and how does it impact the dairy industry**?

3. **Which bacteria found in *VUMicro™ 3.11* are commonly associated with the disease mastitis**?

4. **What is meant by the "shelf life" of milk? What is commonly done to make use of milk that has surpassed its shelf life**?

5. **List two means by which the quality of milk (as reflected by its microbial content) can be determined**.

Name:_____

Section:_____

6. **Fill in this table to describe the meaning of results obtained when milk quality is tested by the dye reduction test**.

| Time for color change | Grade of milk |
|---|---|
| No change | Control using heated milk |
| 10 minutes | |
| 30 minutes | |
| 60 minutes | |

7. **How does the dye reduction test work**?

8. **Fill in this table to explain how bacterial counts are affected in the grading of milk leaving a dairy**.

| Grade of milk | Bacterial content |
|---|---|
| Certified raw milk | |
| Certified pasteurized milk | |
| Grade A raw milk | |
| Grade A pasteurized milk | |
| | Milk not suitable for drinking |

9. **Which bacteria included in *VUMicro™ 3.11* would be considered part of the "normal flora" of milk**?

Name:_____

Section:_____

10. **What are "diseases of milk**?"

11. **Fill in this table with the genera of *VUMicro™ 3.11* microbes associated with each of these milk diseases**.

| Milk Disease | Associated Organisms |
|---|---|
| Bitter milk | |
| Bloody or red milk | |
| Slimy milk | |
| Rancid milk | |

12. **Fill in this table to describe the conditions used for each version of pasteurization listed**.

| Type of pasteurization | Temperature used | Length of exposure |
|---|---|---|
| Holding method (original method) | | |
| Flash pasteurization | | |
| Super pasteurization | | |

13. **List five types of consumer products made by microbial action on milk**.

14. Enter the ***VirtualUnknown™ Microbiology's*** Virtual Lab and select **Dairy Micro** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read through the Case Study. Evidence suggests the problem is found in the milk holding tanks. **Provide an explanation for the presence of the microbe in that location**.

Name:_____

Section:_____

15. **Why is it to be expected that the milk entering the holding tank was contaminated with bacteria**?

16. Record the Gram stain reaction observed. **Based on this information, list eight organisms in *VUMicro™ 3.11* that could be responsible for the spoilage**.

17. **Based on the reference information on these eight bacteria and the information in the Case Study, list the top three possible identities for your unknown**.

18. Perform the lactose fermentation test on the unknown microbe. **Why is the result observed expected for this microbe**?

19. Watch the catalase video and record the results. **At this point, which genera of bacteria are still possible identities for this unknown**?

20. Proceed to identify the microbe, performing all tests necessary to narrow the choice of organisms to a single possibility. **What is the identity of your unknown?**

Name:_____

Section:_____

# 51… INDUSTRIAL MICROBIOLOGY

**The goals for this exercise are**:

- to provide practice in selecting, performing, and interpreting biochemical tests to identify bacteria
- to provide practice in gathering important data from case studies and reference books
- to demonstrate the connection between discussion in lecture of bacterial physiology and the use of bacteria for industrial processes with the case studies and data provided in the software

**Instructions**:

1. Locate information identifying microbial enzymes that have commercial uses and the bacterial that produce them. **List some of the more common microbes and products used for commercial purposes in the table below**.

| Microbe | Enzyme | Use |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

2. Enter the Virtual Lab and select **Industrial** from the list of predefined unknowns. Your instructor will tell you whether to check the box permitting autoinoculation. Read the case study carefully. **What is the identity of the unknown organism**?

3. **Does the unknown organism produce any of the enzymes listed in your table**? **If so, which**.

4. Look at the ***VUMicro™ 3.11*** reference book on Biochemical Tests. **Which enzymes of interest to commercial producers and listed in your table are expressed by bacteria included in this software**?

Name:_____

Section:_____

5. Use the Identification Matrix and your list of enzymes of industrial importance to determine which bacteria listed in the Identification Matrix possess commercially-valuable enzymes. **List below the organisms possessing at least two of these enzymes**. **Which microbe has the most**?

6. Of the 70-Gram negative facultatively anaerobic bacteria in ***VirtualUnknown™ Microbiology***, only *Escherichia coli* is used commercially for producing enzymes. **What problems with production prevent more of these bacteria from being used for this purpose**?

7. Some of the enzymes produced by bacteria are purified to make injectable products for therapeutic use. **Why would Gram negative bacteria pose greater challenges to industry than would Gram positive bacteria**?