

MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES

BACTERIOLOGY LABORATORY EXERCISES

IMPORTANT NOTE: This year the MMID laboratory exercise has been radically changed from the past years. Because of moving the second year students to MDL-7 and loss of the necessary preparation facilities, it is not possible to continue an involved, complex wet laboratory for the entire class. Therefore, major changes have been introduced, and we ask that you bear with us and help us make this transition.

The material that we expect you to learn from this exercise has not changed; it is only the mechanism for delivering the material that has changed. We cannot accommodate the entire class participating in a three day wet lab. Many, if not most, of you have taken microbiology courses with extensive wet labs in your undergraduate educations. Surveys of your predecessors have shown that many of you who have not had microbiology are interested in gaining some hands-on experience with microbiology, and we would like to make such an experience possible. Furthermore, federal and state laws now make it extremely rare, if not illegal, for non-certified personnel to perform diagnostic tests, so most of you will never perform the types of procedures that are part of this laboratory. However, understanding the principles and applications of the tests is essential for your future practice, passing this course, and performing well on certain standardized exams that you will take in the future. Here are the changes made for this year:

1. Everyone must complete the on-line Virtual Microbiology Laboratory found at <http://www.mgm.ufl.edu/~gulig/mmid/mmid-lab> (also linked from the MMID home page). Everyone must submit their results and answer the questions upon completion of the virtual exercise. Note that this virtual laboratory exactly reproduces the physical steps and results that you would experience with the wet lab. If you complete the virtual lab you will have learned everything we expected of your predecessors.

3. Since virtual reality doesn't always look like the real thing, we will have examples of results and demonstration of procedures in MDL-7 October 24 and 25.

2. For those of you who want some hands-on experience, you will have the opportunity to perform specific tests of your choosing on October 24 (observing results on October 25). We can accommodate about half of the class for this portion of the wet lab. Your predecessors had to performed all of the appropriate tests in sequence to arrive at the diagnosis. You will do this in the virtual lab. This wet lab will not be done in sequence, but is aimed at letting you pick and choose the tests you want to perform (e.g., Gram stain, streaking a plate, or a rapid Strep test). You will never be tested for ability to perform these tests for this course, so even those students who have not had microbiology laboratory courses in the past need not participate in the wet lab.

There will be an electronic survey to determine how many students will participate in the wet lab. This laboratory manual is available on the web in hypertext-linked format, complete with videos and images to show methods and results. It is important for you to read and use the manual to learn the essential information and to complete the virtual laboratory.

Your feedback on this modified laboratory exercise is very important to us. Please complete any evaluations and provide us with suggestions, comments, and criticisms.

GENERAL INTRODUCTION

I. Objectives of the laboratory exercises

- A. provide **familiarity with procedures** used for culturing (growing) and identifying microorganisms of medical importance.
- B. aid in **proficiency in submitting specimens** for the identification of infectious disease in your future patients.
- C. provide **clinical cases** for diagnosis of infectious disease and its management.

These goals will be accomplished in the virtual microbiology lab exercise dealing with respiratory infection by identification of microorganisms in samples corresponding to case histories from patients. You will be asked to answer questions concerning the case histories and the general principles of diagnosis and of the etiological agents of respiratory diseases. For those who choose, practical experience can be obtained performing tests of your choosing in the limited wet lab.

II. What is expected of you

Your completion of the virtual lab giving you practical (decision making) and theoretical experience is important because:

1. You will be **tested** for your proficiency at **reading and interpreting** the results (note that the virtual exercise is sufficient, since your exam is electronic as well).
2. You will be **tested** on the **theory** behind the etiological agents and their identification.
3. You are expected to record the results of tests, identify the bacteria in the cultures, and arrive at diagnoses of the diseases.
4. You must submit your identifications and diagnoses along with completing an online (open note, open book) homework assignment.

YOU MAY COMPLETE THE VIRTUAL LABORATORY IN GROUPS, BUT YOU MUST SUBMIT YOUR RESULTS AND COMPLETE THE ONLINE HOMEWORK USING YOUR OWN NAME AND ID.

III. Laboratory Safety Rules - for those participating in the wet lab.

Because pathogenic microorganisms are used in the laboratory, reasonable precautions must be taken to avoid infection. YOU are responsible for YOUR OWN behavior, but should be concerned about the behavior of YOUR COLLEAGUES. If someone is being negligent, you should point this out to them or the instructors so that no one will be jeopardized.

Most important - because MDL-7 is not set up as a wet lab, all laboratory activity must be restricted to the central tables set up for such activity.

1. Wear old clothes or lab coats. The Gram stain is virtually impossible to remove from clothing.
2. Pencils, pens, fingers, and other objects must be kept out of the mouth. **Eating and drinking are strictly prohibited during lab time.**
3. Laboratory tables and desks should be free of books, clothing, and other personal items during lab. **Wipe down the bench surface with disinfectant before and after each laboratory exercise.**
4. Quarantine spills and notify a faculty member, who will help you disinfect it.
5. Do not insert more than the "wire" portion of the loop into tubes containing bacteria - keep the handle clean. Dispose of the loops loop-side down into the disposal buckets.
6. The bacteria on a dried and fixed slide are usually, but not necessarily, dead. Handle such slides with care. Alcohol and Gram's iodine will kill bacteria, so properly prepared Gram-stained slides are safe. All staining should be done on the staining racks provided over sinks to prevent spills on the bench tops and ultimately your possessions.
7. **Don't walk around the lab with contaminated loops, open culture tubes or petri plates, or other externally contaminated materials.**
8. Discard disposable contaminated materials, such as slides and petri dishes, in the covered buckets in each lab. Do not place test tubes in these buckets; leave them in pans provided in the labs. **Nothing even remotely possibly contaminated may be placed in the regular trash.**
9. When finished, wash your working area with disinfectant. **Wash your hands thoroughly after handling inoculated cultures and before leaving the lab.** Do not remove cultures from the lab area. **If you learn to WASH YOUR HANDS between patients, you will have learned the most important lesson about infection control that will save patient lives.**
10. Those of you who choose to wear gloves must exercise care in preventing the contamination of the entire lab. Many people who believe that they are **protecting themselves** by wearing gloves in fact **contaminate the lab** by handling items such as pencils, faucets, and doors with contaminated gloves. For example, do you handle a culture of *Klebsiella pneumoniae* with your gloves, write with your pen, and later put the same pen in your mouth?! **Do not throw your gloves into the regular trash where they will be handled by the housekeeping staff.** This is a violation of the law and common sense.

INTRODUCTION TO DIAGNOSTIC LABORATORY EXERCISES - FOR EVERYONE

I. Bacterial morphology.

Bacteria are 100-1000 times smaller than most mammalian cells; they range from 0.4 to 3 microns (10^{-3} mm) in diameter and several microns in length. To examine them you will need a light microscope with an oil immersion objective (100X). Microorganisms differ widely in shape and size. The majority of bacteria are either spheres (cocci) or rods (bacilli). A few occur as curved rods (vibrios) or in more complex shapes. Specific types of bacteria may also vary in size and grouping (single, clumps, chains, etc.) The shape and arrangement of the cells with their staining properties are used for classifying and preliminarily identifying clinical isolates. In response to a hostile environment, some bacteria adopt a dormant state by generating a spore, easily visualized with the light microscope.

II. Diagnostic Microbiology.

To identify the causative agent in infections, specimens are obtained, and each organism is isolated and identified. Microscopic examination of the specimen or the organisms is a first step. This can be an unstained ("wet mount") specimen or fixed specimen on a glass slide stained to visualize the microorganisms and other cellular elements. **The most commonly used stain is the Gram stain**, although other special stains (e.g., **acid-fast stain**) can be used to tentatively identify certain organisms.

To grow and isolate microorganisms, the specimen is spread ("streaked") onto agar media containing nutrients to yield colonies representative of each of the bacteria. Potentially important organisms are further tested for identification. A variety of methods is used - culture media which select for growth of groups of organisms (**selective media**), media containing indicators which cause different organisms to appear differently (**differential media**), biochemical tests, phage typing, antibody typing, and many others.

II. The Gram Stain.

A. Introduction.

The Gram stain is one of the most valuable and most generally used. The Gram stain divides bacteria into two groups, the **gram-positive** organisms, which stain dark purple to black, and the **gram-negative** organisms, which take on the color of the counterstain, usually red. Bacteria are stained with crystal violet followed by Gram's iodine. These two solutions form a complex which, in gram-positive bacteria, is not washed away with ethanol; gram-negative bacteria rinse clear. To visualize the clear gram-negative bacteria, they are counterstained with a contrasting color. Red stains (e.g., safranin) are usually used, but color blind individuals may have difficulty differentiating gram-stained bacteria. The use of Bismarck Brown as counterstain is a good alternative. Please inform your lab instructor if you are color blind.

The ability of gram-positive bacteria to retain the crystal violet-iodine complex following treatment with ethanol varies with the age and species of bacteria and, to a lesser extent, the environment from which they were obtained.

B. Procedure for Gram staining a specimen.

1. Using a sterile loop, transfer a loopful of tap water to a clean glass slide. Touch a loop to the desired colony, and mix the bacteria in the water on the slide. A VERY SMALL amount of bacteria will suffice.
2. Allow the specimens to dry on the slide at room temperature. Do not heat the slide to speed drying because this can distort the cellular morphology or staining properties of the organism.
3. After the specimen has dried, **heat-fix** the slide. Gently heat the slide by passing quickly through the flame, specimen side up, 3-4 times. It should be warm but not hot to the touch.
4. Stain the bacterial smears by Gram's method as follows: Flood the slides sequentially with solutions a-d for the indicated times.

(a) Crystal Violet-----1 minute

Wash gently in tap water for 2-3 seconds.

(b) Gram's Iodine (I₂-KI)-----1 minute

Wash gently in tap water, shake off excess water.

(c) 95% alcohol-----10 seconds

Do not over-decolorize the specimen with alcohol. **If you're going to screw up the Gram stain, this is the step!**

Wash gently in tap water, shake off excess water.

(d) Safranin (counterstain)-----20 seconds

Wash in tap water and blot dry.

5. Examine with oil immersion optics (not at lower power). Move the condenser almost all the way up to touching the slide. Do not let the high/dry (40X) lens get into the oil. It will be very difficult to clean.
6. Gram-positive organisms will be purple/blue. Gram-negative organisms will be pink to red.

III. Streaking a plate for isolation of colonies.

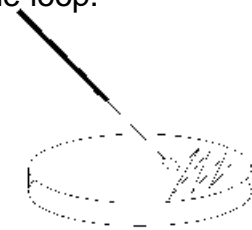
A. Introduction.

The single most important step in analyzing a specimen containing bacteria is to obtain isolated colonies of bacteria that arise from single cells. Attempts to identify bacteria in a clinical sample cannot be done unless isolated colonies are used.

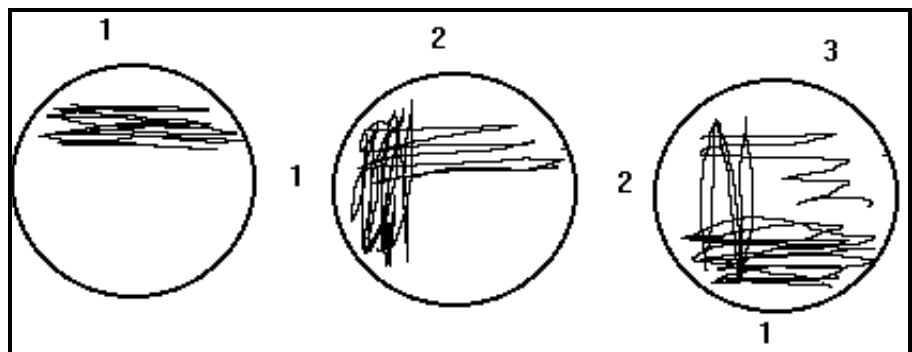
To obtain well-isolated colonies, it is essential to disperse the inoculum (sample) on the surface of an enriched agar plate so that individual bacteria are well separated from each other. Ideally, each of the bacterial species present will produce a distinct colony type. The appropriate technique will be demonstrated by one of the instructors in each laboratory.

B. Procedure.

1. With the loop, spread the inoculum back and forth across the upper 1/4 of the plate, keeping the lines of inoculation very close together (area 1 in figure below). Isolated colonies are not expected in this area. Do not use strong pressure, which will break the surface of the agar. Use the end of the loop, not its side when streaking. Dispose of the loop in the biohazard bucket on the bench.
2. Turn plate approximately 90°. Streak the plate as indicated in the figure (area 2) across about 1/4 of the plate. Dispose of the loop.
3. Repeat step 2 one or two times more. In area 3 and/or 4 single colonies should appear.
4. Label plates on the bottom and incubate inverted at 37E.



Note: in drawings, lines should be closest together in Sec. 1 and progressively further apart in succeeding sections



Part 1
Identification of gram-positive Cocci

I. Introduction.

The **gram-positive cocci** include organisms that are round and that usually occur in **chains or pairs (streptococci)** and those that occur in **clusters or bunches (staphylococci)**. Infections by pathogenic gram-positive cocci are responsible for many bacterial diseases, ranging from superficial skin lesions to severe life-threatening infections. Other members of the group are fairly regular inhabitants of skin and mucous membranes, the so-called "normal flora."

The primary isolation from infectious material is usually made on **sheep blood agar**, a rich medium that supports the growth of many types of microorganisms. The appearance of colonies and **red blood cell lysis** are important diagnostic features. The most common streptococci and staphylococci can be divided into groups on the basis of their reactions on blood agar (examples are shown at <http://www.medinfo.ufl.edu/year2/mmid/imagky.html> on the MMID home page):

Alpha hemolytic - **partial lysis of red blood cells**, producing a **greenish discoloration**. The two most important groups are *Streptococcus pneumoniae* (pneumococcus), a frequent cause of lobar pneumonia, and the viridans group of streptococci, normal inhabitants of the oropharynx that may cause disease (e.g., endocarditis) when they invade the vascular system.

Beta hemolytic - **complete lysis** of red blood cells and **clearing of the medium** around the colony. Common pathogens which produce this reaction are Groups A, B, C and some D streptococci, as well as *Staphylococcus aureus*, the most common pathogenic staphylococcus.

Gamma hemolytic - **no apparent change** in the medium (non-hemolytic is more descriptive). *Staphylococcus epidermidis*, a normal skin inhabitant.

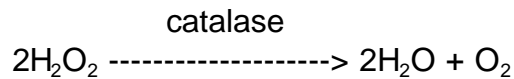
II. General Procedures.

The purpose of this experiment is to make observations of some diagnostic features of the important streptococci and staphylococci.

A. Differentiation of streptococci from staphylococci.

Although microscopic examination of stained smears presumptively permit distinction between these two groups of organisms, **a definitive classification can be made on the basis of the presence or absence of the enzyme CATALASE. Staphylococci contain this enzyme, streptococci do not.**

Catalase test. Place a drop of 3% hydrogen peroxide on a clean microscope slide. Place a heavy loopful of cells from **isolated colonies** into the liquid (you may have to pick up four to five colonies if they are small). Immediate generation of gas bubbles constitutes a positive test. Avoid the inclusion of blood cells from blood agar plates as blood contains catalase.



B. Identification of group A Streptococci.

1. **Bacitracin test.** Commercially available paper disks saturated with a solution containing Bacitracin will inhibit about 97% of all strains of Group A streptococci; other groups of $\hat{\alpha}$ -hemolytic streptococci will not be affected. Streak a blood agar plate with an **isolated colony** of $\hat{\alpha}$ -hemolytic streptococci (you're not looking for isolated colonies now). After inoculation, flame the provided forceps, and aseptically pick up a bacitracin disk (B or A disk). Place the disk on the plate and press gently onto the agar medium to ensure firm contact with the agar. Observe the plates for inhibition of growth after overnight incubation at 37^E.

2. **Phadebact Strep (Coagglutination) test.** The Phadebact brand rapid streptococcal identification system is based on a coagglutination reaction. Bacteria are mixed with a solution that contains antibody to the Group A antigenic determinant of the bacterial cell. The antibodies are bound through their Fc portion to nonviable staphylococcal cells, so that if the antibodies bind at their Fab site to group A streptococci, the staphylococci will be clumped together as a lattice of immune complexes forms (coagglutination). If no Group A streptococci are present, the staphylococci will remain in a homogeneous suspension. A negative control serum with no antibodies to Group A streptococci is also run separately to prevent false positive results if the staphylococci spontaneously clump.

- a. Mix the two reagent solutions by vigorous shaking to suspend the staphylococcal cells.
- b. Using a sterile inoculating loop, pick about 5 colonies of the $\hat{\alpha}$ hemolytic streptococci and smear the cells into two of the oval areas marked on the white cards provided with the kit. Label one of the areas as the test and the other as the negative control.
- c. Add one drop of the test reagent to oval marked "test" and one drop of the control reagent to the other oval.
- d. Stir the cells in the reagent, but be sure to use a fresh loop between the test and control samples to prevent cross-mixing of the reagents. Rock the card to continue mixing the samples.
- e. If the test sample agglutinates, but the control sample does not, the sample is Group A streptococcus (*Streptococcus pyogenes*). If the test and unknown samples do not agglutinate, the test sample is not Group A streptococcus. If, however, both samples agglutinate, no conclusions may be drawn, and the test must be repeated. A demonstration test will be available to aid in interpretation.

C. Differentiation of pneumococci from other alpha hemolytic streptococci.

Optochin test. Pneumococci (but not other α -hemolytic streptococci) are inhibited by optochin. Apply a disk of filter paper containing optochin (O or P disk) to a heavily streaked plate (see procedure for applying the bacitracin disk). If the organism is a pneumococcus, a large zone free of bacterial growth will surround the paper disk after overnight incubation at 37°C. If the organism is another α -hemolytic streptococcus, there will be no zone of inhibition around the disk, or at most a narrow one. Optochin is available commercially as "Taxos P" or "Optochin disk."

D. Differentiation of *Staphylococcus aureus* from non-pathogenic staphylococci.

Coagulase test. The test which distinguishes *S. aureus* from non-pathogenic staphylococci is the coagulase test. Coagulase is a secreted enzyme of *Staphylococcus aureus* that causes plasma to clot (coagulate). The tube test is performed by inoculating an isolated colony into 10% rabbit plasma. After incubation at 37°C for 2-4 hours, examine the tube for the presence of coagulation. If the reaction is negative, incubate overnight and re-examine the tube.

Part 2 Gram-negative enteric rods

I. Introduction.

This part of the exercise will focus on the family *Enterobacteriaceae*, which includes several genera of medical importance. This is a large and diverse group, and the laboratory methodology for their identification has evolved over many years. You will work with *Klebsiella pneumoniae* and *Escherichia coli*.

The choice of medium for the initial isolation from a clinical specimen may depend on the specimen source. Usually specimens are cultured initially both on blood agar and a number of **SELECTIVE** media (e.g., **MacConkey agar, which excludes the growth of gram-positive organisms because it contains bile salts**). Media such as MacConkey also permit an assessment of the ability of the organisms to **ferment lactose (DIFFERENTIAL media)**, which provides one of the key branch points in the diagnostic scheme.

These days systems are used which enable the simultaneous inoculation of many media and the evaluation of numerous biochemical characteristics. In this laboratory exercise, we will use the more classical (old fashioned) techniques because they form the foundation for all metabolic identification systems.

II. General Procedures

A. **MacConkey agar plate.** Streak samples for isolated colonies on MacConkey agar using same procedure as for Part 1. MacConkey agar is an example of a selective medium; it permits growth of gram-negative enterics but inhibits the growth of gram-positive bacteria.

After 24 hours incubation, examine the plates to distinguish the two different colonial types. The MacConkey medium provides evidence as to whether each organism ferments lactose. Colonies which ferment lactose are red. This reaction is due to the action of acids produced by fermentation of lactose on the bile salts and the subsequent absorption of neutral red, a pH indicator, from the medium. Colonies of non-fermenters of lactose appear colorless.

E. coli and *K. pneumoniae* are **usually** lactose-positive. To make life interesting, you will also be given a lactose-negative *E. coli* as part of this exercise.

B. Citrate slant.

Some bacteria can use citrate as a source of carbon, while others cannot. Streak the surface of the citrate tube with an **isolated** colony from the MacConkey plate. Incubate the tube with the **lid loose**. Utilization of citrate as a carbon source results in a color change from green to blue. The indicator is bromthymol blue, which turns from green to blue at low pH. Read the test at 24 hour incubation at 37E.

***K. pneumoniae* is citrate-positive, while *E. coli* is usually citrate-negative.** Both of your *E. coli* strains are typical for citrate utilization.

SPECIFIC INSTRUCTIONS - USEFUL FOR THE VIRTUAL LAB AND WET LAB.

These instructions are written to aid in the orderly completion of the virtual laboratory (there are questions about which tests to perform next based on having obtained specific results). For those participating in the wet lab, you cannot perform the tests in order, since a three day lab would be required. On October 24, you may perform any tests you desire on whichever cultures you choose (but you should make sure you choose only appropriate tests which will yield meaningful results). On October 25 you will observe and interpret the results of the tests.

Forms to record results are provided at the end of the handout.

Objectives:

1. Familiarize students with gram-positive cocci and gram-negative enteric rods and basic diagnostic microbiology related to respiratory infections
2. Teach Gram stain technique
3. Teach plate streaking technique for isolated colonies
4. Use enteric bacteria to demonstrate differential metabolic media and selective media

Procedures:

Part A1: Analysis of cultures corresponding to **cases 1-4** for **gram-positive cocci**.

Part A2: Analysis of cultures for **cases 1-4** for **gram-negative enteric rods**.

Part B: Analysis of **student throat flora**.

Day 1.

A1,A2: Streak one of the cultures from cases 1-4 on a blood agar plate and a MacConkey agar plate for isolation. Tip the tube to move the liquid to near the opening so that only the "wire" portion of the loop enters the culture tube.

B. Swab the back of the throat of someone with the swab provided. Streak a blood agar plate for isolation of single colonies. Use the swab provided for first section, then use the loop for sections 2, 3, and 4 as indicated in instructions.

Day 2.

- A1.
1. Observe hemolysis and colony morphology from the mixed culture.
 2. Perform a Gram stain on representative isolated colonies from the blood agar plate and the MacConkey plate.
 2. Perform the catalase test on gram-positive cocci.
 3. a. For gram-positive, catalase-positive cocci, (*Staphylococcus spp.*) - perform coagulase test.
 - b. for gram-positive, catalase-negative cocci, (*Streptococcus spp.*)
 - i. á hemolytic
M streak colony onto blood agar plate and drop on a bacitracin disk.
M perform Phadebact (coagglutination) strep test.
 - ii. á hemolytic
M streak colony onto blood agar plate and drop on optochin disk.

- A2.
 1. Observe colonies on the MacConkey plate. Record Lac+ or Lac-.
 2. Make a smear and Gram stain of representative colonies based on Lac+/- and colony morphology.
 3. Choose one well isolated colony of each type you find from the MacConkey plate, and streak the surface of a citrate slant to differentiate *K. pneumoniae* from *E. coli*.

- B.
 1. Observe hemolysis and colony morphology from throat culture.
 2. For any α -hemolytic colonies, perform a Phadebact test for Group A strep.

Day 3.

- A1.
 1. Read results of bacitracin and/or optochin sensitivity tests by examining inhibition of growth.
 2. Read coagulase test.
 3. Perform the Phadebact test if not done the previous day and if it is indicated by the results to date.

- A2.
 1. Read the results of the citrate tube to differentiate *E. coli* from *K. pneumoniae*.

Combine the results of parts A1 and A2 and propose a diagnosis based on the culture data and the case presentation. Note that the name of a bacterium is not a diagnosis. Name the disease as well.

Submit your results and diagnoses and complete the online homework questions.

Case histories

Case 1.

A 60 year old male who had influenza a week earlier reported to the emergency room with a 104° fever and shaking chills, a productive cough with a yellowish sputum, and chest pain. CBC revealed 30,000/mm³ WBC, predominantly PMN. Chest X-ray showed pleural effusion with a patchy infiltrate and 1-2 cm nodules with cavitation. The sample provided was obtained from sputum.

Case 2.

A 45 year old female with previous rhinorrhea, pharyngitis, and cough visits her doctor with a 102° fever which appeared abruptly after a sudden shaking chill episode. He has chest pain and a productive cough with rust colored sputum. Auscultation demonstrates inspiratory rales and "tubular" breath sounds in the right lung. X-ray shows diffuse lobar consolidation of the right lung. Sample is from sputum.

Case 3.

A 38 year old associate professor at the University of Florida College of Medicine, who is otherwise healthy, notes a mild sore throat before going to bed. The next morning his throat is extremely painful making it difficult to eat or drink (but he didn't mind because he needed to lose a few pounds, anyway). Dedicated to his science and students he goes in to work. As the morning progresses the pain rapidly worsens, he has chills and feels achy. Even his faithful technician tells him he looks bad. By the afternoon he calls it quits and heads home. But first he makes a swab of his pharynx, using expert technique that he is about to teach second year medical students. At home his temperature is 99.8, so he begins taking ibuprofen and throat anesthetics. Examining his throat in the mirror, he notes highly erythematous tonsils and pharyngeal region with pus. The submandibular and anterior cervical lymph nodes are tender. The sample is taken from the pharyngeal swab.

Case 4.

A 55 year old male who is diabetic and alcoholic is admitted to the hospital for head trauma when he tripped during a drunken stupor. After three days in the hospital he developed a fever of 102°C with chills, a cough producing a current jelly-like sputum. Gram stain of the sputum revealed numerous PMNs, epithelial cells, gram-positive cocci, and gram-negative rods. Chest X-ray reveal abscess formation in posterior segments of the right upper lobe. The sample is from the sputum.

Case #1

Type of sample _____

Results of tests:

Part A1.

organism 1

organism 2

Blood agar plate results

hemolysis

gram stain

Catalase test

Bacitracin sensitivity

Rapid strep (coagglutination)

Optochin sensitivity

Coagulase test

Identification of organisms _____

Part A2.

organism 1

organism 2

MacConkey agar (lactose +/-)

Gram stain

Citrate slant (+/-)

Identification of organisms _____

Diagnosis of illness _____

Case #2

Type of sample _____

Results of tests:

Part A1.

organism 1

organism 2

Blood agar plate results

hemolysis

gram stain

Catalase test

Bacitracin sensitivity

Rapid strep (coagglutination)

Optochin sensitivity

Coagulase test

Identification of organisms _____

Part A2.

organism 1

organism 2

MacConkey agar (lactose +/-)

Gram stain

Citrate slant (+/-)

Identification of organisms _____

Diagnosis of illness _____

Case #3

Type of sample _____

Results of tests:

Part A1.

organism 1

organism 2

Blood agar plate results

hemolysis

gram stain

Catalase test

Bacitracin sensitivity

Rapid strep (coagglutination)

Optochin sensitivity

Coagulase test

Identification of organisms _____

Part A2.

organism 1

organism 2

MacConkey agar (lactose +/-)

Gram stain

Citrate slant (+/-)

Identification of organisms _____

Diagnosis of illness _____

Case #4

Type of sample _____

Results of tests:

Part A1.

organism 1

organism 2

Blood agar plate results

hemolysis

gram stain

Catalase test

Bacitracin sensitivity

Rapid strep (coagglutination)

Optochin sensitivity

Coagulase test

Identification of organisms

Part A2.

organism 1

organism 2

MacConkey agar (lactose +/-)

Gram stain

Citrate slant (+/-)

Identification of organisms

Diagnosis of illness _____