

Lab Notes for Exam 3 Section

STAPHYLOCOCCUS IDENTIFICATION

These tests are used to differentiate between the three most common **Staphylococcus species**. These tests may be used for other purposes as well. The table below summarizes the characteristics of each species.

Laboratory Tests for Differentiation of Staphylococcal Species			
Test	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>
Mannitol Salts Agar:			
Growth	+	+	+
Fermentation	+	–	+ or – *
DNase	+	–	–
Coagulase	+	–	–
Novobiocin Sensitivity	Sensitive	Sensitive	Resistant
Pigmentation	Golden yellow	White	White

*Indicates variation between strains. Some *S. saprophyticus* species may be negative for mannitol fermentation, while others may be positive. The strain we use is typically mannitol +.

Ex. 4-4: Mannitol Salt Agar

Organisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*

Media: One MSA (mannitol salt agar) plate per pair

Procedure:

1. Divide plates into three sections. Label each section with the name of a different organism.
2. Using aseptic technique, do a straight line inoculation, with a loop, of each organism into the appropriate section of the plate. Incubate the plate, upside down at 37° C.
3. Next lab period: Record your observations. Remember that MSA is both selective and differential. Look for the growth on the surface of the agar and make note of the amount of growth (scant, medium, or abundant). Growth on the surface indicates salt tolerance. A yellow color indicates the ability to ferment the sugar mannitol.

Ex. 5-16: DNase Test for the presence of the DNase exoenzyme, which breaks down DNA into nucleotides

Organisms: : *S. aureus*, *S. epidermidis* and *Staphylococcus saprophyticus*

Media: One DNase Test Agar plate; this medium does NOT contain the methyl green dye described in the lab manual. Instead, we will use 1M HCl as the indicator.

Procedure:

1. Divide the plate into thirds and do a single streak inoculation of each organism. Incubate at 37° C for 24 hours.
2. Next Lab period: To see the reaction, add 1M HCl to the plate, just like you added the iodine to the starch plate in Ex. 5-13. If the organism hydrolyzes DNA (it produces the DNase enzyme), a clear zone will appear around the line of inoculation. The absence of a clear zone in the agar indicates a negative result and the inability of the organism to produce DNase.

EX. 5-27: Coagulase Test

Purpose: The coagulase test is one of several tests that can be used to confirm the identity of *Staphylococcus aureus*. Coagulase is a virulence factor that increases the pathogenicity of an organism by causing the host blood to clot. When the pathogen invades a host, the clot will protect the organism by walling it off from the host's immune system. Coagulase (+) *Staphylococcus aureus* is a BSL 2 (Biosafety Level) organism.

Organisms: *S. aureus*, *S. epidermidis* and *Staphylococcus saprophyticus* - broth cultures

Materials: 3 tubes of rabbit plasma

Procedure:

1. To a tube of rabbit plasma, add 0.25 ml of a broth culture (the first mark on the sterile pipette above the joint). Mix and incubate the tube at 37° C for 24 hours. Do the coagulase test for all three species of *Staphylococcus*.
2. Next Lab period: Check the rabbit plasma for solidification. If the plasma forms a clot, then the reaction is positive and the organism is *Staphylococcus aureus*. If the plasma remains liquid, then the reaction is negative and further tests need to be performed to identify the *Staphylococcus* species. If these tubes are incubated for more than 48 hours, the results are unreliable and are considered invalid.

Ex. 5-24 Novobiocin Test

Organisms: *Staphylococcus aureus*
Staphylococcus epidermidis
Staphylococcus saprophyticus

Materials Needed:

3 Mueller-Hinton plates
3 tubes TSB to dilute cultures
Sterile swabs
Novobiocin discs

Procedure:

1. Use the following technique (Kirby-Bauer test, Ex. 7-3) to inoculate each Mueller-Hinton plate with one of the three *Staphylococcus* species: Prepare a diluted stock culture by transferring 0.25 ml of stock culture into a labeled, sterile tube containing 5 ml TSB (regular TSB tube). Mix well by finger vortexing. Dip a sterile swab into the diluted culture and remove the excess inoculum by pressing the saturated swab against the inner surface of the tube. The swab should be moist, but **not** dripping. Use the swab to streak the surface of the plate in a horizontal direction, then turn the plate 90° and streak the surface in the other direction.
2. When the surface of the plate appears dry, place a Novobiocin disc in the center. Incubate the plates for 24 hours.
3. Next Lab Period: Measure the zone size. Any measurement equal to or less than 16 mm indicates resistance to Novobiocin. A zone 17 mm or larger indicates susceptibility to Novobiocin.

STREPTOCOCCUS IDENTIFICATION

These tests are used to differentiate between **Streptococcal species**. Streptococci are organized into Lancefield groups based on serological properties. The Lancefield groups are designated by Group A, Group B, Group C, Group D, etc. The table below summarizes the characteristics of each species.

Laboratory Tests for Differentiation of Streptococcal Species					
Lancefield Group:	A	B	D		K, H, N
	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>E. faecalis</i> Enterococci	<i>S. bovis</i> Nonenterococci	<i>S. salivarius</i> <i>S. mitis</i>
Hemolysis	β	β	γ	α	α
Bacitracin	sensitive	resistant	resistant	resistant	resistant
Bile Esculin Agar (BEA)	–	–	+	+	–
6.5% NaCl	–	–	+	–	–

EX. 4-3: BILE ESCULIN AGAR (BEA) TEST – Bile esculin agar (BEA) is selective for Group D streptococci and enterococci. Group D organisms produce the enzyme esculinase, which metabolizes the carbohydrate esculin. The ferric citrate indicator will turn black in the presence of the esculin breakdown product.

Organisms: *S. pyogenes*, *Streptococcus bovis*, *Enterococcus faecalis* (formerly *Streptococcus faecalis*)

Media: 1 BEA (bile esculin agar) plate

Procedure:

1. Divide the BEA plate into 3rds and inoculate using a straight streak with *S. bovis*, *S. pyogenes*, and *E. faecalis*. Incubate at 37° C until next lab period.
2. **Next lab period:** Look for a black precipitate in the agar around the area of growth. *S. bovis* may produce just a faint gray haze because it grows so poorly on this medium. This test identifies Group D organisms.

Ex. 5-24: BACITRACIN SENSITIVITY TEST

Purpose: The bacitracin test is used to identify *Streptococcus pyogenes*, the most pathogenic of *Streptococcus* species.

Organisms: *Streptococcus pyogenes*
Streptococcus mitis OR *Streptococcus bovis* OR *Enterococcus faecalis*

Materials Needed:

- 2 blood agar plates (BAP)
- 2 tubes TSB to dilute broth cultures
- Sterile swabs
- Bacitracin discs
- Forceps

Procedure:

Perform the Bacitracin Sensitivity Test using the Kirby Bauer procedure described previously in Ex. 7-3.

1. Prepare a diluted stock culture by transferring 0.25 ml of stock culture into a labeled, sterile tube containing 5 ml TSB (regular TSB tube). Mix well by finger vortexing.
2. Two blood agar plates should be inoculated with a lawn, one for each species of *Streptococcus*. Dip a sterile swab into the diluted culture and remove the excess inoculum by pressing the saturated swab against the inner surface of the tube. The swab should be moist, but **not** dripping. Use the swab to streak the surface of the plate in a horizontal direction, then turn the plate 90° and streak the surface in the other direction. Leave an uninoculated margin around the edge of the plate. This will allow you to observe any hemolysin reaction that may occur simultaneously.
3. With the lid on, allow the plate to dry for 5 minutes. Place the Bacitracin disc on the center of the plate. Incubate the plates at 37°C.
4. **Next lab period:** Look for a zone around the disc. A zone of inhibition will appear dark red because the RBCs are not hemolyzed, while the lawn on the rest of the plate may appear clear if of β-hemolysis has occurred. A dark red zone of inhibition around the disk indicates bacitracin sensitivity.

Ex. 5-25: BLOOD AGAR

Purpose: To differentiate *Streptococcus* species using the three different hemolysin reactions.

Organisms: *Enterococcus faecalis*, *Streptococcus mitis*, *Streptococcus bovis*, and *Streptococcus pyogenes*

Media: One blood agar plate per pair

Procedure:

1. Divide the plate into three sectors. Make a straight line inoculation of the organism in the appropriate section of the plate. Incubate the plate, inverted at 37° C.
2. **Next lab period:** Observe the BAP for hemolysin activity. Alpha- hemolysis will make the agar appear greenish in color with partial clearing of the red blood cells; the red hemoglobin is converted into green biliverdin. Beta-hemolysis will lyse the red blood cells and break down the hemoglobin completely, leaving the agar clear and yellow. Gamma-hemolysis will not change the agar, with white culture growth on the surface of the agar.

NaCl BROTH

Purpose: Differentiate between enterococcus and nonenterococcus Group D Streptococcus.

Organisms: *Enterococcus faecalis* and *Streptococcus bovis*

Media: Two tubes 6.5% NaCl broth (purple broth)

Procedure:

1. Inoculate one tube of 6.5% NaCl broth with *S. mitis* and inoculate the other tube with *E. faecalis*. Incubate both at 37° C.
2. **Next lab period:** Observe for color change. A yellow color is a positive reaction for *Enterococcus faecalis*. A purple color is a negative reaction.

DICHOTOMOUS KEYS

Purpose: Many different types of species can be identified using a dichotomous key. Dichotomous keys tend to consist of pairs of opposing statements about the morphology of a group of organisms, thus the dichotomy. Keys may rely on superficial characteristics that do not necessarily reflect evolutionary or functional relationships. Bacterial species can be identified using a combination of cell structures, biochemical reactions (presence or absence of specific enzymes), and serology.

Procedure:

1. Use the 8 pastas provided to construct your own dichotomous key. Start with the most general characteristic that can separate the different pasta types into two main groups. Don't expect your key to be identical to other students' keys. The groupings will depend on which criteria you choose for your broadest group.
2. Work on your dichotomous keys for the Culture Identification Exercise. You will need to have your dichotomous keys ready for the next lab period.

UNKNOWN CULTURE IDENTIFICATION

Purpose: Solve the identity of an unknown bacterial specimen by creating a dichotomous key and using the staining, culturing and biochemical identification procedures you have learned about and performed during the semester.

Possible Organisms:

Alcaligenes faecalis
Enterobacter aerogenes
Enterococcus faecalis
Escherichia coli
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella arizoniae
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus saprophyticus
Streptococcus bovis
Streptococcus pyogenes

Rules:

1. This lab involves **independent** work. You may direct general questions to the lab instructor, but he/she will NOT interpret results for you or tell you what tests to perform.
2. You may discuss ideas with other students, but only you should perform tests on your own culture.
3. You may use any resource to develop your dichotomous key and to write your report, but you must reference all information. This includes the lab tests described in the lab manual.
4. You may only perform one biochemical test at a time.
5. You must justify the use of a test by showing the lab instructor your dichotomous key.
6. You may only repeat a test with the instructor's approval.
7. You are allowed one confirmatory test.

Day 1:

1. Arrive to class with a dichotomous key (flow chart) that will allow you to identify any of the organisms on the above list. You must have a rough draft of your dichotomous key to receive your unknown culture.
2. Each student will receive a 24 hr old TSA slant of a pure bacterial culture.
3. You will do three inoculations today from your stock culture: a streak plate for single colonies, a broth, and a slant. The streak plate and broth will be observed for growth characteristics. The slant will be your stock culture for future tests. Incubate the streak plate and the tubes at 35°C for 24 hrs.

Day 2:

1. Observe the streak plate and tubes for cultural characteristics. See Exercise 3 to review the terms that are used to describe the appearance of growth on the plate and the broth. **Record your observations & results on the Culture ID Data form.**
2. **Do a Gram stain from each the streak plate, slant culture, and the broth culture.** All three of your Gram stains should give identical results. If you have conflicting results, let the instructor

know immediately. *Use the Culture ID Data Form to record your observations & results.* The form is on the last page of these notes.

3. Perform your first biochemical test. Remember to show the instructor your dichotomous key to justify your decision and to pick up materials for your test. *Record all your observations and results, making reference to the specific laboratory exercise used for each test.*

Days 3-6:

1. Perform appropriate tests and make observations/results. Remember to perform a confirmatory test.
2. Don't get stressed out...breathe...

The Report

The report that you submit for this exercise will consist of three parts. The first part of the report will be a dichotomous key that YOU constructed to determine your unknown organism. The second part will be a compilation of the tests you have carried out with the observations and results to be submitted on the Unknown Culture Identification form included with this packet. The third part will consist of a research paper about the organism based on information you have obtained from a variety of references. When handing in your report, do not use a binder, plastic cover, or other bulky covering. A simple cover page with everything stapled together is sufficient.

Part I. Dichotomous key (15pts)

1. You must write up your OWN dichotomous key for all the possible unknown organisms listed on page 1 (e.g. Gram negative bacillus). Writing this key requires you use the same type of reasoning used in the Dichotomous key lab.
2. DO NOT simply copy the dichotomous key from you lab manual or from other sources (e.g. Bergey's manual). The organisms in the dichotomous key in Ex. 71 are not the same as the ones used in this exercise. Also, because of genetic variability between organisms, the results may not be same as in our lab book or in other sources.
3. The key must include the source of your information. You can use footnotes in your key or just include the information right next to test name. Since the tests we use come from either the lab manual or the Lab Notes, reference each test by citing the exercise number or the week from the Lab Notes. Example: "6.5% NaCl Broth, Lab Notes Week 11" or "Nitrate Reduction Test, Ex. 5-7".
4. Your dichotomous key must be typed, font size no larger than 12. You may draw in your own arrows. The chart must be legible and portray the information in a logical order.
5. The 15 points will be distributed as follows:
 - 3 points = Key is dichotomous (each step depends on two choices)
 - 2 points = Each test is referenced
 - 10 points = Key is correctly identifies all 12 organisms, not just your unknown

Part II. Compilation of tests (15pts)

1. When you have identified the organism, fill in the information on the Culture ID Data Form. In the space provided at the top of the sheet, write the identity of your unknown using its complete scientific name written in the correct form. For example, *Staphylococcus aureus*, not *S. aureus*. Do not forget to include the culture number.

2. In the space labeled **Appearance & Observations**, indicate the appearance of the organism and/or the medium after inoculation onto the various types of media you have used. For example, in the Indole test, a positive organism could be described as ***“a magenta color developed in the layer above the agar after addition of Kovac’s reagent”*** .
3. In the **Results** section indicate whether the organism was positive or negative for a reaction. It is sufficient to write the word "positive" or "negative" in this space.
4. Be sure to confirm your results with the appropriate test. For example, the EMB test will confirm *E. coli* or a coagulase test will confirm *Staphylococcus aureus*.
5. The 15 points will be distributed as follows:
 - 2 points = Correct identification of organism
 - 3 points = Correct identification of Gram Stain reaction and cell morphology
 - 5 points = Appropriate choice of biochemical tests according to your dichotomous key
 - 5 points = Correct interpretation of biochemical tests

Part III. Research paper (20pts)

1. In addition to providing the above information, each student is expected to write a research report of at least **two pages double-spaced** about the organism you have identified. Don't forget to use *italics* when using the scientific name of an organism. Your research paper should include the following topics:
 - a. (5 pts) General characteristics of the organism. Describe basic characteristics of your organism. You may include types of metabolism, classification, ecological roles, and results of biochemical tests and staining.
 - b. (6 pts) Relation to human health. Discuss how your organism is pathogenic or beneficial to humans.
 - c. (6 pts) Your choice. Every organism is unique! Provide some interesting facts or details you find fascinating about your organism. Some ideas are historical information, disease outbreaks, useful applications, or personal encounters with the organism. Have fun here!
 - d. (3 pts) References
2. References: Your source of information can include reference books, journal articles, most internet resources, but not popular magazine articles or Wikipedia. ***You should have a minimum of three sources.*** Do not count the course textbook or the lab manual as a source, although they may be a good place to start researching your organism. References should be at the end of your report on a separate page in a numbered list using the appropriate format. Each reference should be cited in the body of the report using the number of the reference enclosed in parentheses at the end of a sentence but before the period. Footnotes are not used in scientific writing. Some examples of standard methods for citing printed references in a scientific paper:
 - For journal articles: M. Jeffrey et al, *J. Comp. Pathol.* 124, 280 (2001)
 - For books: Maynard Smith, E. Szathmary. *The Major Transitions in Evolution* (Freeman, New York, 1995)

Be cautious when using the Internet. The extension associated with the URL indicates the type of website you are accessing:

- “.com” indicates a commercial website
- “.gov” indicates a government website
- “.edu” indicates a education institution, like a university or college

- “.org” indicates a not-for profit organization. These websites may belong to advocacy groups with a particular bias.

Examples of web references:

- Whitman, D. B. Rev. 2000 April. Genetically modified foods: harmful or helpful? Genetically Modified Foods, Cambridge Scientific Abstracts, <http://www.csa.com/hottopics/gmfood/overview.html> (with date you accessed the website)
- Webber, G. D. 1996 July 22 (last rev.) Regulation of genetically engineered organisms and products. Biotechnology Information Series, North Central Regional Extension, Iowa State University, http://www.nal.usda.gov/bic/Education_res/iastate.info/bio11.html (with date you accessed the website)
- Sullivan, S. 2000. Is Genetically Modified Food Safe? <http://www.ac.wvu.edu/~sullivs2.gm.htm> (with date you accessed the website)

If a website has no specific author, list the reference by the website sponsor (ex. National Institutes of Health, or Mayo Clinic)

3. The paper is to be written in your own words, which means you do not copy writing directly from the source. ***That is plagiarism and the entire unknown report will be rejected if this is done.*** Direct quotes are not used in science writing, so you must paraphrase.
4. ***The report must be typed with margins no greater than one inch, spacing no greater than double, and font size no greater than 12.*** Do not take up valuable space with the title of the report – all that is necessary at the top is the name of organism and your name, nothing else. Also figures or pictures that you might include in your report do not count as part of the one page minimum. It is expected that you will use a Spell-Checker and correct English.
5. The 20 points will be distributed as shown above. In each section I will be looking for 1) accuracy of information and 2) good writing mechanics including proper grammar and spelling. Don't forget to use *italics* when using the scientific name of an organism.

BACTERIAL CULTURE IDENTIFICATION DATA FORM

Name _____

Culture Number _____

Organism _____

		Appearance & Observations	Results
Morphological Characteristics			
Cell shape			
Cell arrangement			
Gram stain reaction			
Cultural Characteristics			
Colony morphology			
Slant morphology			
Broth growth			
Biochemical Tests			
	Test Name	Appearance & Observations	Results
	Test 1		
	Test 2		
	Test 3		
	Test 4		
	Confirmatory Test		

Additional notes or observations:

EX. 11-7: ELISA

Scientists have learned to use the immune response of animals to make antibodies that can be used as tools to detect and diagnose diseases. Animals such as chickens, goats, rabbits, and sheep can be injected with an antigen and, after a period of time, their serum will contain antibodies that specifically recognize that antigen. If the antigen was a disease agent, the antibodies can be used to develop diagnostic tests for the disease. In an immunoassay, the antibodies used to recognize antigens like disease agents are called primary antibodies. Primary antibodies confer specificity to the assay. Other kinds of antibody tools, called secondary antibodies, are made in the same way. In an immunoassay, secondary antibodies recognize and bind to the primary antibodies, which are antibodies from another species. Secondary antibodies are prepared by injecting antibodies made in one species into another species. It turns out that antibodies from different species are different enough from each other that they will be recognized as foreign proteins and provoke an immune response. For example, to make a secondary antibody that will recognize a human primary antibody, human antibodies can be injected into an animal like a rabbit. After the rabbit mounts an immune response, the rabbit serum will contain antibodies that recognize and bind to human antibodies. The secondary antibodies used in this experiment are conjugated to the enzyme horseradish peroxidase (HRP) which produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

PROCEDURE

You will be provided the tools to perform an ELISA. You will be given a simulated body fluid sample that you will share with your classmates. One of the samples in the class has been “infected”. You will also be provided with positive and negative control samples. Then you and your fellow students will assay your samples for the presence of the “disease agent” to track the spread of the disease through your class population. The assay will involve:

- 1) Addition of your shared sample plus control samples to the wells in a microplate strip. Incubation will allow all the proteins in the samples to bind to the plastic wells via hydrophobic interaction. This is an immunosorbent assay because proteins adsorb (bind) to the plastic wells.
- 2) Addition of anti-disease antibody (primary antibody) to the wells. The primary antibody will seek out the antigen from the many proteins bound to the well. If your sample was “infected”, the antibodies will bind tightly to the disease agent (antigen) in the wells.
- 3) Detection of the bound antibodies with HRP-labeled secondary antibody. If the primary antibodies have bound to the antigen, the secondary antibodies will bind tightly to the primary antibodies.
- 4) Addition of enzyme substrate to the wells. If the disease antigen was present in your sample, the wells will turn blue. This is a positive diagnosis. If the wells remain colorless, the disease antigen was not present in your sample and the diagnosis is negative.



MATERIALS

- Yellow tubes - Student samples (0.75 ml) (1 per student)
- Violet tube - Positive control (0.5 ml)
- Blue tube - Negative control (0.5 ml)

- Green tube - Primary antibody (1.5 ml)
- Orange tube - Secondary antibody (1.5 ml)
- Brown tube - Enzyme substrate (1.5 ml)
- 12-well microplate strips
- Disposable plastic transfer pipets
- 70-80 ml wash buffer in beaker

PROCEDURE: EXCHANGING OF FLUIDS

1. Write down the number on your yellow tube at the top of your data sheet. These are your “body fluid” samples that will be shared randomly with your classmates.
2. When you are told to do so, find another student and use a plastic transfer pipet to transfer all of your sample into the tube of the other student. (It does not matter whose tube is used to mix both samples.) Gently mix the samples by pipetting the mixture up and down. Then take back half of the shared sample (about 750 µl) to your own tube.
3. Write down the name and ID# of the student you just exchanged with on the data sheet for “Round 1.” Do not go on to the next round until the entire class is done with this round.
4. When you are told to do so, do round 2. Stop and wait until everyone is done with round 2 before going on to round 3.

PROCEDURE: USING ELISA TO TEST IF YOU CONTRACTED A DISEASE

1. Work in groups of 2.
2. Label the outside wall of each well of your 12-well strip. Two students may share a strip of 12 wells. On each strip, label the first three wells with a “+” for the positive controls and the next three wells with a “-“ for the negative controls. On the remaining wells write your and your partner’s initials. For example, Florence Nightingale and Alexander Fleming would label their shared strip like this:



3. Bind the antigens to the wells: Transfer 50 µl of the positive control antigen from the violet tube into the three “+” wells. Use a fresh pipet and transfer 50 µl of the negative control antigen from the blue tube into the three “-” wells. Use a fresh pipet and transfer 50 µl of your and your partner’s samples into the initialed wells.
4. Let stand for 5 min to allow antigens to bind to wells.
5. Wash the unbound samples out of the wells: Tip the microplate strip upside down onto the paper towels so that the samples drain out, then vigorously tap the strip upside down on the paper towels. Make sure to avoid samples splashing back into wells. Discard paper towel.
6. Use a fresh transfer pipet filled with wash buffer from the beaker to fill each well with wash buffer taking care not to spill over into neighboring wells. The same transfer pipet will be used for all washing steps. Tip the microplate strip upside down onto the paper towels so that the wash buffer drains out, then vigorously tap the strip a few times upside down on the paper towels.
7. Repeat step 6. Discard paper towels.
8. Use a fresh pipet to transfer 50 µl of primary antibody (PA) from the green tube into all 12 wells of the microplate strip.
9. Wait 5 minutes for the primary antibody to bind.

10. Wash the unbound primary antibody out of the wells by repeating wash step 6 two times.
11. Use a fresh pipet to transfer 50 μ l of secondary antibody (SA) from the orange tube into all 12 wells of the microplate strip.
12. Wait 5 minutes for the secondary antibody to bind.
13. Wash the unbound secondary antibody out of the wells by repeating wash step 6 three times. Use a fresh pipet to transfer 50 μ l of enzyme substrate (SbB) from the brown tube into all 12 wells of the microplate strip.
14. Wait 5 minutes but not more than 20 minutes. Observe and record your results. A blue color means a positive reaction, while no change means negative.
15. We will analyze the results of the class data below to figure out who was first infected.

DATA SHEET

Your ID Number: _____

Are you infected? Circle **YES** or **NO**

Names and ID#s of those you exchanged:

Round 1 _____ ID#: _____

Round 2 _____ ID#: _____

Round 3 _____ ID#: _____

ID Number and Name of those infected	Contacts		
	Round 1	Round 2	Round 3
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

STAPHYLOCOCCUS RAPID IDENTIFICATION

Purpose: Identify *Staphylococcus aureus* using the agglutination kit, Staphytest Plus.

Background: Staphytest Plus is a slide agglutination test used to identify *Staphylococcus aureus*. The test detects the clumping factor, Protein A, and certain polysaccharides found in some *S. aureus* strains. This allows the differentiation of *S. aureus* from those staphylococci that do not possess these properties. The developer contains latex beads coated with fibrinogen and IgG antibodies that bind Protein A or the polysaccharides. The latex beads and *S. aureus* will agglutinate together due to the binding of the IgG on the bead and Protein A on the bacterial cell surface. The agglutination of the white latex beads is a reaction that is visible to the naked eye when seen against a dark background.

Materials: Oxoid's Staphytest Plus kit
1 reaction card/group
Staphytest Plus Test reagent (Test Latex Reagent)
Sterile plastic loops

Organisms: *S. aureus* and *S. epidermidis*

Procedure:

- 1) The instructor will announce how to divide into groups.
- 2) Dispense one drop of test latex reagent onto each of the circles on the reaction card.
- 3) Using a loop, pick up and smear the equivalent of 5 average-size Staphylococcal colonies onto one of the circles from one of the culture media plates. Mix this into the Test Latex Reagent. Spread to cover the circle. Discard the loop.
- 4) Repeat step 3 with the cultures from the other plate onto the other circle.
- 5) Pick up and rock the card for up to 20 seconds and observe for agglutination.
- 6) When test is done dispose of reaction cards in biohazard bags.

STREPTOCOCCUS RAPID IDENTIFICATION

Purpose: Rapid identification of *Streptococcus pyogenes* using the Sure-View® Strep A test kit.

Background: The Sure-View® Strep A test is an ELISA based on the same principle as a home pregnancy test. The test strip is coated with an antibody that specific to a Strep A (*Streptococcus pyogenes*) antigen. During testing, a throat swab specimen reacts with the antibody to produce a red line in the test region if Strep A is present (a positive result). No line is produced in the test region if Strep A is not present (a negative result). For demonstration purposes, we will be testing stock cultures grown in the lab rather than throat swabs.

Above the test region is a control that should always turn red, regardless if the test specimen is positive or negative. If the control line does not appear, then the test is invalid.

Organisms: *Streptococcus pyogenes* and *Streptococcus bovis* or another non-Strep A organism

Materials: 2 Sure-View® Strep A test strips
2 sterile swabs
2 clean glass test tubes
Reagent A
Reagent B

Procedure:

1. Hold the reagent A bottle upright and add 4 full drops to the test tube. Reagent A is red.
2. Hold the reagent B bottle upright and add 4 full drops to the test tube, being careful not to touch the tip of the bottle to any surface to avoid cross contamination. Reagent B is colorless. Adding Reagent B should turn Reagent A from red to yellow. Tap the tube gently to mix the reagents.
3. Wet a sterile swab in the liquid portion of the blood agar slant. Transfer the swab to the test tube containing Reagents A and B. Rotate the swab in the reagents vigorously 10 times, then leave in the tube for 1 minute.
4. After 1 minute, press the swab against the side of the tube so the liquid remains in the tube. Discard the swab in a Biohazard bag.
5. Remove the test strip from the sealed foil pouch, handling the strip by the end with the picture of the hand, arrows pointing down. Place the strip in the test tube with the inoculated reagents. If the procedure was followed correctly, the liquid should be at or just below the MAX line at the bottom of the strip.
6. Leave the strip in the tube and read after 5 minutes. The result is invalid after 10 minutes.

About your results:

POSITIVE: two distinct lines appear. The intensity of the color of the bottom line in the test region (T) will vary with the amount of Step A antigen present in your sample. Any shade of red is considered positive.

NEGATIVE: one line appears in the control region (C). No red or pink line appears in the test region (T).

INVALID: no line appears in the control region(C).

Ex. 5-30: ENTEROTUBE II

Purpose: Use of a rapid identification procedure to identify a microorganism.

Materials Needed: Enterotube tube
SIM tube

Procedure:

1. Remove both caps. Note that one end of the wire is bent and is used as the handle. The other end of the wire is pointed and is used to pick up the inoculum. Do not flame the wire.
2. Pick up a large inoculum from the culture plate. The inoculum should be visible at the tip of the needle.
3. Inoculate the tube by twisting the wire while pulling it through all twelve compartments at the same time. Do not pull the wire all the way out of the tube.
4. Push the wire back through, and break the needle off at the notch as demonstrated by the instructor. Discard the broken needle in the autoclave bag. Replace the caps on both ends.
5. With a flamed, sterile needle, punch a hole through the thin plastic that covers the air inlets in each of the last eight compartments. This is done so that air can enter these compartments. Incubate at 37° C with the tube on its flat surface in the tray labeled for your lab section.
6. Stab inoculate a SIM tube using a needle. Incubate at 37°C and test for indole production. You will need this result to interpret your Enterotube. We will not be adding indole to the Enterotube itself.

INTERPRETING YOUR RESULTS

1. Use the **BLUE** Enterotube II key and manual to help you interpret all the reactions except the indole test results. The indole test results will come from the SIM tube you inoculated.
2. The VP test is not necessary for identification.
3. Use the printed ID strip to record the results of each test. Circle any positive result.
4. Add the value of each circled number and record in the appropriate section. This will give you a 5-digit ID value.
5. Find the five-digit number in the Coding Manual. This number will indicate the possible genus and species of the organism and any additional tests needed to confirm the identification.