

**BIOL 3702L:
A Survey of
the Cocci**

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<http://www.aviano.af.mil/News/Article-Display/Article/724769/mda.armstrong-success-through-support/>

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Purpose of These Exercises

- These exercises shall focus on selected members of the cocci
 - **Gram-positive:** species of *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Micrococcus*
 - **Gram-negative:** *Moraxella catarrhalis*
- The Gram-negative bacilli, *Escherichia coli* and *Enterobacter aerogenes*, are used in some tests for comparison
- This is NOT a comprehensive study of all cocci, just an introduction

<https://www.livestrong.com/article/121176-bacteria-cell-functions/>

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Purpose of These Exercises (cont.)

- **Learning objectives:**
 - *Understand the underlying chemical and/or physiological mechanisms of each test* [the pertinent information can be found in the laboratory instructions for each exercise]
 - Know how to properly conduct each of the tests and when to employ them
 - Accurately interpret test results
 - Comprehend how the results distinguish between different types of bacteria

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Tests To Be Conducted This Week

Students must know and understand the underlying basis for each test in addition to knowing what constitutes a positive test results as well as a negative test result.

- Hemolysis
- Oxidase Test
- Gelatinase Test
 - Tube method
 - Plate method
- DNase Activity
 - With dye
 - Without dye
- Coagulase Test
- Catalase Test
- Esculin/PYR Test
- Esculin Hydrolysis (plate assay)
- Temp./Salt Tolerance
- Antibiotic Susceptibility

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Some Important Details

- Activities shall be assigned to specific groups:
 - Tables 1 and 2 shall work together on all tests*
 - Tables 3 and 4 shall work together on all tests* (*Except for Coagulase Test; see below)
- **HOWEVER**, each **INDIVIDUAL** is responsible for knowing how to conduct a given test and how to interpret the results.
- Some activities **REQUIRE** that someone from a group return to complete the test during an open laboratory period.

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Special Permission

- Because many of the results generated in these tests must be shared with other members of the assigned lab group, students will be permitted to use their cell phones to take pictures of the results.
- This is a **ONE-TIME** permission.
- **HOWEVER, BEFORE AND AFTER USE, CELL PHONES MUST BE DISINFECTED WITH AN ALCOHOL WIPE!!! NO EXCEPTIONS!!!**


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Hemolysis Test

- Streak *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis* onto separate blood agar plates
- Incubate 24 hours at 37°C.
NOTE: For best results, *S. pneumoniae* and *S. pyogenes* should be incubated in the presence of CO₂.




Alpha, Beta, and Gamma Hemolysis as Indicated by the Greek Letters.

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Hemolysis Test (cont.)

- **YOU MUST RETURN WITHIN 18-24 HOURS TO OBSERVE THE RESULTS!!!**
- Examine and record the patterns of hemolysis for each organism.



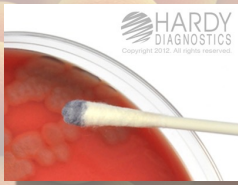
Alpha, Beta, and Gamma Hemolysis as Indicated by the Greek Letters.

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Oxidase Test

- Use separate OxiSticks™ Oxidase Swabs to touch several colonies of *Moraxella catarrhalis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Micrococcus luteus*
- Observe for development within 10-20 seconds of a blue/purple color on the swab (positive result)



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Gelatinase Test

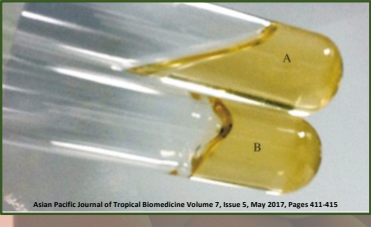
Tube Test

- Inoculate a separate nutrient gelatin deep with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, and *Escherichia coli*.
- Incubate the four tubes plus an additional uninoculated nutrient gelatin deep (control) at 37°C for 24-48 hours.
- Place the tubes in the refrigerator (or on ice) for 15-30 minutes.
- Observe each tube for fluidity.

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Gelatinase Test (cont.)



A) Positive gelatinase reaction
B) Negative gelatinase reaction

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Gelatinase Test (cont.)

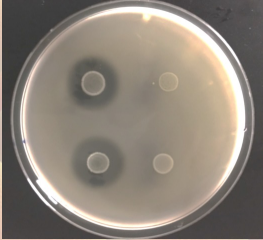
Plate Assay

- Divide a BEA agar plate into quadrants.
- Use a cotton swab to inoculate separate quadrants with a dime-size area with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, and *Escherichia coli*.
- Incubate the plate at 37°C for 24-48 hours.
- Flood surface of plate with 4 M ammonium sulfate.
- Observe for the presence of clear zones around the growth on the plate.

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Gelatinase Test (cont.)



Gelatinase agar plate after being treated with ammonium sulfate.

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DNase Test

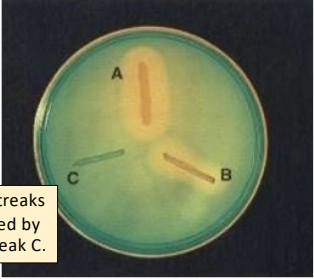
- Use a swab to inoculate separate halves of a **DNase/toluidine** plate with *Staphylococcus aureus* and *Staphylococcus epidermidis*
- Use a swab to inoculate separate halves of a **DNase/no dye** plate with *Staphylococcus aureus* and *Staphylococcus epidermidis*
- Incubate both plates at 37°C for 18-24 hours

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DNase Test (cont.)

- Observe DNase/toluidine plate for a clear zone (positive reaction) around patches




DNase Activity for Bacterial Streaks A and B, But None is Expressed by the Bacterium Comprising Streak C.

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DNase Test (cont.)

- To DNase/no dye plate, flood surface with 1N HCl for 5 minutes
- Drain off the HCl and observe the plate for a clear zone (positive reaction) around patches



Positive DNase Activity

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Coagulase Test

- Thaw three (3) tubes of Coagulase Cryo™ reagent
- Tube Method
 - Inoculate one tube with *Staphylococcus aureus*
 - Inoculate a second tube with *Staphylococcus epidermidis*
 - Incubate both tubes at 37°C for 4 hours (could occur in less time)
 - Examine tubes for clotting ('gelling') of the rabbit plasma

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Coagulase Test (cont.)




Top tube: Positive coagulase reaction
Bottom tube: Negative coagulase reaction

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Coagulase Test (cont.)


- Slide Method
 - From the remaining (third) reagent tube, place a drop of coagulase reagent to the left and right sides on two separate glass slides
 - On one slide, place a drop of sterile distilled water or saline in the reagent and mix well [This is the negative control reaction]
 - On the same slide, transfer some growth of *Staphylococcus aureus* and mix well

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Coagulase Test (cont.)

- Slide Method (cont.)
 - On the second slide, place a drop of sterile distilled water or saline in the reagent and mix well [This is the negative control reaction]
 - On the same slide, transfer some growth of *Staphylococcus epidermidis* and mix well
 - Incubate slides at room temperature for 5 minutes and observe if clumping occurs. Clumping is indicative of a positive reaction.


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Coagulase Test (cont.)




Control (left image) and Positive (right image) coagulase test results using *Staphylococcus aureus*

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Catalase Test

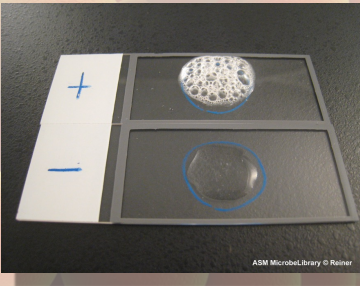
- For each of the following species, transfer some growth to the surface of a glass slide: *Streptococcus pyogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Micrococcus luteus*.
- To each smear, add 3-5 drops of 3% hydrogen peroxide.
- A positive reaction results in immediate production of bubbles.


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Catalase Test (cont.)

- A positive reaction results in immediate production of bubbles.




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Esculin/PYR Test

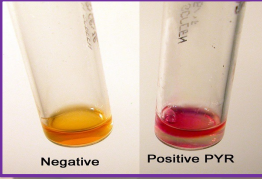
- Resuspend the pellet in three (3) vials of ESC/PYR using sterile water.
- Heavily inoculate separate tubes *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*
- Incubate tubes at 37°C for two (2) hours
- Observe for color change
 - Yellow indicates a positive esculin hydrolysis
 - Other color indicates negative esculin hydrolysis

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Esculin/PYR Test (cont.)

- Test now for PYR hydrolysis by adding PEP reagent (3-5 drops)
- Incubate at 37°C for 15 minutes
 - Yellow/peach color = negative reaction
 - Red/pink color = positive reaction



http://www.microbiologynotes.com/pyr-1-pyrrolidonyl-%CE%B2-naphthylamide-test-principle-uses-procedure-result-interpretation-examples-and-limitation/

https://www.thermofisher.com/blog/protectomics/hostpathogen-protectomics-mycoplasma-pneumoniae/


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Esculin Hydrolysis: Plate Assay


- Label four plates of BEA agar, one each for *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli*
- Streak each plate with the respective organism.
- Incubate plates at 37°C for 24-48 hours
- Observe for darkening of agar

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Esculin Hydrolysis: Plate Assay (cont.)



Positive Esculin Hydrolysis Result

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Temperature/Salt Tolerance

- Used to distinguish between streptococci, staphylococci, and enterococci
- Inoculate two tubes of BHI broth for each of the following representative organisms:
 - *Streptococcus agalactiae*
 - *Enterococcus faecalis*
 - *Staphylococcus aureus*
- Similarly, inoculate two tubes of BHI broth containing 6.5% NaCl for each of the above organisms.

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Temperature/Salt Tolerance (cont.)

- Incubate one tube of each pair at 37°C and the remaining tube at 45°C.
- After 36-48 hours of incubation, examine the tubes for growth. Record the results as positive (“+”) or negative (“-”). There should be definitive differences in temperature and salt tolerance among the different genera.

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Antibiotic Susceptibility

- Susceptibility of Cocci to Antibiotics
 - Novobiocin resistance can distinguish among *Staphylococcus* species
 - Bacitracin and SXT susceptibility can be used to distinguish *Streptococcus pyogenes* from other streptococci and *Enterococcus*
 - **Novobiocin susceptibility test:** make patches of *Staph. saprophyticus* and *Staph. epidermidis* on blood agar, then place a disk of novobiocin on each patch

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Antibiotic Susceptibility (cont.)

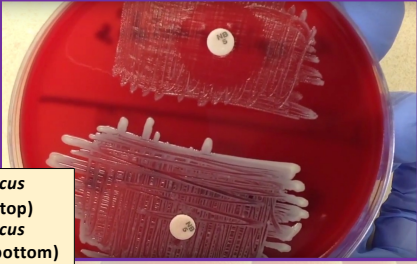
- **Bacitracin and SXT susceptibility tests:** make patches of *Staphylococcus aureus*, *Micrococcus luteus*, *Streptococcus pyogenes*, and *Enterococcus faecalis* on blood agar plates, then place disks of Bacitracin and SXT on these patches
- Incubate all plates for both novobiocin and bacitracin/SXT tests at 37°C for 18-24 hours
- Observe and record results.

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Antibiotic Susceptibility (cont.)



Staphylococcus epidermidis (top)
Staphylococcus saprophyticus (bottom)


<https://youtu.be/ASDQitQAcU>

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Antibiotic Susceptibility (cont.)



Streptococcus pyogenes susceptibility to bacitracin (A) and trimethoprim-sulfamethoxazole (SXT)

https://www.labce.com/50615135_group_a_strep_a_disktest.aspx

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Lab Report Expectations

- Complete each of the report sheets for each given exercise. Share data where appropriate, but do not plagiarize!
- Remember, where appropriate, **YOUR ANSWERS MUST BE CLEAR, CONCISE, AND GRAMMATICALLY SOUND SENTENCES!**
- Retain these laboratory exercise reports. Your laboratory instructor may not call for them at this time, but may do so in the near future.

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https://www.huffingtonpost.com/barbara-jacoby/asking-questions-is-really-hard_b_7052722.html



Questions?

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