About Science Prof Online PowerPoint Resources

• Science Prof Online (SPO) is a free science education website that provides fully-developed Virtual Science Classrooms, science-related PowerPoints, articles and images. The site is designed to be a helpful resource for students, educators, and anyone interested in learning about science.

• The SPO Virtual Classrooms offer many educational resources, including practice test questions, review questions, lecture PowerPoints, video tutorials, sample assignments and course syllabi. New materials are continually being developed, so check back frequently, or follow us on Facebook (Science Prof Online) or Twitter (ScienceProfSPO) for updates.

• Many SPO PowerPoints are available in a variety of formats, such as fully editable PowerPoint files, as well as uneditable versions in smaller file sizes, such as PowerPoint Shows and Portable Document Format (.pdf), for ease of printing.

• Images used on this resource, and on the SPO website are, wherever possible, credited and linked to their source. Any words underlined and appearing in blue are links that can be clicked on for more information. PowerPoints must be viewed in *slide show mode* to use the hyperlinks directly.

• Several helpful links to fun and interactive learning tools are included throughout the PPT and on the Smart Links slide, near the end of each presentation. You must be in *slide show mode* to utilize hyperlinks and animations.

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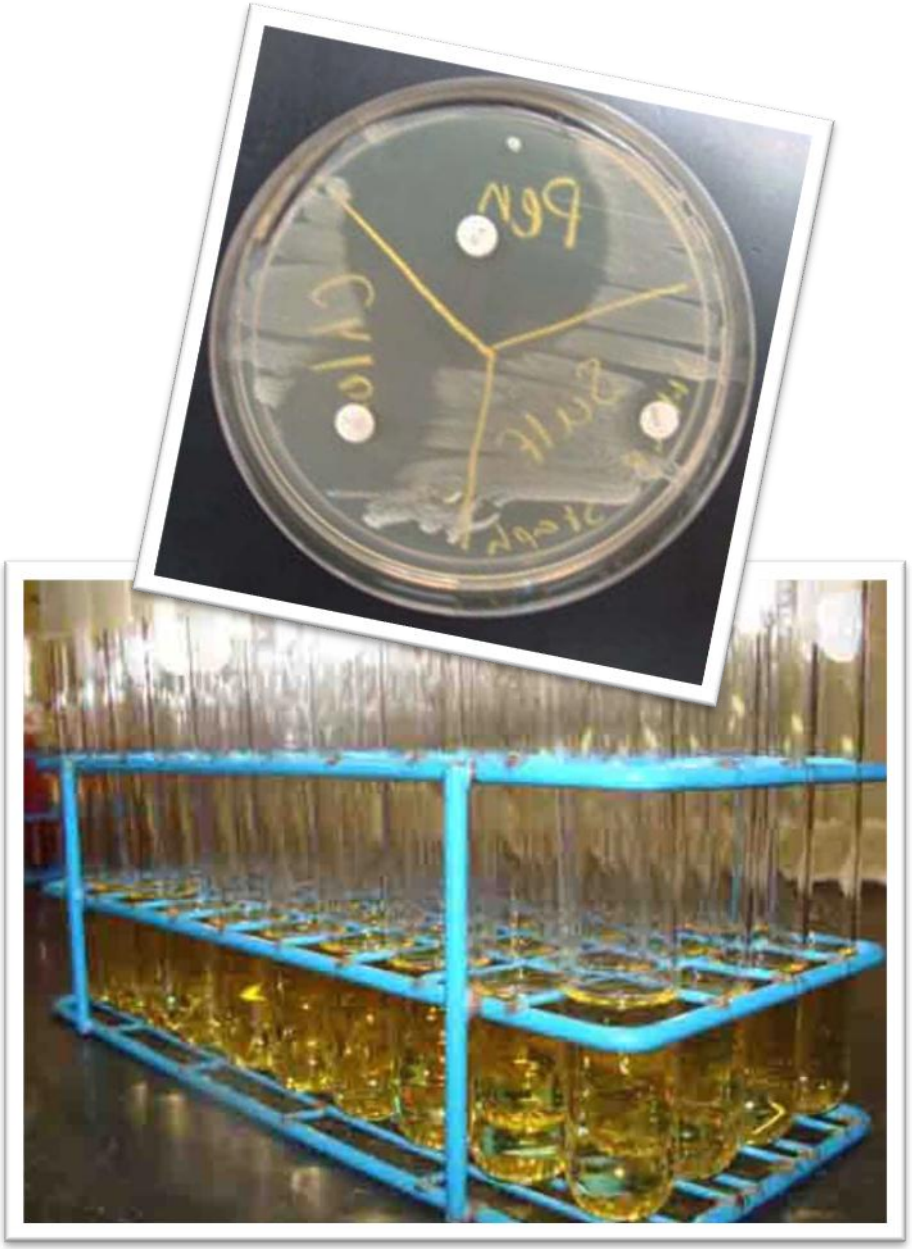
Image: Compound microscope objectives, T. Port

Laboratory Project 4

Urine Cultures & Bacterial Identification

- Colony Counts
- Serial Dilutions
- API-20E
- Antibiotic Sensitivity Testing



Images: Antibiotic sensitivity tests; Test tubes filled with liquid media, both by T. Port

Urine Cultures

Session 1

Inoculating Media

***Q:** Why are you inoculating this medium?*

***Q:** What do you hope to learn about your sample?*

1.TSY

2.MAC

Images: TSY agar with inoculation loop; sterile Blood Agar; sterile MacConkey's Agar, all by T. Port

Urine Cultures

Session 1

Inoculating Media

Pattern that you will use to inoculate your plates:

Images: MacConkey's Agar inoculated with a lactose fermenting bacteria, T. Port

Urine Cultures

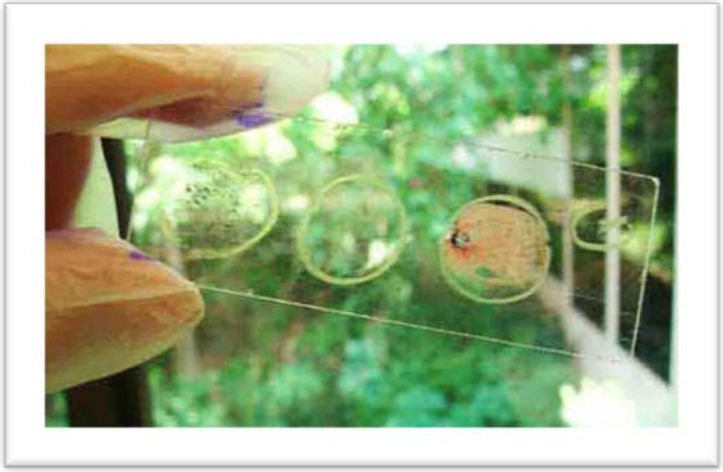
Session 2

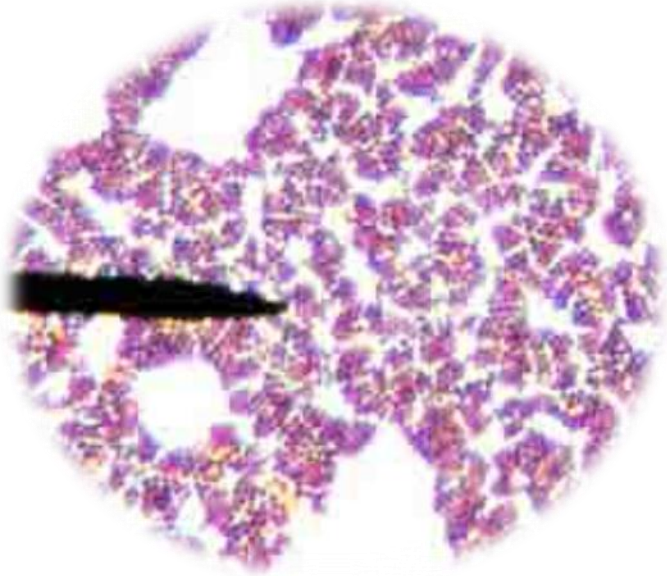
Tests to Identify Unknown Bacteria in Urine Culture

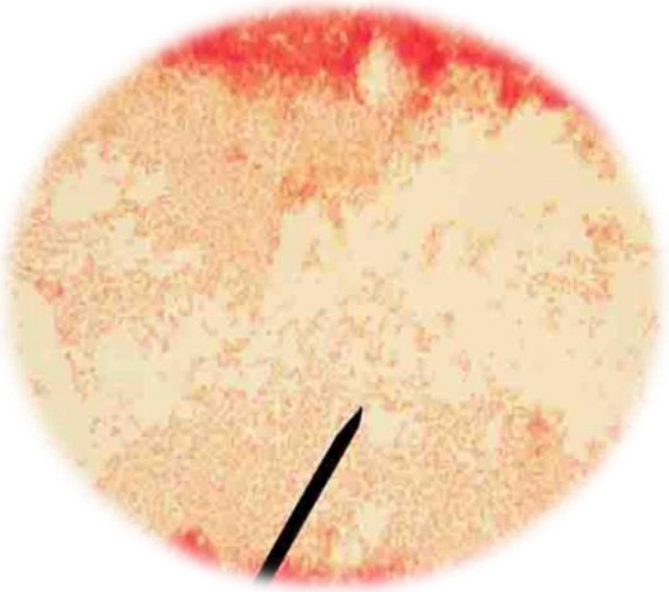
Which tests do I do?

1. First use Gram stain to determine cell wall structure and cell morphology of unknown.

2. Then see Lab Project 4 instructions for the proper tests to order for your patient.







Images: Gram stained microscope slide with controls; Gram-positive bacteria @ 1000xTM; Gram-negative bacteria @ 1000xTM, all by T. Port

Urine Cultures

Session 2

(Interpret in Session 3)

Bacitracin Test

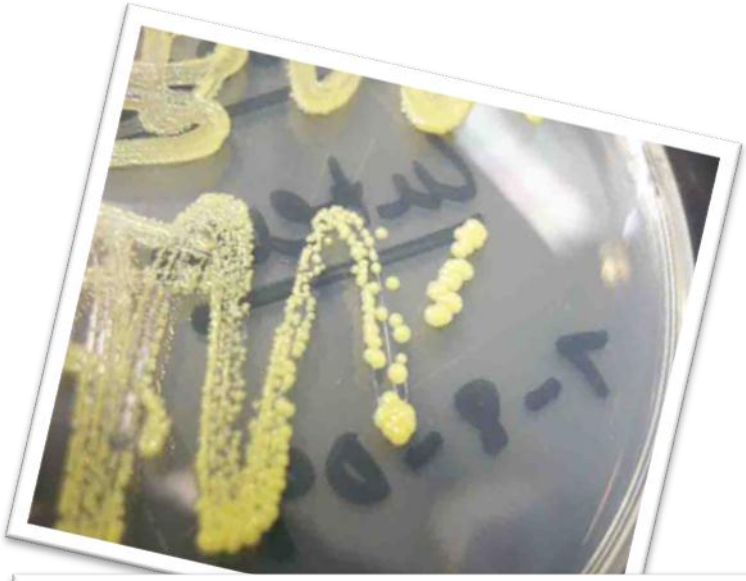
Bacitracin is a an antibiotic that does not work well orally, but is very effective topically (on eye and skin).

MOA is to interfere with construction of the thick Gram-positive peptidoglycan cell wall.

Bacitracin Test is an antibiotic sensitivity test utilizing a bacitracin infused disc.

The test helps identify *Micrococcus luteus*. *Micrococcus* is especially sensitive to Bacitracin, and will show a zone of inhibition > 30 millimeters.

Other bacteria in our laboratory stock cultures will have zones of inhibition < 30 millimeters.



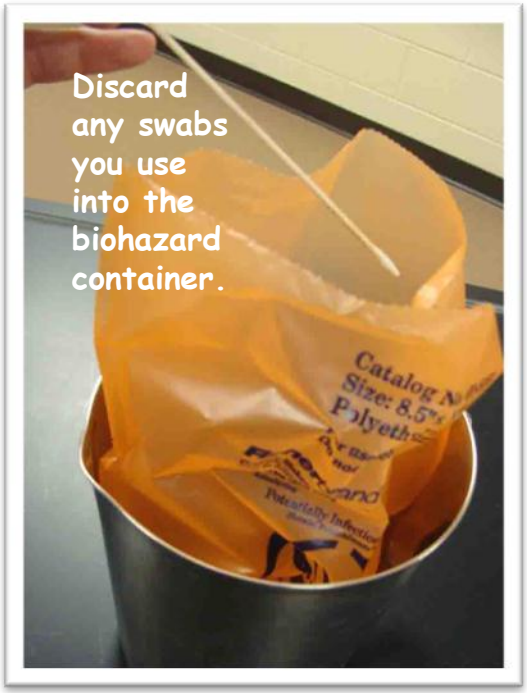
Images: *Micrococcus luteus* colonies growing on TSY agar; Bacitracin test on *Staph epi* (left) & *M. luteus* (right), T. Port

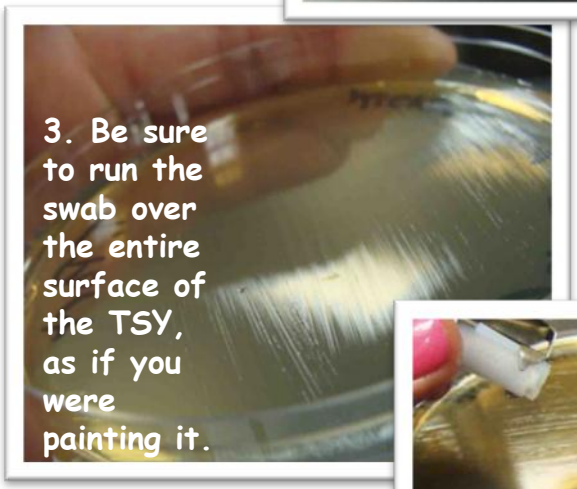
Urine Cultures

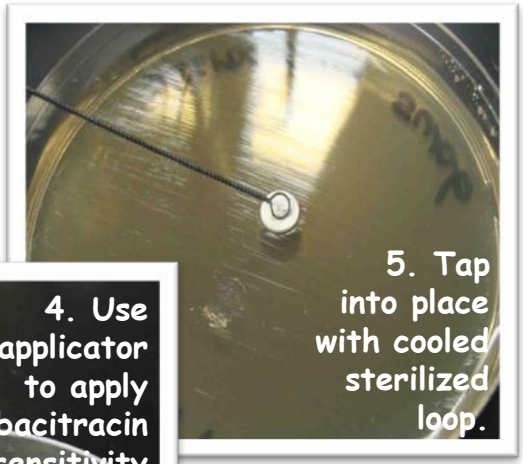
Session 2

Bacitracin Test

These photos correspond to the Lab **Project 4, Session 2 Procedure** for doing the Bacitracin Test.







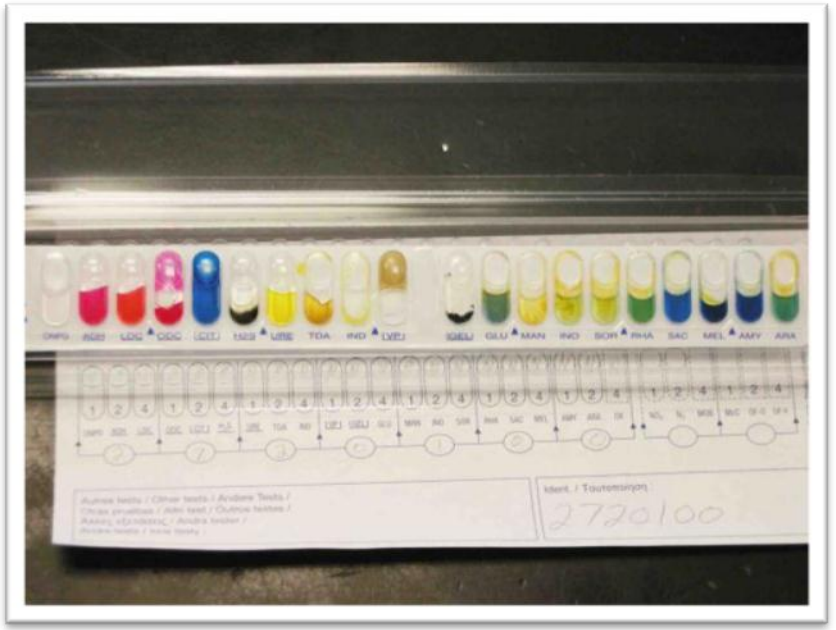
Images: All Bacitracin Test photos by T. Port

Urine Cultures

Session 2

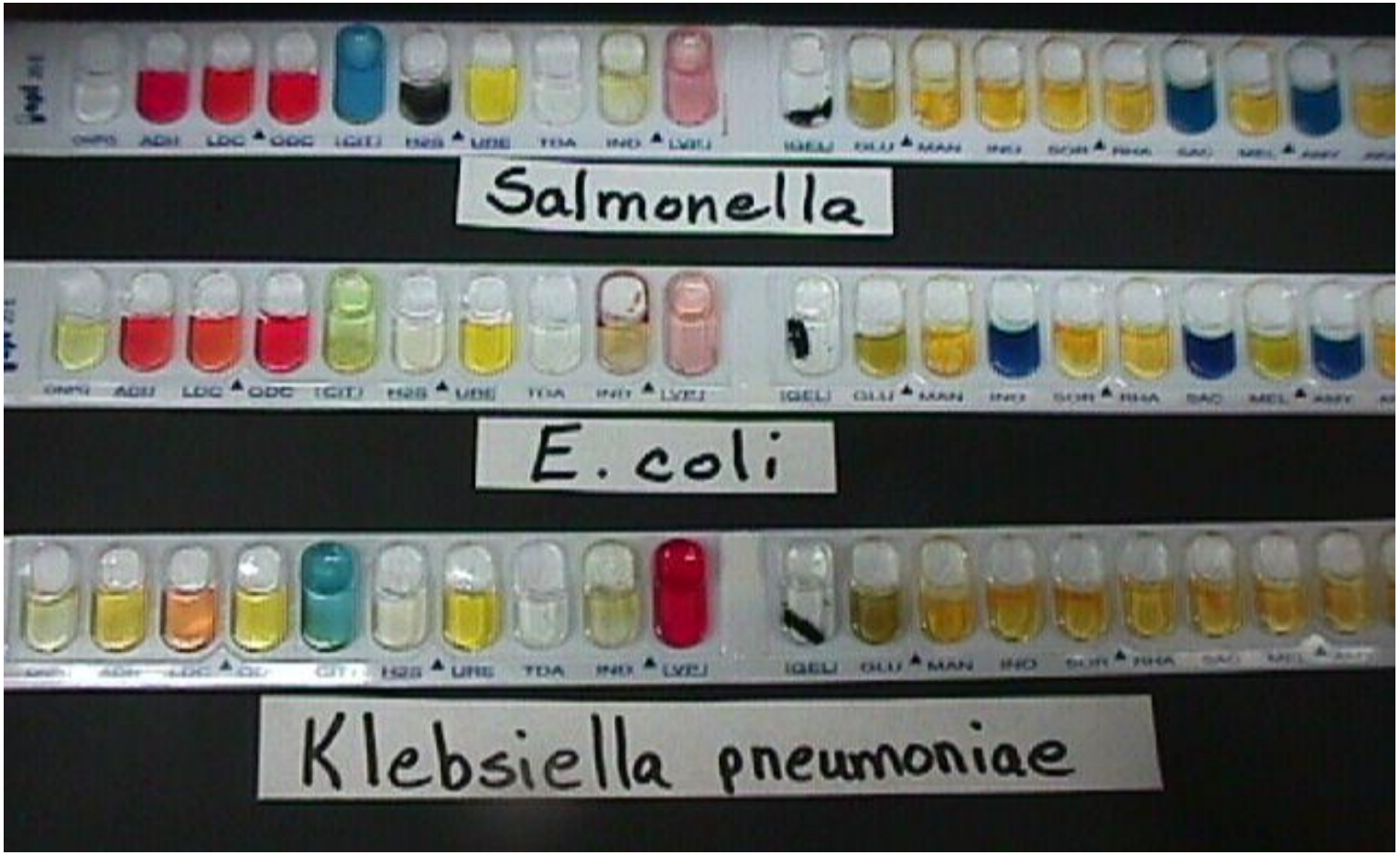
(Interpret in Session 3)

api®20E



- The **API-20E** test is used to ID Gram-negative bacilli from the family Enterobacteriacea.
- ***Q:*** *So what test would someone need to do on a bacterial sample before utilizing the api®20E?*
- The api®20E is a system of 20 individual, miniaturized tests used to determine the **metabolic capabilities** of the organism.
- From identification of metabolic capabilities, we can zero in on the identification of the genus and species.
- The tests allow us to come up with a numerical 7-digit profile, based on which tests are positive and which are negative. You then look up that magic number, to find the species identification of your sample.

Images: API-20E with results sheet, T. Port



Required Reading on api20e:

- Lindquist, J. (2010) "API-20E Enteric Bacteria Identification System" from *An Introduction to Bacterial Identification*.
- API-20E Animation from Microbe Library.
- Reading the API-20E, a YouTube video from Dr. Kimmitt.

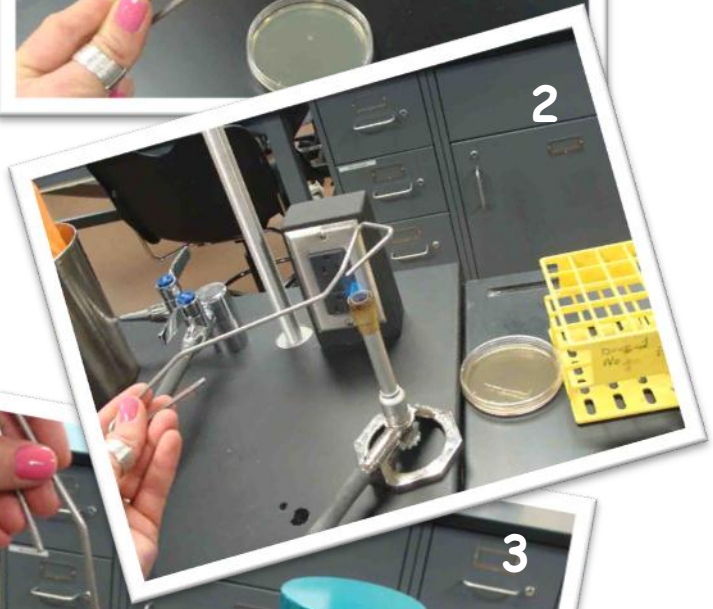
Image: API-20E results for different species of bacteria, Dr. Charles Gerba, University of Arizona

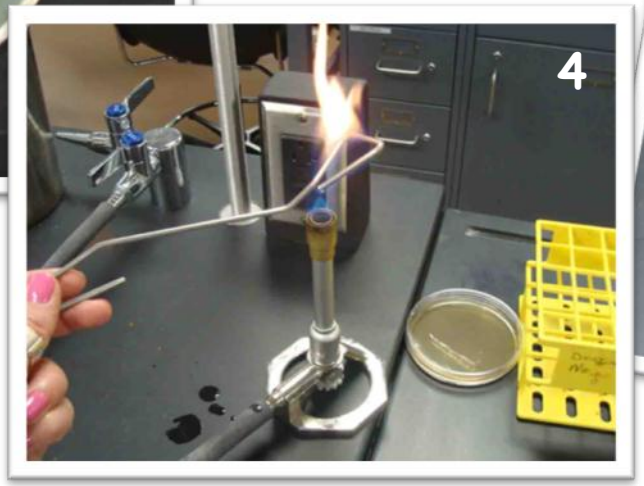
Urine Cultures

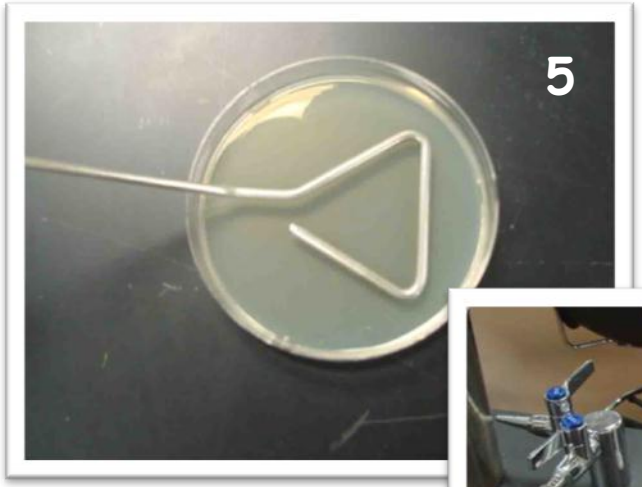
Session 2

Antibiotic Testing

These photos correspond to the Lab **Project 4, Session 1 Procedure** for doing the Anitbiotic Sensitivity Testing Procedure (antibiotics other than Bacitracin). Read instructions carefully.







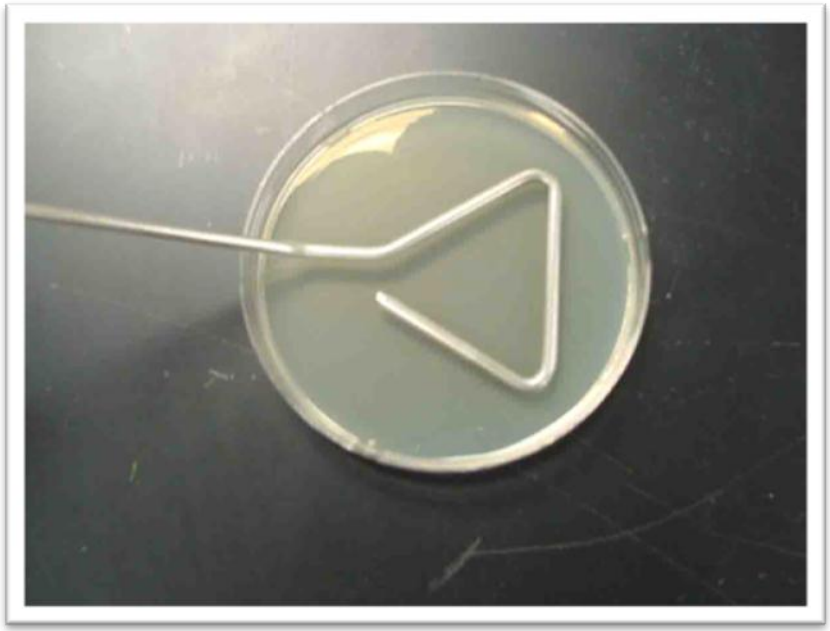
Images: Sterilizing a microbiology spreader, TSY used as heat sink for

Urine Cultures

Session 2

Antibiotic Testing

Heat Sink



Let spreader cool for **5 seconds** before touching it to anything (alcohol or media)!!

Images: TSY heat sink touched with spreader that was too hot, note melting of agar; Spreader and TSY agar, T. Port

Urine Cultures

Session 2

(Interpret in Session 3)

Antibiotic Testing

- **Antimicrobics** are drugs used in the treatment of infectious disease.
- **Sensitivity disks** can show us which antimicrobic will be most effective in controlling a microbe.
- The disks that we will be using are impregnated with an antibiotic.
- A nutrient agar plate is uniformly inoculated with bacteria and the disks are placed on the media.
- Over the incubation period, the antibiotic diffuses out from the disk.
- If the microbe is sensitive to the antibiotic in question, a **zone of inhibition** (an area without bacterial growth) will occur around the disc.

Image: Antibiotic sensitivity disks in applicator; Zones of bacterial inhibition from antibiotic disks, T. Port

Urine Cultures

Session 3

Antibiotic Zones of Inhibition

- After incubation you can collect **quantitative data** on the effectiveness of an antimicrobial drug by measuring the diameter of the **zone of inhibition**.
- Do not open the plate to do this! The measurement can be obtained the bottom of the plate.
- Measure in millimeters (mm).

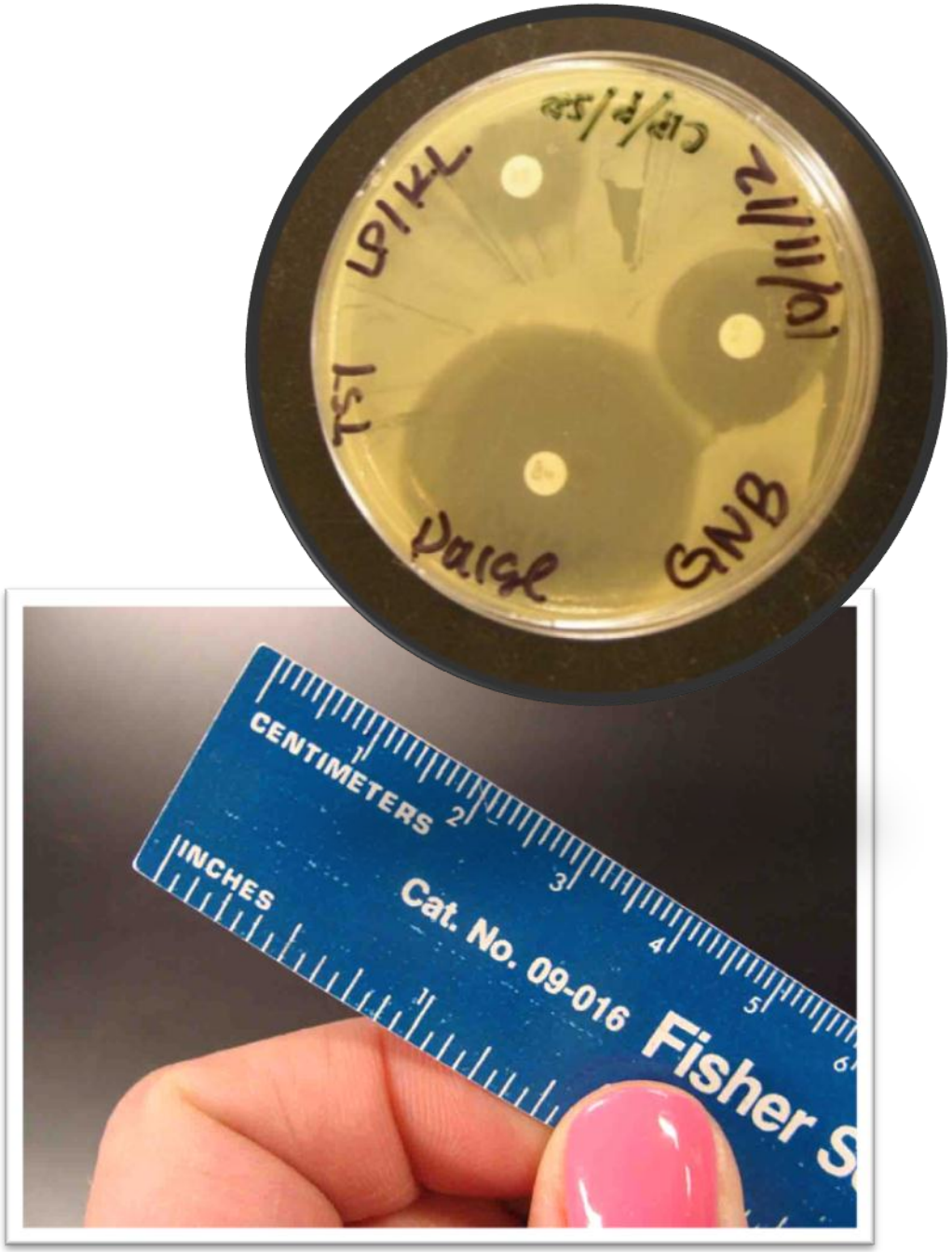
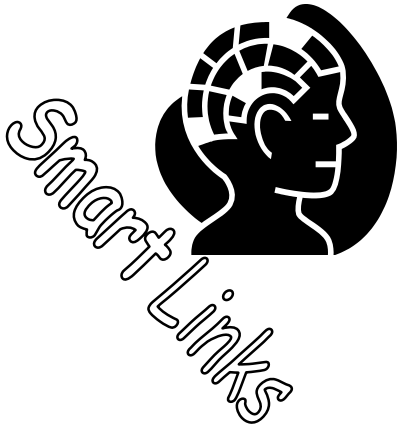


Image: Zones of bacterial inhibition from antibiotic disks; Ruler showing inches & centimeters, T. Port

Confused?



Here are links to fun resources that further explain microbiology media & culture:

- **Urine Cultures & Bacterial Identification** Laboratory Main Pages on the Virtual Microbiology Classroom of Science Prof Online.
- How to Interpret: MacConkey's (MAC), Mannitol Salt (MSA) and Blood Agar (BAP) videos from Science Prof Online.
- Bacterial growth video and narration, YouTube, Dizzo95..
- Gram Stain Interactive Tutorial. This is an extremely useful tutorial that shows, step-by-step, what happens in Gram-positive and Gram-negative cells during Gram staining.
- Acid-fast Stain Animated Tutorial. The staining procedure depicted in this tutorial differs a bit from how we do it in lab, but this tutorial is still very useful. Shows the steps of the staining procedure and the resulting color of Acid-fast and Nonacid-fast cells.
- Endospore Stain PowerPoint. Although this is just a PPT, it does have useful information and images for students learning about the endospore stain.
- API-20E Animation from Microbe Library.
- Reading the API-20E, a YouTube video from Dr. Kimmitt.

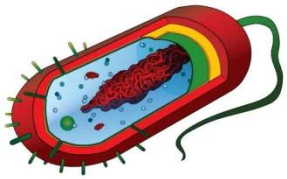
(You must be in PPT slideshow view to click on links.)

Are microbes intimidating you?

Do yourself a favor. Use the…

Virtual Microbiology Classroom *(VMC)* !

The VMC is full of resources to help you succeed, including:



- practice test questions
- review questions
- study guides and learning objectives

You can access the VMC by going to the Science Prof Online website
www.ScienceProfOnline.com

Images: Staph, Giant Microbes; Prokaryotic cell, Mariana Ruiz