



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.
- This digital resource is licensed under Creative Commons Attribution-ShareAlike 3.0:
<http://creativecommons.org/licenses/by-sa/3.0/>

Alicia Cepaitis, MS
Chief Creative Nerd
Science Prof Online
Online Education Resources, LLC
alicia@scienceprofonline.com

Tami Port, MS
Creator of Science Prof Online
Chief Executive Nerd
Science Prof Online
Online Education Resources, LLC
info@scienceprofonline.com

Laboratory Exercise 2

How to Aseptically Pour Bacterial Growth Media & Prepare an Isolation Streak Plate

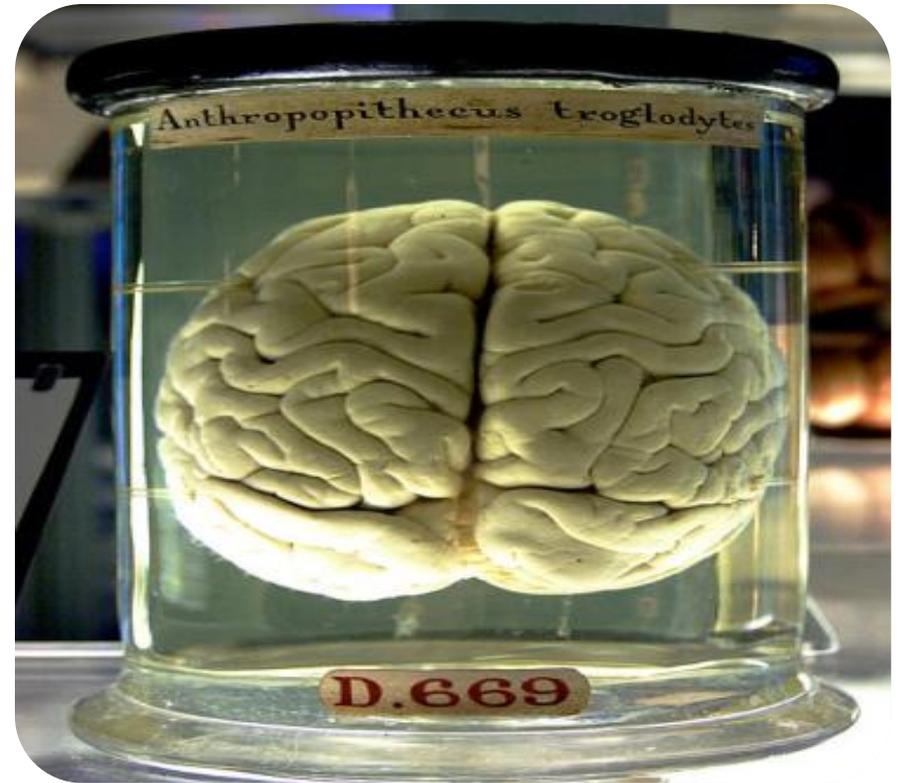


Plug in and turn on microincinerator now!

What am I going to learn from Lab Topic #2?

Isolation Streak Plate Method

- You will learn how to aseptically pour bacterial growth media.
- You will practice performing [isolation streak plates](#) using aseptic technique.



Please plug in your microincinerators.

Growth Media

- Bacteria and other microbes have particular requirements for growth.
- In order to successfully grow bacteria in lab, we must provide an environment suitable for growth.
- **Growth media** (singular = medium) are used to cultivate microbial growth.
- **Media** = mixtures of **nutrients** that the microbes need to live. Also provides a **surface** and the necessary **moisture** and **pH** to support microbial growth.
- **Tryptic Soy Agar** (TSY) is the medium that we most often use. Complex nutrient media which supports the growth of a wide variety of microbes.



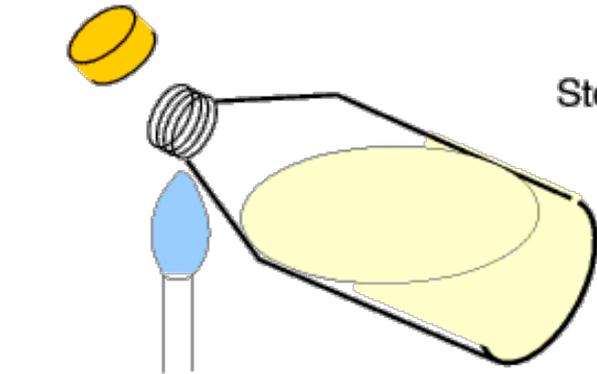
How is media made?

- When lab personnel make media they measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH**.
- They pour the media into bottles, cap it and **autoclave**.
- This is a process similar to home canning techniques in food preservation.
- The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is **autoclaved** it is considered sterile (all life forms killed).



"Pouring a Plate"

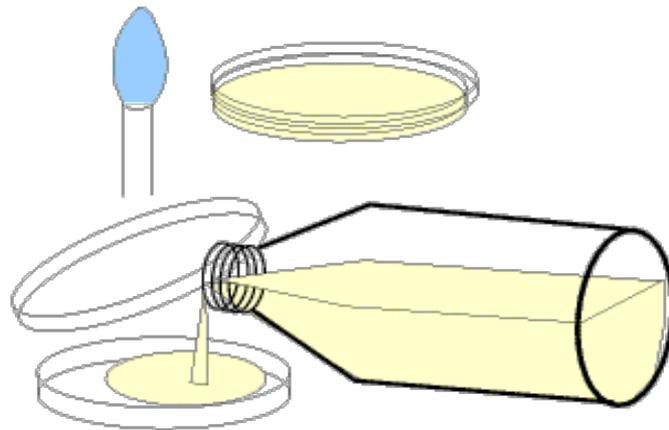
Sterilised molten agar is poured in and left to set.



Neck of agar bottle is passed through flame



Petri dish lid is opened as little as possible, angled and kept over the base.



Each Petri dish hold about 20 ml, so 200ml will do for 10.

Watch
VIDEO:

[How to Aseptically Pour
Bacterial Growth Media](#)

R.G. Steane



Labeling Plates

All Petri plates for this and future lab exercises should be labeled and stored in the following manner:

1. Make certain that all plates are labeled on the **bottom half** (i.e. the portion of the Petri plate that contains the media).
2. You can label **plastic** with a **sharpie**; **glass** with a **wax pencil**.
3. Include the following:
 - a. Your initials or identifying mark
 - b. Date
 - c. Type of specimen
4. All plates are incubated in the green storage bin (which is identified as "SAVE") in the **"upside down"** position.

"Upside down" means that the $\frac{1}{2}$ of the Petri plate with media faces up. The empty $\frac{1}{2}$ of the Petri plate is down.

We **do not** use rubber bands to hold lids in place.
(Except for the plates that you may transport home)

Plates will be incubated at 37° C for 24 hrs, then stored at room temperature until next week, when you will observe for results.



Images: Positive & negative differential reaction on [Mannitol Salt Agar](#), T. Port

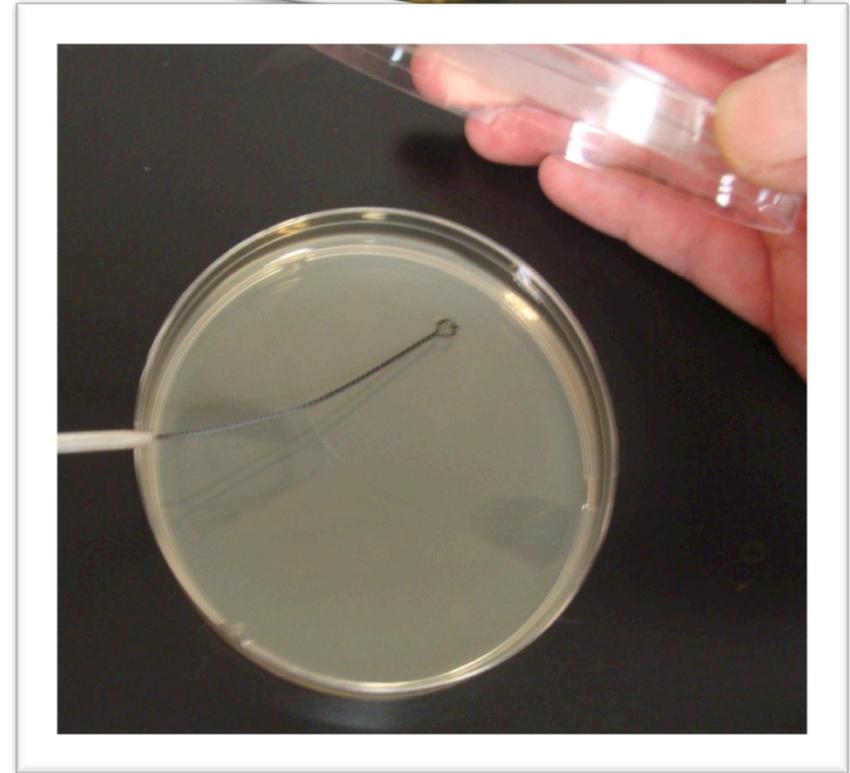


1843 - 1910

Germ Theory of Disease

Robert Koch

- Experimented with **medium** to grow bacteria on.
- He tried **gelatin**, but it did not work.
- Wife of colleague recommended **agar** (a gelatin-like product derived from seaweed).
- Didn't melt, and bacteria couldn't digest it.
- He could also add various **nutrients** necessary to grow certain organisms.
- Koch (*pronounced Coke*) originated use of a two part dish for growing bacteria (**Petri dish** named after Julius Petri, a German bacteriologist), and a technique for isolating pure **bacterial colonies**.

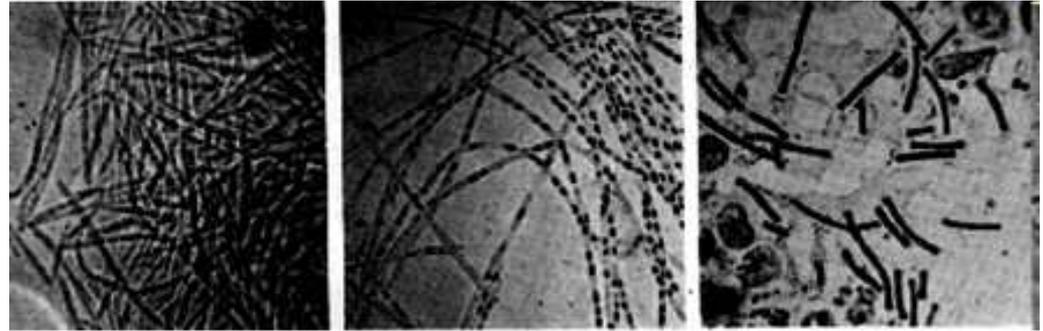


Disease, Please!



Anthrax

Gram + bacteria
Bacillus anthracis



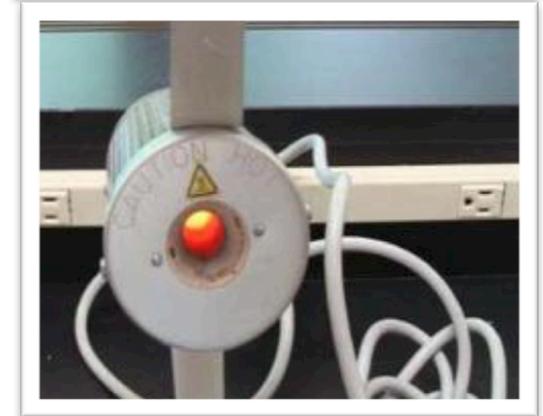
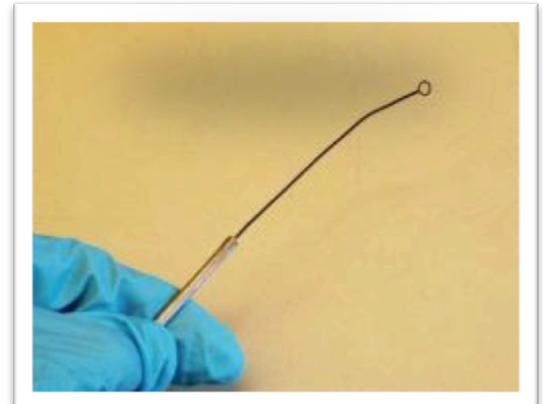
Robert Koch's original micrographs of the anthrax bacillus.

- Endospore-producing bacterium. (Genera *Bacillus* & *Clostridium* examples of endospore producing bacteria.)
- *Bacillus anthracis* first bacterium proven to be the cause of a disease.
- Anthrax was killing European livestock Farm animals, apparently healthy in the morning, might die by the end of the day, **blood turned black**. Human working closely with livestock could catch anthrax (a.k.a. wool-sorter's disease).
- In 1877, Robert Koch grew *Bacillus anthracis* in pure culture (meaning that he had to do many isolation streak plates to isolate the different types of bacteria sampled from the dead animals).
- He then produced experimental anthrax by injecting one of the isolates into animals.
- These experiments resulted in Koch formulating guidelines, called Koch's Postulates, for linking specific organisms with specific diseases.



Isolation Streak Plates & Aseptic Technique

- You'll be using an **unknown bacteria** that you will be identifying in the next lab.
- To help identify our unknown we will culture it onto [MacConkey's](#) & [Mannitol Salt](#) using **streak plate method**.
- To do a [streak plate technique](#), we will use an [inoculation loop](#) (aka smear loop, inoculation wand or microstreaker).
- Simple tool used to retrieve an inoculum from a culture of microorganisms.
- Always sterilize in [microincinerator](#) until loop becomes red hot **before** and **after** each use.
- By doing this, the same tool can be reused in different experiments without fear of cross-contamination.
- Be sure that your inoculation loop has **cooled** before using it to retrieve inoculum or to streak a plate!
- If you hear medium **sizzle** when you touch it with loop, the loop is too hot!



When obtaining a bacterial sample from a tube or plate of media do so **gently!** The bacteria is growing as a thin film on top of the media! Don't scrape so hard that you have pieces of agar in your sample!



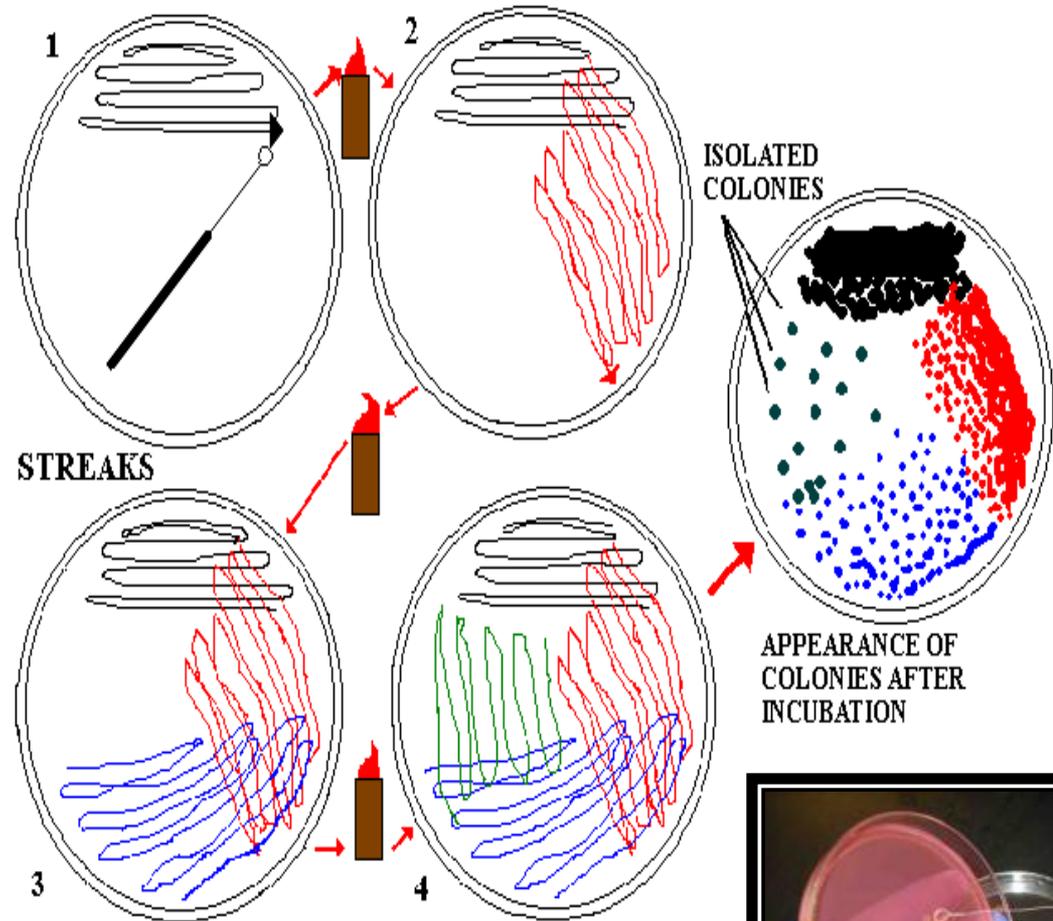
If obtaining bacterial sample from slant tubes:

- never pick up test tube by the cap.
- do NOT set cap down on lab bench
- flame neck of the test tube before & after obtaining sample.

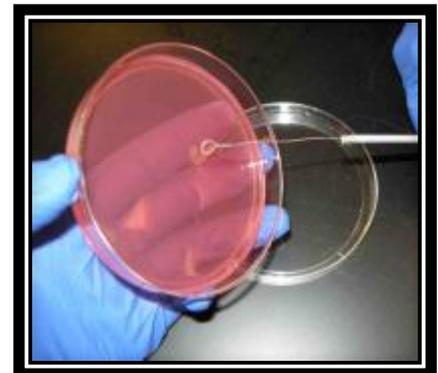
Image: [E. coli](#) growing on TSY agar in slant tube and in Petri dish, T. Port

Isolation Streak Plates & Aseptic Technique

- Streak plating is used to isolate a single type of bacteria.
- This technique spreads out original "parent bacteria" in a sparse pattern that, after growth, results in individual colonies.
- After incubation, the 4th quadrant of your plate should have dots.
- These small "dots" are individual colonies, and represent millions of bacteria of the same type.



* **IMPORTANT!!!**: Be very gentle when streaking the sample onto the plate. Try not to gouge the surface of the medium with your inoculation loop.



Discard Bin at Back of Lab



Confused?

Here are links to fun resources that further explain streak plate technique and differential staining:

- [Gram Stain & Isolation Streak Plate Technique](#) Lab Main Page on the Virtual Microbiology Classroom of [Science Prof Online](#).
- [Streak Plate Procedure](#) Animation with narration from Sinauer Associates.
- [Streak Plate](#) Interactive Animation from MSU. Test your skill to see if you can do a virtual streak plate procedure that produces isolated colonies.

Smart Links



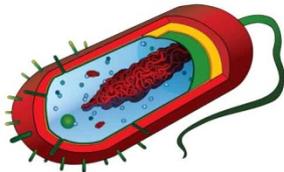


Are microbes intimidating you?

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