BIOL 3702L: Aseptic and Streak Plate Techniques

http://www.aviano.af.mil/News/Article-Display/Article/724709/mdss-airmen-ensure-success-through-support/

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Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the basic tenets of aseptic technique;
- Correctly use a sterile inoculating loop and needle;
- Perform the transfer of microbes from one medium to another without contamination; and
- Isolate colonies of individual microbes via the streak plate method.

https://www.shutterstock.com/video/clip-537799-stock-footage-researchers-working-in-lab-with-microscope-close-up.html

2

Today's Techniques

- Your success in this laboratory course is directly dependent upon mastering:
  - Aseptic technique
  - Streak plate isolation of pure culture
- You must learn to handle microbial cultures without introducing contamination.
- The ability to isolate pure cultures by streak plating is essential to identifying microbes.
- **BE METICULOUS AND DILIGENT IN PEFORMING BOTH METHODS!!!**

https://i.ytimg.com/vi/bRadiLXkqoU/maxresdefault.jpg

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Aseptic Technique

- The following videos provide excellent overviews of proper aseptic transfer.
- The first demonstrates aseptic transfer using a Bacti-Cinerator.
- NOTE: A Bacti-Cinerator must be allowed to "warm up" for 10 minutes or so before being used to sterilize loops/needles.

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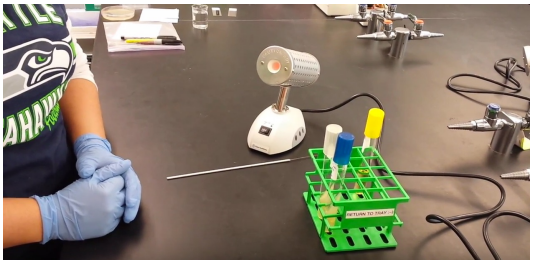
Aseptic Transfer Using a Bacti-Incinerator



https://youtu.be/78Ky7e_du2c

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Aseptic Technique (cont.)

- The second video demonstrates the proper use of a Bunsen burner for sterilizing transfer loops.
- **USE EXTREME CAUTION!!!** BE AWARE THAT A PROPER BUNSEN BURNER FLAME IS LIGHT BLUE IN COLOR TO ALMOST NOT VISIBLE!!! (Take note in this video.)
- In BIOL 3702L, it is preferred that use of a Bunsen burner be kept to a minimum.

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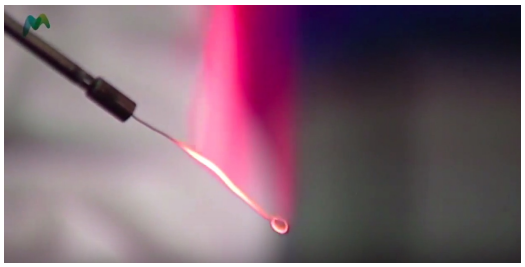
Aseptic Transfer Using a Bunsen Burner



https://youtu.be/-3AX6MYGqms

and proud.

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Aseptic Technique (cont.)

- Key points to being successful in aseptic technique:
  - NEVER remove and set a cap on the bench. This invites contamination.
  - NEVER carry a tube by its cap. The caps may not be tightly fitted to the tube.
  - NEVER turn a tube containing broth at such an angle that the broth reaches the cap. Caps may not be tightly fitted to the tube.

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Aseptic Technique (cont.)

- Key points (cont.):
  - NEVER shake a tube containing broth. Again, caps may not be tightly fitted to the tube.
  - Learn dexterity by handling one or more tubes, caps, and the inoculating loop/needle with both hands and not setting any of these items on the bench top.

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Streak Plate Technique

- The streak plate technique is a type of "dilution" gradient method that physically separates cells from one another in a mixture.
- The section initially streaked should, unsurprisingly, result in confluent growth.

and proud.

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Streak Plate Technique (cont.)

- As this growth is diluted by using proper technique, the amount of confluent growth lessens.
- An assumption is made that one colony is derived from a single cell.

and proud.

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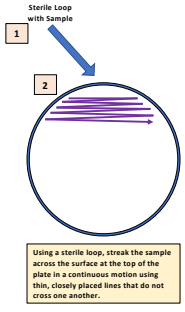
Three-Phase Technique



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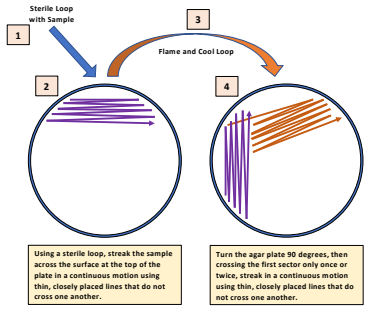
Three-Phase Technique (cont.)



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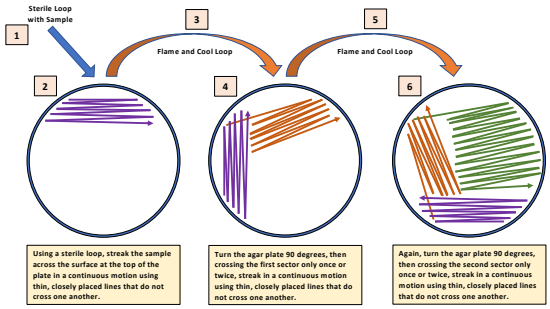
Three-Phase Technique (cont.)



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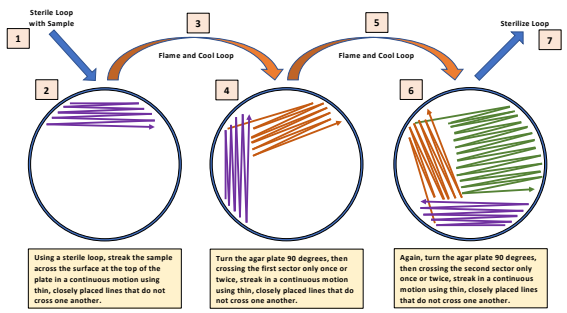
Three-Phase Technique (cont.)



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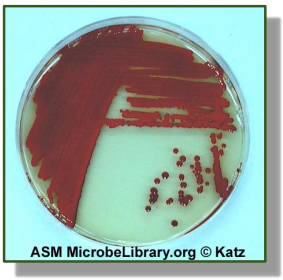
Streak Plate Technique (cont.)

- The following video demonstrates how to perform a proper streak plate using a type of "three phase" technique.



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Making a Streak Plate

https://youtu.be/pxqF-5QibQk

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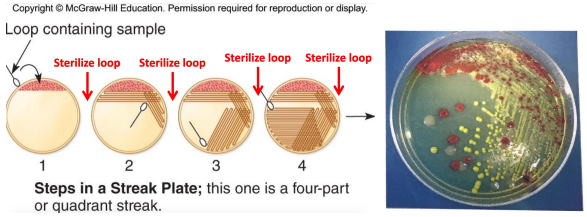
Streak Plate Technique (cont.)

- Some microbiologists use four quadrants.



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Streak Plate Technique (cont.)

- The number of quadrants used is not important, but these key elements are:
  - Use the entire surface of the agar plate;
  - Use the tip of the loop – do not use it lying flat on the agar surface;
  - Keep the streak lines "tight", i.e., close together;
  - Do not cross over a prior quadrant more than once or twice; and
  - Be sure to sterilize the loop between streaking a new quadrant.

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Today's Exercise

- ***Each person*** must perform the following selected portions of today's exercise:
  - Perform the following
    - Part B: Practice Sterilizing a Loop/Needle by Using the Bacti-Cinerator
    - Part D: Aseptic Transfer from Broth to Broth
    - Part E: Aseptic Transfer from Broth to Agar Slant
    - Part G: Isolation of Colonies from an Agar Plate
  - DO NOT PERFORM PARTS A, C, AND F: These methods are nonetheless important, so be sure to review them for possible future use.

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Today's Exercise (cont.)

- Aseptic Transfer
  - Part A – Do not perform
  - Part B – Practice sterilization of a loop/needle using a Bacti-Cinerator
  - Part C – Do not perform
  - In the following order, use a loop to transfer *Chromobacterium violaceum*, *Escherichia coli*, and then *Staphylococcus aureus* from TSB cultures to:
    - (Part D) fresh un-inoculated TSB .
    - (Part E) fresh un-inoculated TSA agar slants.

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Today's Exercise (cont.)

- Aseptic Transfer (cont.)
  - Part F – Do not perform
  - Part G – In the following order, use a loop to transfer *Chromobacterium violaceum*, *Escherichia coli*, and then *Staphylococcus aureus* from a streak plate onto fresh un-inoculated TSA agar slants.

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Today's Exercise (cont.)

- Aseptic Transfer (cont.)
  - Incubate all tubes at 37°C for 24-48 hours (the specific incubation location shall be assigned by the laboratory instructor)
  - Return and make observations; record results as appropriate

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Today's Exercise (cont.)

- Streak Plate
  - Using the the TSB mixed culture of *Escherichia coli* (white colonies), *Chromobacterium violaceum* (purple colonies), and *Staphylococcus aureus* (golden/yellow colonies), perform your best streak technique on two separate TSA plates.
  - Incubate this TSA plate at 37°C for 24-48 hours, *then refrigerate as directed.*
  - Your laboratory instructor will provide constructive criticism.

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Lab Report Expectations

- From both assigned exercises, print the relevant report pages.
- Record/draw your observations as indicated.
- Answer ALL Discussion Questions for ***those assigned parts*** of the exercises. Where appropriate, USE CLEAR, CONCISE, AND GRAMMATICALLY SOUND SENTENCES!

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