BIOL 3702L: Smear Preparation and Simple Staining

YSU

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

1

Today's Techniques

- The purpose of today's laboratory exercise is three-fold:
  - To gain experience in preparing smears of the appropriate density;
  - To learn how to properly stain cells with a simple dye so that they can be visualized under the microscope; and
  - To perform simple staining to assess cell morphology.

https://embryology.med.unsw.edu.au/embryology/index.php?title=File:Bacteria_-_gram-stained_vaginal_smear_01.jpg&

Y and proud.

2

Smear Preparation

- Making a heat-fixed smear is more of an "art" than it is science.
- The goal is to make a smear that is dense enough, but not too dense, to permit both cell visualization and proper staining
  - Too dense – hard to distinguish cells and staining patterns can be affected. AVOID MAKIG DENSE SMEARS!!!
  - Too disperse – difficult to find cells under the microscope

Y and proud.

3

Three Smears of Differing Thickness

(marked by white wax ring)

- Left and right smears: **too thick**
- Center smear: **correct thickness**

https://hicksmicro.blogspot.com/p/staining-problems.html

4

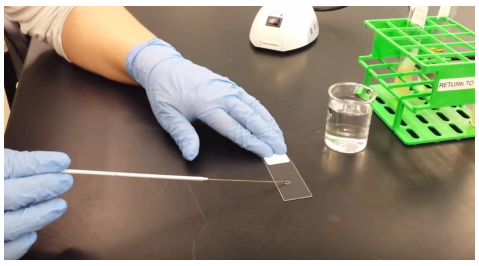
Smear Preparation (cont.)

- Two types of cultures can be used to make smears and each requires slightly different approaches
  - Solid cultures – aseptic transfer of a very *small amount* of cells to a *small volume* of water on the surface of a microscope slide
  - Broth cultures – aseptic transfer of a one or more drops of culture fluid spread out on the surface of a microscope slide

Y and proud.

5

Making a Bacterial Smear – Solid Culture

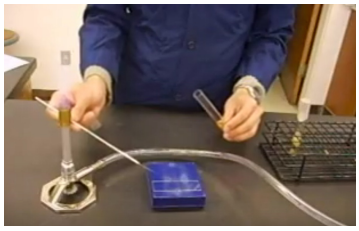


https://youtu.be/gM5_Y2QgXq0

Y and proud.

6

Making a Bacterial Smear – Broth Culture



https://youtu.be/HxjN2mBLGCE

What obvious error is being committed during this procedure?

and proud.

7

Smear Preparation (cont.)

- Following the preparation of the smear and allowing it to air dry, the cells must be heat-fixed by one of the following methods:
  - (PREFERRED METHOD) Pass the slide through the top blue portion of the Bunsen burner flame – three to six passes at no more than one second each
  - Place on a hot plate set to the highest temperature for no more than three seconds

USE EXTREME CAUTION WITH HEAT SOURCES!!!

and proud.

8

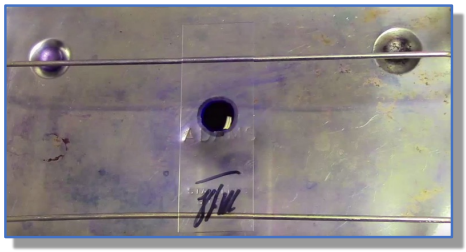
Smear Preparation (cont.)

- Once heat-fixed, the smears are now ready for staining
- **STAINING MUST TAKE PLACE ONLY ON THE PROVIDED RACKS SET OVER THE BENCH SINKS!!!** (This is one of the few times that chemical reagents are permitted to flow into the sinks.)

and proud.

9

Simple Staining of a Bacterial Smear



https://youtu.be/n5fXIpJUgD4

and proud.

10

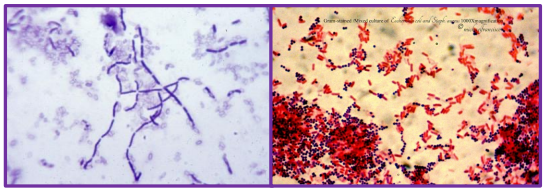
Staining Slides

- Stains can be used not only to visualize cells and distinguish cell shape, but with the appropriate staining procedure (e.g., Gram stain), distinct cell types can be observed.
- Simple staining involves a single dye placed on a smear, then washed away prior to observation under a microscope.
- The types of dyes and the length of staining times differ with a given procedure.

and proud.

11

Staining Slides (cont.)



Simple stain (left) and Gram stain (right)

https://www.studyblue.com/notes/note/n/microbio-lab-practical/deck/3837183
and https://www.flickr.com/photos/micodude/6500465759

and proud.

12

Today's Exercise

- Smears from solid cultures:
  - On two (2) separate slides, place a small drop of water from the bench-top water bottle. Use these slides to prepare a single smear each of
    - *Staphylococcus aureus* from a TSA slant culture
    - *Escherichia coli* from a TSA slant culture
  - Allow the slides to dry, then heat fix both slides.

13

Today's Exercise (cont.)

- Smears from a broth culture:
  - Completely suspend the cells from the TSB mixed culture of *Escherichia coli* and *Staphylococcus aureus* by rolling the tube between your hands
  - Prepare a smear from the TSB mixed culture.
  - Allow to dry, then heat fix the slides.

14

Today's Exercise (cont.)

- Staining with Crystal Violet (CV):
  - Place the heat-fixed slides on the staining rack
  - Flood the area of the smear on each slide with CV; allow the CV to remain on the slides for 60 to 90 seconds
  - Drain the CV from the slides and briefly wash (3-4 seconds) the slide with water from the bench-top bottle.

15

Today's Exercise (cont.)

- Staining with Crystal Violet (CV): (cont.)
  - Drain the water from the slides and carefully blot the slides dry using **PAPER TOWEL**. (Bibulous paper is not available.)
  - Examine the slides under oil immersion and record your observations on the exercise data sheet. NO COVERSLIP IS NEEDED!!!!

16

Today's Exercise (cont.)

- Special Notes/Hints:
  1) All staining can be done at the same time.
  2) Slides that are heat-fixed or stained can be stored in slide boxes for later processing.
  3) To make a smear from a broth culture, the number of loopfuls is dependent upon the density (turbidity) of the culture.

17

Today's Exercise (cont.)

- Special Notes/Hints: (cont.)
  4) Broth cultures that have sat for a time should be thoroughly mixed by rolling the tube between your palms or sharply thumping the tube several times.
  5) No coverslips are needed on fixed, stained smears. To observe cells, place the immersion oil directly on the smear.

18

Lab Report Expectations

- Be sure to complete laboratory worksheet.
- Retain this lab report. Your laboratory instructor may call for its submission in the near future.

and proud.

19

20