Staining

Isolation of Bacteria

- Most bacterial samples have numerous different bacterium
- Identification requires testing on an individual type of bacteria
- Two methods for isolating are the Streak plate and the Spread plate

Streak Plate

- Transfer bacteria to a small area of agar plate
- Get increasingly smaller amounts of bacteria on successive sections of the plate by sterilizing the loop and spreading the previous area
- When you get a small enough quantity of bacteria in an area, they will be able to grow in individual colonies

Streak Plate *continued*

- Objective: isolate single colonies of bacteria from a mixed culture
- Draw procedure diagram off of board
- Flame loop between each quadrant, but do NOT dip the loop back in the broth tube
- Each student will do their own. This is worth 3 points (1 pt labeling, 1 pt technique, 1 pt isolation)

Spread Plate

- Transfer a big drop of bacteria to the plate, then spread in all over
- Series of dilutions required to get a sample with few enough bacterial cells to produce individual colonies.
  - We will not perform the dilutions in this lab. We will just learn the spreading technique.

Spread Plate *continued*

- Objective: isolate single colonies of bacteria from a mixed culture (or at least learn the technique that you would use)
- After transferring a couple of drops mixed culture to the agar plate, sterilize spreader
  - Dip in ethanol
  - Flame (do NOT dip back in ethanol)
  - Let cool

Stain Categories

- Morphological – size, shape, arrangement
  - Simple stain and Negative stain
- Differential – cell wall composition
  - Gram stain and Acid-fast stain
- Structural – cell structures
  - Endospore stain and Capsule stain

Stains

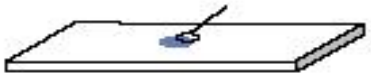
- Basic stains (+) – react with acidic (-) parts of the cell
  - ex. crystal violet, safranin, methylene blue
    - i.e. stains that get inside the cell
- Acidic stains (-) – are repelled by the negatively charged cell surface
  - Ex. Congo red and india ink
  - Stains the background, not the cells

Simple Stain

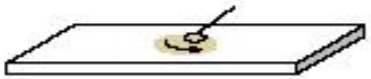
- Objective: Determine morphology and arrangement
- All bacteria will be stained
- Make a smear prep
  - Method of getting bacteria adhered to the slide
  - see next slide for procedure
- Each pair of student will do a simple stain on *M. luteus*

Smear Prep

Procedural Diagram
Bacterial Smear Preparation

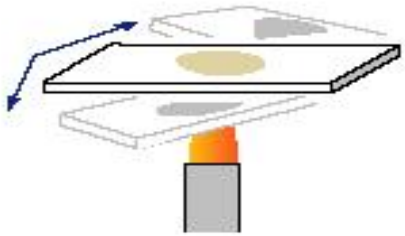


1. Place a small drop of water (not too much) on a clean slide using an inoculating loop.



2. Aseptically add bacteria to the water. Mix in the bacteria and spread the drop out. Avoid spattering the emulsion as you mix. Flame your loop when done.

3. Allow the smear to air dry. If prepared correctly, the smear should be slightly cloudy.



4. Using a slide holder, pass the smear through the upper part of a flame two or three times.This heat-fixes the preparation. Avoid overheating the slide as aerosols may be produced.

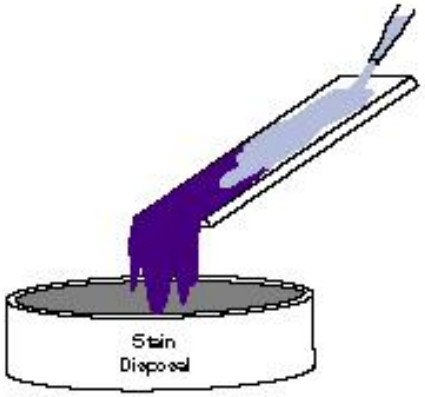
5. Allow the slide to cool, then continue with the staining protocol.

Simple Stain

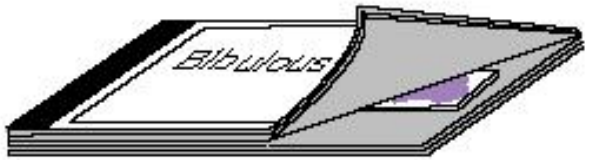
Procedural Diagram
Simple Stain Preparation

1. Begin with a heat-fixed emulsion.

2. Cover the smear with stain.
Use a staining tray to catch excess stain.



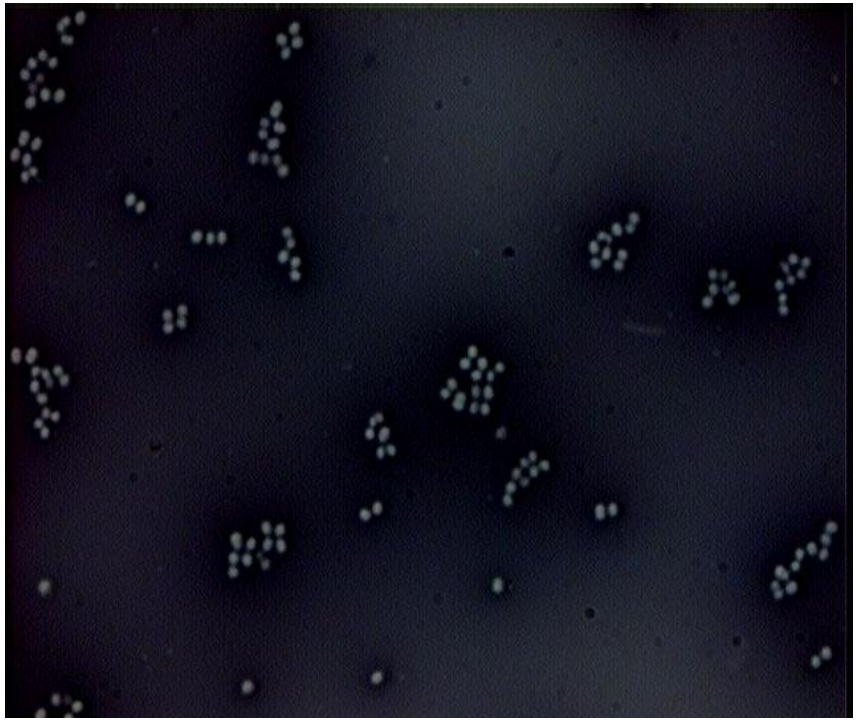
3. Grasp the slide with a slide holder.
Rinse the slide with water.
Dispose of the excess stain
according to your lab practices.



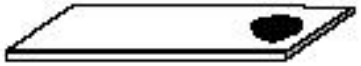
4. Gently blot dry in a tablet of bibulous paper or paper towels. Do not rub.
Observe under oil immersion.

Negative Stain

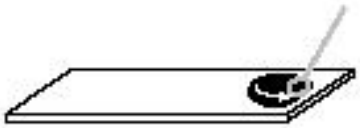
- Objective: Determine morphology and arrangement
- India ink used to stain background, not cells
- Gives a good view of morphology
  - Not heat fixed, so cells are not distorted
- Prepare negative stain with *M. luteus*



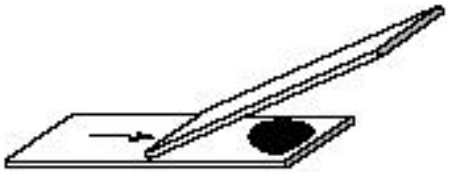
Procedural Diagram
Negative Stain



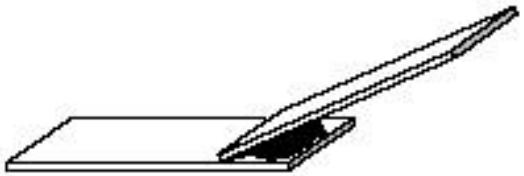
1. Begin with a drop of acidic stain at one end of a clean slide.



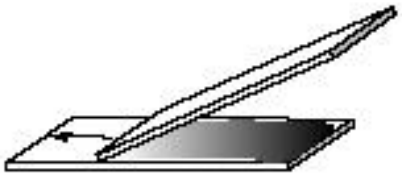
2. Aseptically add organisms and emulsify with a loop. Do not over-inoculate and avoid spattering the mixture. Sterilize the loop after emulsifying.



3. Take a second clean slide, place it on the surface of the first slide, and draw it back into the drop.



4. When the drop flows across the width of the spreader slide...

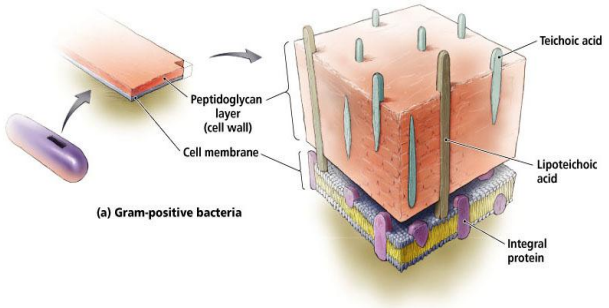


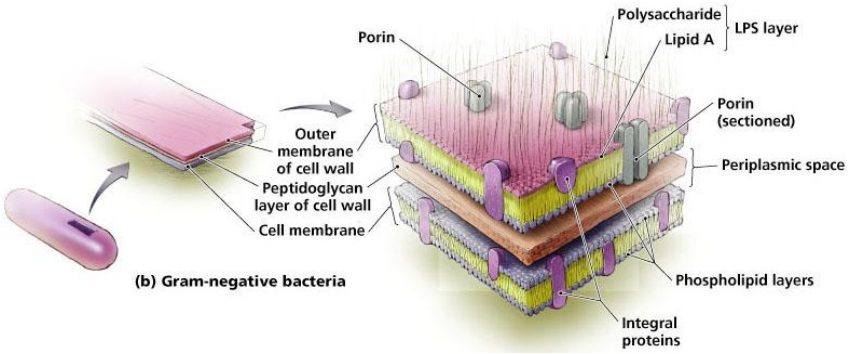
5. ...push the spreader slide to the other end. Dispose of the spreader slide in a jar of disinfectant or Sharps container.

6. Air dry and observe under the microscope. Do NOT heat fix.

Gram Stain

- Objective: determine if bacteria is gram positive or negative
- Initial procedure to determine unknown bacteria
- Takes advantage of differences in cell wall composition (differential stain)
  - Gram positive has thick layer of peptidoglycan
  - Gram negative has thin layer of peptidoglycan

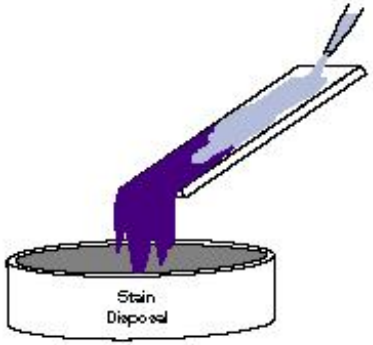




Procedural Diagram
Gram Stain Preparation

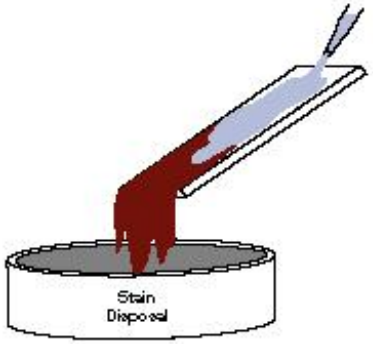
1. Begin with a heat-fixed emulsion.
(See Fig. 3-35.)

2. Cover the smear with Crystal Violet stain for 1 minute.
Use a staining tray to catch excess stain.

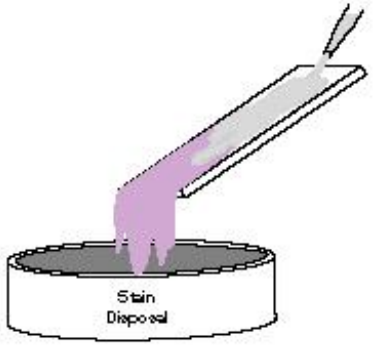


3. Grasp the slide with a slide holder.
Gently rinse the slide with distilled water.

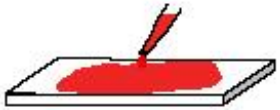
4. Cover the smear with Iodine stain for 1 minute.
Use a staining tray to catch excess stain.



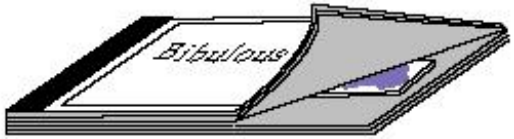
5. Grasp the slide with a slide holder.
Gently rinse the slide with distilled water.



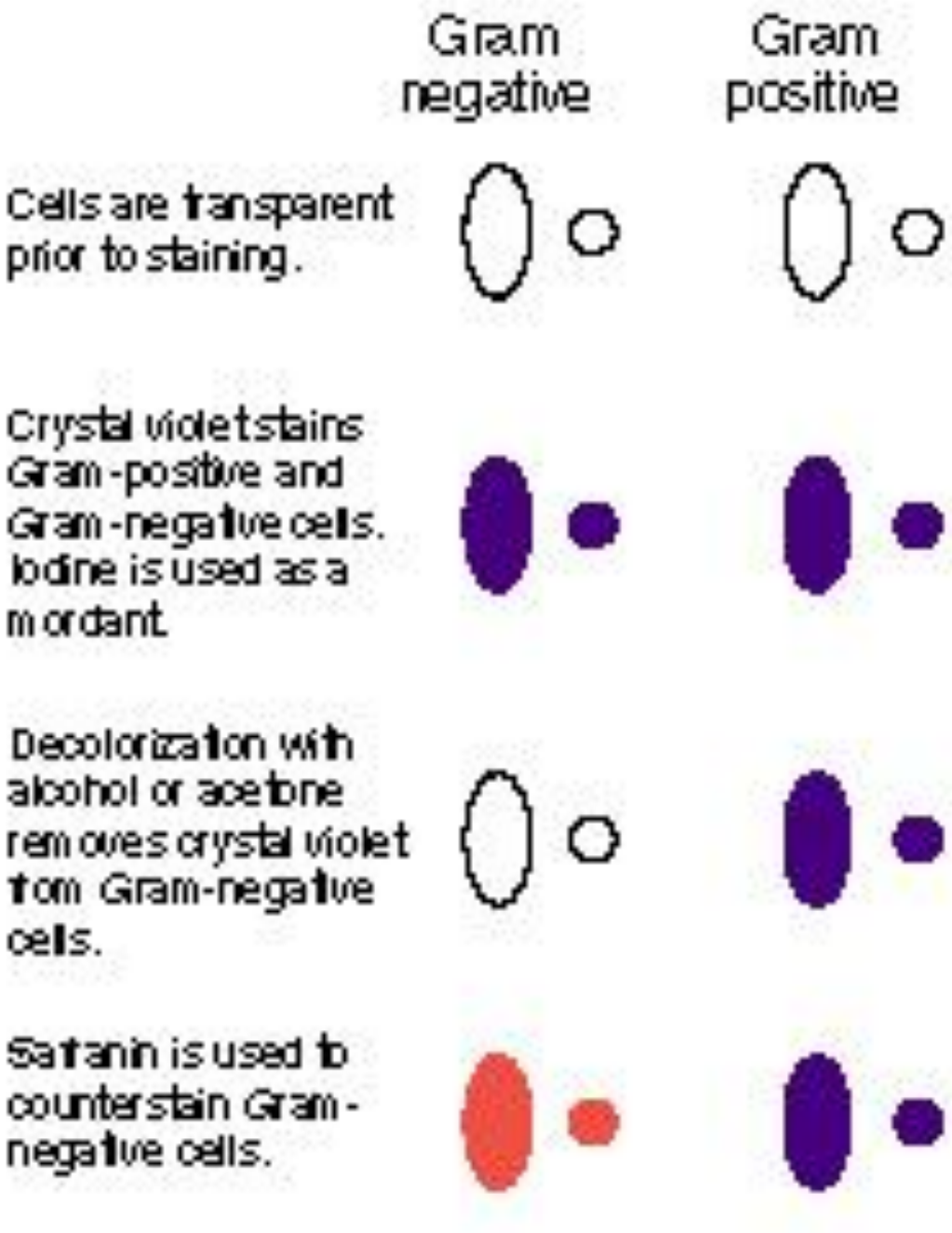
6. Decolorize with 95% ethanol or ethanol/acetone
until the run-off is clear.
Gently rinse the slide with distilled water.



7. Counterstain with Safranin stain for 1 minute.
Rinse with distilled water.



8. Gently blot dry in a tablet of bibulous paper.
Do not rub.
Observe under oil immersion.


Gram negative
Gram positive
Cells are transparent prior to staining.
Crystal violet stains Gram-positive and Gram-negative cells. Iodine is used as a mordant
Decolorization with alcohol or acetone removes crystal violet from Gram-negative cells.
Safranin is used to counterstain Gram-negative cells.

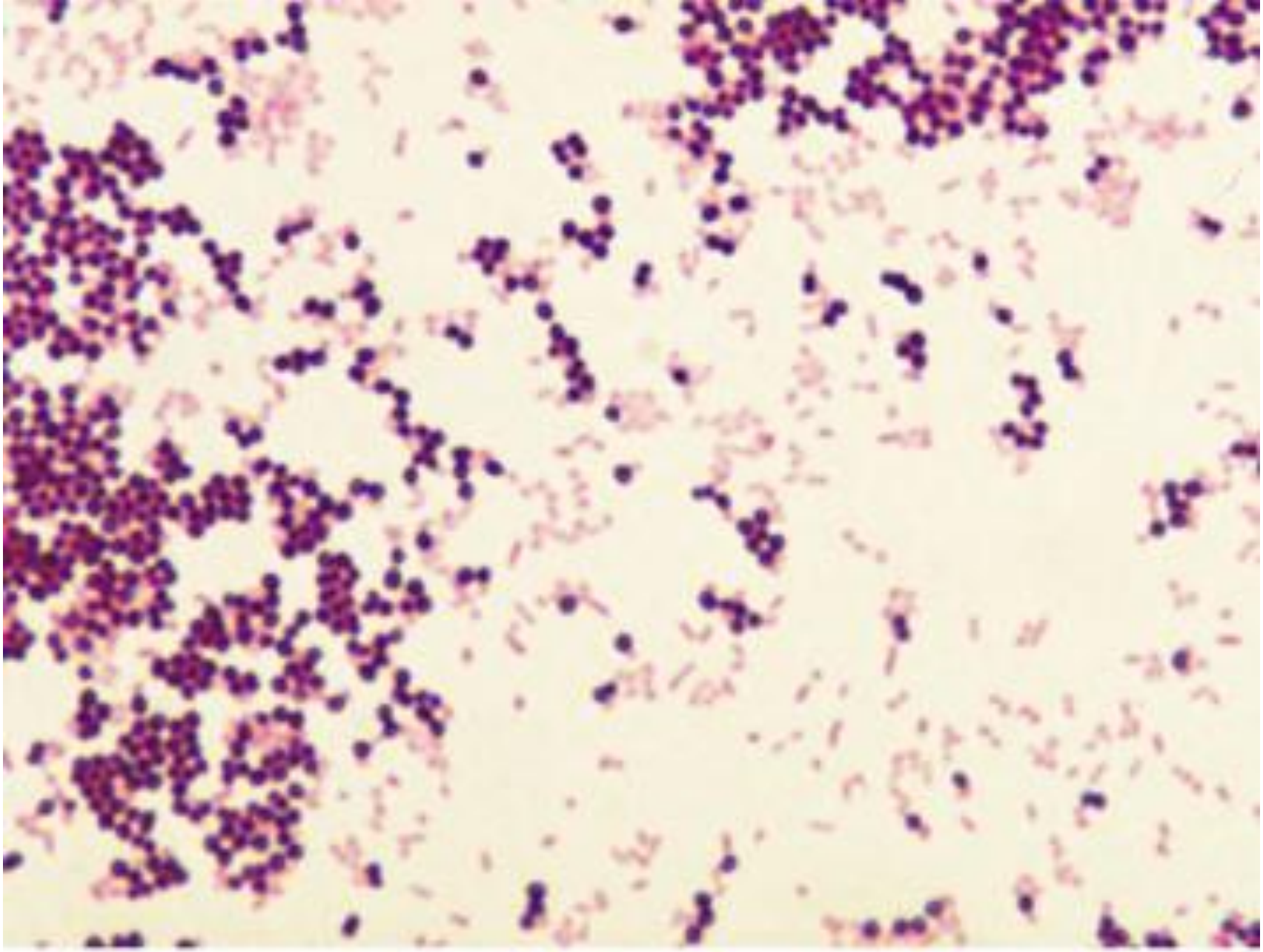


Figure 3-41

Gram Stain

- Gram stain:
  - *E. coli*
  - *S. aureus*
  - *E.coli/S. aureus* mixture
- Remember, next week you will be doing a gram stain for points, so get it down today!