Gram staining protocol

How might we identify bacteria?

Background

Discovered in 1884 and named after bacteriologist Hans Christian Gram, Gram staining is a tried and true method of identifying an unknown and/or unidentified bacterial species. After a sequence of stain, destain, and counterstain, different bacterial cell walls will retain different stains, leaving us with Gram-positive (purple) and Gram-negative (pink) cells.While all bacterial cell walls contain peptidoglycan, a complex, cross-linked polysaccharide, there is a difference between the amount of peptidoglycan in different types of bacteria. There is also the presence or absence of an outer membrane, which is a difference between bacteria classified as either Gram negative or Gram positive. A common practical application of Gram staining remains the ability to quickly distinguish bacteria classification present in an infection, and potentially determine an appropriate antibiotic to prescribe.

Materials

crystal violet
distilled water
wooden clothespins (optional)
ethyl alcohol, 95%
disposable pipettes
gram iodine (Lugol's)
bunsen burner
wax pencil (china marker)
safranin
compound microscope
microscope slides
inoculating loop

Heat-Fixing Bacterial Slides

1. Using the wax pencil, create two different circles on your slide, each about the size of a pencil eraser.
2. Label 1 circle with a minus sign, and the other as "mix"
3. Using a sterile inoculating loop, select a colony of E. coli bacteria from a plate and smear a thin layer of the colony onto the "-" circle microscope slide.
4. Allow 5 minutes to air dry, smear and then heat-fix slide by passing the slide, smear side up over a candle or Bunsen burner five or six times.
5. Repeat steps 3-4 utilizing the liquid of a greek yogurt onto the "mix" circle.

Staining Bacterial Slides

1. Apply crystal violet stain one drop at a time to both circles on the slide until you have a bead that covers the whole circle then allow it to stand for 1 minute. Gently dip into beaker of clean water to remove excess stain or rinse slide over a SLOW running faucet (do not put stream directly onto bacteria circles).
2. Apply iodine (fixes the dye) one drop at a time to both circles on the slide until you have a bead that covers the whole circle then allow it to stand for 1 minute. Gently dip into beaker of clean water to remove excess stain or rinse slide over a SLOW running faucet (do not put stream directly onto bacteria circles).
3. Decolorize slides with 95% ethyl alcohol by applying 1 drop at a time until no more color runs off (approx. 30 seconds). Gently dip into a beaker of clean water to remove excess stain or rinse slide over a SLOW running faucet to stop the action of the alcohol decolorizer.
4. Apply safranin counterstain one drop at a time to both circles on the slide until you have a bead that covers the whole circle then allow it to stand for 30 seconds. Gently dip into a beaker of clean water to remove excess stain or rinse slide over a SLOW running faucet (do not put stream directly onto bacteria circles). Blot slide dry with paper towel and view under microscope.
    1. Gram-**p**ositive bacteria will appear to be stained **p**urple color
    2. Gram-**n**egative bacteria will appear pi**n**k in color.

A Closer Look at Insects

Gram Staining Lab Assignment

1. What is a Gram stain?

2. What are the two differences between Gram negative and Gram positive bacteria cells?

3. What was the order of the stains we used in the Gram stain lab?

    1)
    2)
    3)
    4)

4. What stain was removed from the 'decolorizer' step (ethanol/alcohol)?

5. Observe your slides from the lab on the microscope.
    What color are your *E. coli* cells?

    What color are the yogurt bacteria cells?

6. Draw the membranes and cell walls of these cells below: (a google image search will suffice)

Gram positive	Gram negative

7. It is understood that Gram negative cells are more pathogenic (disease causing). Form a hypothesis as to why this is the case.

8. From a google search, find two Gram positive and two Gram negative types of bacteria that are found in our microbiome and add to the table below.

Gram status	Bacteria species	Location(s) on our body
+		
-		

9. What are the applications of gram staining?