

Lab 2. Skin Microbiome and Plate Culture. S&F Ch. 2, pp 41-59; Ch. 4; Belkaid & Segre (2014).

The human body contains many “normal biota,” microbes that normally coexist with us and aid the function of our own bodies. Some species occur normally on all of us; but in addition, different individuals harbor many diverse strains. We will culture bacteria from our own skin epithelium. We will streak-plate three known species of bacteria. We will observe bacteria under a microscope using Gram Stain and wet mount.

- **Assume all bacteria could be pathogenic.**
- **If you are immunocompromized: Inform instructor before class.**

A. Skin sample. Perform in the restroom across from HIG 322.

- To obtain specimen: Swab a region of skin with swab soaked in saline solution (0.09 M NaCl).
- Smear swab back and forth across a lactose MacConkey agar plate (Mac), and on Tryptic Soy Agar (TSA). Look up MacConkey: What are the composition and use of this medium?
- Perform a control by smearing untouched saline on Mac and TSA plates.
- Incubate skin plates and controls at 37°C.

B. Streak plates. Observe demonstration of three-streak procedure. **Remember, after culture loop pulls bacteria from one streak, never go back.** Streak back and forth to disperse unseen bacteria.

- Obtain shared plate cultures of *Bacillus megaterium*, *Salmonella enterica*, and *Micrococcus luteus*. For each species, use three-streak procedure to inoculate Mac plate and TSA plate.
- Incubate all streak plates at 30°C. Include controls (mock inoculation, without bacteria).
- Pour plates for future use. Perform with Ms. Blankenhorn, at some point during the lab period. Obtain 250-ml flasks of liquid agar, recently sterilized in autoclave. Obtain stack of four sterile petri plates. Pour agar, till bottom surface just covered. Let stand.
- Scoop dust into an extra TSA plate. Incubate on your lab bench till next week.

C. Bacteria observation. Photograph all organisms at 1000X with immersion oil. **Use oil ONLY for 1000X. Never observe 1000X without oil. Never get oil on other lenses.** State full magnification for each sketch, and estimate cell sizes based on micrometer conversion.

- Stage Micrometer: Observe and photograph at 400X, and at 1000X (oil). Determine number of pixels per micrometer (µm).
- Obtain drop of water on slide, then toothpick tip of *Micrococcus luteus*. Perform a Methylene blue stain as demonstrated in class: Air dry; methanol, air dry; methylene blue, 60 sec; water rinse; air dry.
- Perform a Gram Stain on *Bacillus megaterium*, *Salmonella enterica*, and *Micrococcus luteus* (procedure on back). State color and size for each species, and whether you interpret it as Gram-negative or Gram-positive.
- Attempt to observe and photograph live wet mount of each species (drop of water with bacteria on toothpick).

Gram Stain Procedure

1. From a plate colony, smear a tiny amount of bacteria on the slide, in one drop of water. (Skin specimen in saline does not need additional water.) Let specimen air dry, on slide tray.
2. Add methanol to “fix” the specimen onto the slide (methanol denatures proteins, exposing reactive groups to attach the glass.) After 1 minute, the methanol is drained off, and the slides are air-dried.
3. Flood the slide with crystal violet. Allow to remain 30 seconds. Rinse with water. Caution: Squirt water away from the specimen, then allow to drain through for about 10 sec. Avoid over-rinsing, because crystal violet is not bound to the cell until Gram’s iodine is added.
4. Flood the slide with Gram’s iodine. Allow to remain 30 seconds, then rinse with water.
5. Decolorize the slide with decolorizer (alcohol) for 15 seconds. Rinse with water, about 10 sec. Caution: Avoid prolonged decolorization or excessive rinsing. These can wash the crystal violet-iodine complex from gram-positive cells.
6. Flood the slide with safranin for 30 seconds. Rinse with water, about 10 sec. Blot with bibulous paper (do not rub), then allow to air-dry completely before using microscope.

Friday afternoon:

- Observe skin plates and streak plates. Line them up and photograph together (using cell phone camera). Label plate information in your report.
- Where do you see isolated colonies? What species, do you think? If colonies were not isolated, how might your technique be improved?
- What do you conclude from comparing your MacConkey results with your Gram stain? What about the color of the colonies?
- Explain how Gram stain may be affected by (1) species; (2) environment or growth condition of source.
- Crop and/or downsize photos as needed for your report.
- Use Word, Insert, Textbox to label interesting features.

Reference

Yasmin Segré and Julia A. Belkaid. 2014. Dialogue between skin microbiota and immunity. *Science* 346: 954-959.