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- Future Beltsville-NPDN diagnostician training.
- Lab accreditation progress.
- National meeting update.
- Lepidoptera workshop in the planning stage.

The next conference call will be held on July 17, 2008.

Diagnostic Tip of the Month: Obtaining Pure Bacterial Colonies and an Alternative to the Gram Stain

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Starting with an axenic bacterial culture is a requirement for any sort of valid identification work. Individual colonies can look pure but actually be a mixture of different isolates of entirely different genera that grow at different rates – the contamination may not show up for weeks, but by then the plates have usually been thrown out.

In our lab, we work with a lot of slow growing bacteria and it is essential that we get good separation of colonies when we streak plates. To reduce the amount of contaminating and competing bacteria, we have adopted a technique that quickly allows us to obtain well separated pure bacterial colonies.

The method is simple: we add a water dilution step each time we re-streak our isolates. Specifically, from a colony we desire to purify, we will touch our flamed and cooled inoculation loop to the colony to pick up cells, and then dislodge the bacteria into a tube of 3 ml sterile deionized water. We then vortex the tube, and streak out as usual doing a standard dilution streak. We do this a total of three times, and usually by the last streak we have well separated pure colonies.

In the rare instance where the colonies still appear mixed, we will continue with the water/dilution streaks until a single colony type is present. We can verify purity when we do the Gram stain, as mixed cultures are often indicated by different morphologies.

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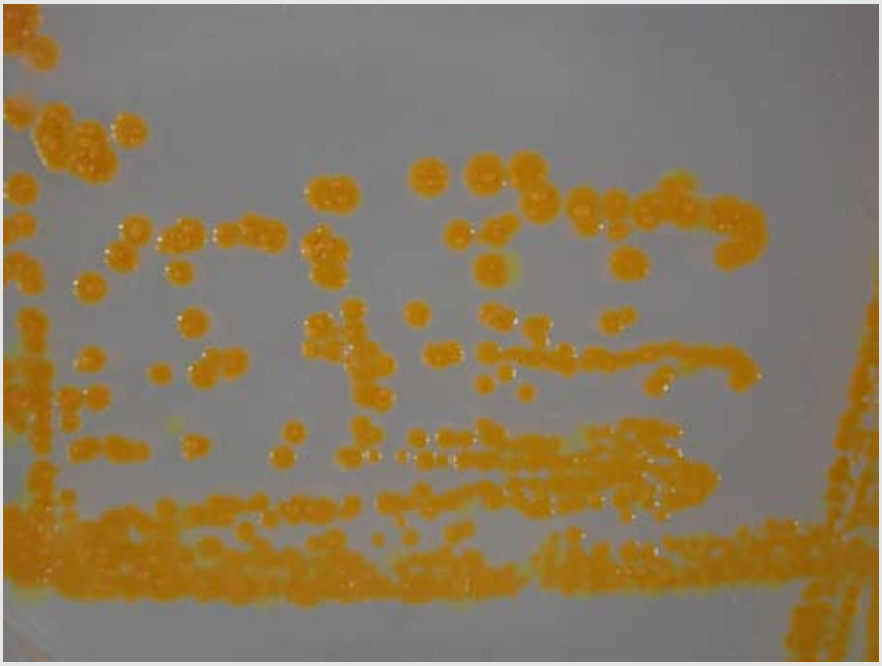

Close-up of a mixed bacterial colony. Photo Melodie Putnam

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For those of you who don't like to do Gram stains, there is another method that can be used to check the Gram reaction, which uses KOH instead of dyes. This test (as does the Gram stain) relies on the differences in the cell wall chemistry between the two types of bacteria. Gram negative cell walls are lysed by 3% KOH and release their contents, including the DNA. The DNA/KOH mixture is viscous and will form a thin, mucoid strand when a needle or other slender object touches and then is pulled away from the disrupted cell mass (photo). Gram positive cells are not affected by the dilute alkali solution and will not lyse, hence no viscous string will form.

This test requires a visible number of cells (a good clump), a sterile toothpick or inoculating loop, a clean slide, and 3% KOH. Cultures should be less than five days old (Halebian, et al., 1981).

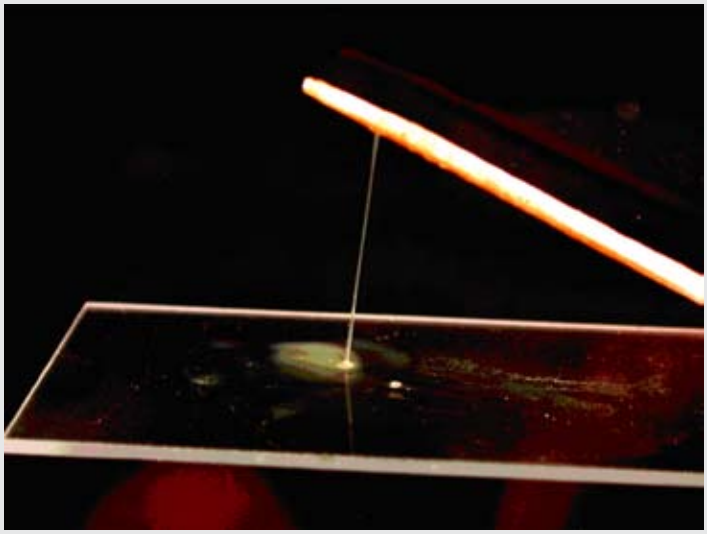
The procedure:

1. Place two drops of 3% KOH on a clean glass slide. Slides with wells in them work well for this.
2. Pick up a load of bacteria from a culture plate using a sterile toothpick or flamed and cooled inoculating loop.
3. Mix the bacteria in the KOH by stirring, and lift the toothpick or loop 1-2 cm from the slide after about 15 seconds.
4. Production of a viscous string within 30 seconds is indicative of Gram negative bacteria. Gram positive bacteria will not form a viscous slime.

NOTE: This assay is not reliable for characterization of certain anaerobic Gram negative bacteria (e.g. species of *Bacteroides*, *Fusobacterium*, *Leptotrichia*, and *Veillonella*). Other methods should be used for typing anaerobic bacteria.

Reference cited

Halebian, S., Harris, B., Finegold, S.M., and R.D. Rolfe. 1981. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. Journal of Clinical Microbiology 13:444-448.



A viscous string of slime from Gram negative bacterial cells treated with a few drops of 3% KOH. The cells were added to the KOH on a slide, and mixed with a sterile toothpick. Gram positive cells will not form this type of ropy slime. Photo Melodie Putnam