Q&A ATCC® *Excellence in Research* Webinar "The Biology of Anaerobic Bacteria and Predominant Propagation Practices"

1. Will we be able to download the presentation?
   This presentation will be available to watch on demand on the ATCC website, or click here.

2. I followed the ATCC Product Sheet for propagating my organism, but it only grew in the primary inoculation broth, not on my agar plate. What should I do next?
   Many times, the organisms are put under stress during lyophilization or freezing. If the organism is sensitive, it might require growth to be well established in the primary broth, then subsequent transfers to agar and additional broth tubes can be made.

3. Does the cryopreservative (10% final glycerol) and the method of preservation (flash freeze) affect the growth (recovery) of an anaerobe?
   Yes. Cryoprotectants are essential to protect the integrity of the cell during intracellular and extracellular ice crystal formation. ATCC uses numerous cryoprotectants depending on the method of preservation. More information about cryoprotectants and preservation can be found in the ATCC® Bacterial Culture Guide. We highly recommend that anaerobes are kept in an anaerobic atmosphere, which can be achieved by suspending the vials in liquid nitrogen vapor before preservation, specifically nucleation. We have found that some anaerobes do not recover as well from serum vials, and will often change the preservation format to frozen.

4. Some anaerobes do not grow on agar, only in broth. Aside from visual turbidity, what are the other ways to check if there are good/live cells?
   In this case, a live/dead stain may be useful. Using a broth culture, you can approximate the percentage of live cells in the culture. For best results, prepare the slide in an anaerobe chamber and then bring it out to the microscope.

5. In your experience, what was the longest time it took for an anaerobe to grow?
   Spore-formers tend to survive longest in culture, provided they are subcultured often enough. If not, they will run out of nutrients and may lose viability due to H_2S gas produced by the anaerobe itself. You will probably have more success keeping cultures that are grown in broth or slants since plates dry out easily. A word of cautions, multiple passages can cause a loss of antibiotic resistance; keep this in mind if you are working with antibiotics.

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6. There are a number of techniques used to reduce media. Which method is the best?
   For broth media, out-gas with an appropriate gas mixture and then add the reducing agent. Choose the reducing agent already present in the media formulation. For best results, allow the media to sit overnight in the dark. Media should always be protected from light. For agar media, place plates in a jar with a sachet and seal. Place the jar in the dark. The more plates present in the jar, the longer it will take; plan on at least 24 hours. Slants can be reduced this way, but make sure the caps are loose.

7. Do we really need to add cysteine before using the media?
   Yes. A simple way to think of this is that out-gassing makes the head space anaerobic while cysteine (or another appropriate reducing agent) makes the broth anaerobic. Another technique is to bubble with gas until the indicator in the broth becomes clear.

8. Is there a recommended media to grow microorganisms from the seafloor (about 30m deep)?
   There is a wide variety of media used for this purpose. These organisms tend to have very specific requirements. We generally use the formulation the depositor provides when we accession these organisms. We recommend referencing published literature.

9. I freeze *Bifidobacterium* cultures in milk and glycerol. For other anaerobes, should I also use milk?
   We highly recommend using 10% glycerol. We have found that to be superior to milk.

10. How many serial transfers from the main anaerobic culture stock are advisable?
    It's difficult to give an exact number of passages due to the range of viability among anaerobic organisms. The fewer passages, the better. This prevents genetic drift and contamination.

11. How do you transfer your lyophilization vials from the anaerobic chamber to the lyophilizer?
    At ATCC, we prepare the vials in the anaerobe chamber. Once they are ready, we place them in container designed for liquid nitrogen use and place a metal rack and liquid nitrogen inside. The vials are placed on the rack, suspended in the liquid nitrogen vapor, and transported to the lyophilizer. The liquid nitrogen vapor ensures the vials are kept in an anaerobic environment.

12. Can you please discuss the preparation of an anaerobic Gram stain?
    We prepare and dry our slides in the anaerobe chamber. We then bring them out and fix them by either the flame or methanol method.

13. At what temperature is it safe to inoculate molten agar for roll tubes?
    After melting the agar, it is recommended to place the roll tubes in a 42°C - 46°C water bath until they reach that temperature. The tubes can then be removed and inoculated. A good rule of thumb is that they should be cool enough to handle. The agar will solidify quickly once removed, so inoculation should be prompt.

14. What is the best medium for growing sulfate-reducing bacteria?
    ATCC Medium #1249: Modified Baar's for Sulfate Reducers is used for anaerobic sulfate reducers.

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