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# A Comparison of Methods for Enumeration of Bacteria from Natural Water Samples: A Multi-Week Laboratory Project

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A common laboratory exercise in microbiology courses is enumeration of bacteria. The process of counting bacteria is often performed by serial dilution of a sample (often a pure culture) followed by spread plating on a non-specific, generally supportive medium and incubation. Students then perform calculations from colony numbers to determine the viable count. In my microbial ecology class, bacterial enumeration was expanded to a multi-week investigative laboratory project. Students worked in teams to enumerate bacteria from two types of natural waters: groundwater and water from a river that is characterized by high nutrients and suspended sediments to which bacteria attach. Students devised their enumeration plans using procedures broadly described in *Standard Methods for the Examination of Water and Wastewater*. In addition to gaining experience with natural samples, students came to realize that for samples with low bacterial concentrations, such as groundwater, concentration by membrane filtration must be used for the viable count. They gained an appreciation for the roles that media choice and incubation conditions can play in the results. As we discussed their data, they came to understand that a typical viable count (here, a heterotrophic plate count) is based on a definition of viability as the ability to reproduce. To better understand that only some viable bacteria divide to form colonies under given conditions, teams next compared the heterotrophic plate count to a direct total bacterial count. The latter involved sample filtration onto black polycarbonate 0.2 micron membranes, staining with acridine orange, visualization by epifluorescence microscopy, and enumeration of the cells in fields of known size. Although there are other methods that students can use to obtain a direct total bacterial count, the high concentration of particulate matter in our river samples makes epifluorescence microscopy essential. For our river samples, the difference between the heterotrophic plate count and the direct total microbial count was often several orders of magnitude. Students then used these data as the basis for another question: How can the viability of bacterial cells that do not divide on a nutrient medium be determined? They attempt to determine viability of individual cells through tools such as respiratory indicator dyes. Through these experiments, students gained laboratory skills and grew in their ability to design experiments, adapt lab procedures, and evaluate methodology and data in a way that is truly analytical.

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