

ASSAY FOR COLIFORM CONTAMINATION IN AMBIENT WATER (OR ICED TEA)

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http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Coliform_assays/Coliform_Enumeration.htm

RELATED PROTOCOLS:

*Commonly Used Media, Sterile Delivery of Liquids by Pipet,
Plate Spreading Technique*

Aristotle taught Alexander the Great to bury feces and boil drinking water. He recognized the dangers of drinking fecally contaminated water. The fecal-oral route spreads many major diseases such as cholera, salmonellosis, dysentery, shigellosis, polio and many others. Mammalian fecal wastes carry large numbers of gram negative rod-shaped bacteria which are capable of using lactose (milk sugar) as a carbon source. Bacteria with these properties are collectively termed **coliform bacteria**. The presence of coliform bacteria is widely used as an indicator of potential fecal contamination. *Total* coliform grow at 35°C, but only *fecal* coliform grow at 44.5°C. The most famous member of this group is *Escherichia coli*.

Levine EMB Agar is a medium which is both selective and differential for the enumeration of coliform bacteria (see the protocol on *Commonly Used Media*) It contains nutrients, 1% lactose, agar and two dyes, Eosin and Methylene Blue. These dyes serve two purposes: first, as a **selective medium**, they inhibit the growth of gram positive bacteria (only Gm- bacteria grow), and second, as a **differential medium**, because they cause colonies which ferment the lactose ("*lac*⁺") to turn purple while the "*lac*⁻" colonies will be pink or uncolored. Further, *E. coli* typically forms purple colonies with a green sheen.

MacConkey Agar is a second medium which may be used to enumerate coliform. It contains 1% Lactose, 0.15% Bile Salts, and the dyes Neutral Red and Crystal Violet. The dyes and bile salts prevent Gm⁺ bacterial growth. On MacConkey agar, *lac*⁺ appear red, while *lac*⁻ appear white.

By spreading a known aliquot of an aqueous sample (often 0.1 mL) on one of these media and incubating until colonies have formed, the number coliform in the aliquot will be equal to the number of *purple (lac*⁺*)* colonies, and is proportional to the degree of potential fecal contamination.

MATERIALS LIST PER STUDENT:

- 2 sterile screw-capped markable culture tubes
- 4 Levine-EMB plates (room temp) per student
- 4 MacConkey Agar plates (room temp) per student

INCUBATORS FOR CLASS:

- 35°C incubator
- 44.5°C incubator

FIVE SPREADING STATIONS, ETC:

- two 200 uL displacement pipetters
- box of 200 uL sterile pipet tips
- two spreader, EtOH beaker, turntable
- flame
- used pipet receptacle
- conductivity meter

1. **SAMPLE:** Precisely record *where* and *when* you collect your samples. Collect the sample as close to plating time as possible, and/or keep refrigerated until plated. Use sterile screw-capped culture tubes (10 or 20 mL), fill 3/4 full from midstream of a *flowing* stream. *Do not contaminate with sediment from stream bottom.* Cap immediately. Write in *pencil* on the tube's frosted area: collection location, date, and your initials. Samples from above and below sewage treatment plants can be interesting, demonstrating whether raw or improperly treated sewage is contaminating the river. Restaurant iced tea, because of mishandling, has been heavily contaminated for many years. Enter your sources in the class spreadsheet.
2. **RECORD your sources and data** in the provided table (page 52b). Mount it in your book once all the data is collected and recorded.
3. **LABEL EACH PLATE** with five items: 1) seat number, 2) initials, 3) plate number, 4) source and 5) aliquot volume.
4. **PLATE OUT ON THE DIFFERENTIAL AGARS:** Choose an aliquot volume to spread, based on the anticipated level of contamination in your specimen: 0.05 mL to 0.2 mL per plate. Using sterile technique as outlined in *Plate Spreading Technique*, spread the aliquot on EMB and on MacConkey agar.
5. **READ CONDUCTIVITY:** record in microsiemens on your data table of each sample.
6. **INCUBATE** at 35°C for 48 hrs for total coliform, or 44.5°C for fecal coliform.
7. **SCORE THE PLATES AFTER 48 HOURS:** Count, score (as specified above) and record the total colonies and the number of coliform colonies/plate. Calculate the number of each category/100 ml of sample, enter into your data table. Especially interesting are *Lac* negative colonies. Circle them to note the ones with unusual colony morphology. Save the plate for single colony isolation (page 53a).
8. **ENTER INTO THE CLASS DATA SHEET.** Enter your data in the spreadsheet on the computer. After class data have been collected, mount the table in your note book.