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Isolation of Fecal Indicator Bacteria from Water Samples by Membrane Filtration
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Corresponding Video Article Title: Quantifying Fecal Indicator Bacteria in Water Using the Membrane Filtration Technique

Environmental Science Education Title: Isolation of Fecal Indicator Bacteria from Water Samples by Membrane Filtration

Overview:

The quality of water destined for use in agricultural, recreational, and domestic settings is of great importance due to the potential for outbreaks of waterborne disease. Microbial agents implicated in such events include parasites, bacteria, and viruses that are shed in high numbers in the feces of infected people and animals. Transmission to new and susceptible hosts may then occur via the fecal-oral route upon ingestion of contaminated water. Therefore, the ability to monitor water sources for the presence of pathogenic microorganisms is significant in order to ensure public health.

Due to the sheer number and variety of potential fecal-oral pathogens that may be present in water and their variable concentrations, it is impractical and expensive to assay directly for each one of them on a regular basis. Therefore, the microbiological assays for water quality monitoring employ coliform indicator bacteria. Coliforms comprise, in part, the normal intestinal microflora of warm-blooded mammals, are non-pathogenic, and are consistently excreted in the feces. Therefore, the detection of coliform bacteria in water means that a fecal release occurred, and that harmful pathogenic microorganisms may also be present.

Principles:
The membrane filtration technique is used to assess the microbiological quality of water by assaying for fecal indicator bacteria. A quantity of water (e.g. 100 ml) is passed through a specialized membrane filter with a minimal mean pore size of 0.45 μm, facilitating the capture of bacteria, as they are approximately 1 μm in size. Following filtration, the membrane is carefully applied to a specialized agarose culture medium, and incubated under the conditions appropriate to culture the target microorganisms.

When applied for use in water quality monitoring, membrane filtration is most ideal for low turbidity sources such as drinking water, swimming pools, and natural recreational waters such as lakes and reservoirs. Waters high in particulate matter (e.g. raw sewage) will result in fouling of the filter; therefore, only smaller volumes (e.g. 100 ml) can be analyzed. Membrane filtration is also not practical for water sources with large numbers of background (or non-coliform) bacteria, which can increase the difficulty of enumerating the target coliform bacteria on the agarose medium following incubation.

This video demonstrates the collection of drinking water and environmental water samples, the membrane filtration of the samples, and the enumeration of several types of fecal indicator bacterial colonies using specialized agarose growth media including total

coliforms, fecal coliforms, and fecal streptococci. Tests conducted further in order to verify presumptive colonies are also shown.

Procedure:

1.   Water Sample Collection

> **1.1.**   Collect several 1L water samples from the test water source (e.g. water fountains, swimming pools, reservoirs, water distribution systems, raw or treated sewage), and transport on ice to the laboratory for microbial analysis.

2.   Water Sample Processing

> **2.1.**   Sterilize or sanitize the membrane filtration manifold assembly prior to use by autoclaving, exposure to UV radiation (2 minutes), or ethanol-flame ignition.

> **2.2.**   Upon cooling of all parts, properly connect the manifold to a vacuum pump and vacuum filtration waste flask containing bleach.

> **2.3.**   Ethanol-flame sterilize a pair of forceps and with them, remove a sterile, gridded membrane filter from its packaging. Membranes measuring 47mm in diameter with a pore size of 0.45 µm are typically used. However, alternate diameters and pore sizes can be employed provided that the pore size can sufficiently entrap the target microorganism(s), and at least 70% of the filter area is comprised of pore space.

> **2.4.**   Place the filter onto the center of the membrane filtration area of the manifold, and apply a sterile filter funnel to the unit and secure it into place.

> **2.5.**   Measure a desired volume of test water (e.g. 100-ml) and add it to the funnel (a 100-ml line mark is visible on the funnel).

> **2.6.**   Apply a partial vacuum [pressure differential of 34 to 51 kPa (kiloPascals)] in order to draw the test sample through the filter. Total suspended solid material, including bacteria and decaying organic matter, greater than the filter mean pore size of 0.45 µm are trapped on the filter. Smaller particles including viruses and dissolved solids such as small amounts of organic matter and salts, will pass through the membrane and into the waste container vacuum flask containing bleach.

> **2.7.**   Following complete passage of the sample through the filter, rinse the interior of the funnel with two to three 20-30 ml volumes of sterile water.

2.8. Power-off the vacuum and remove the funnel from the manifold upon closure of the final rinse.

2.9. With ethanol-flame sterilized forceps, immediately remove the membrane filter from the unit and promptly place it onto the appropriate growth agarose medium for the target microorganism (**Table 1** lists the recommended growth media and incubation conditions for each).

2.10. Apply the membrane filter to the agarose surface with a roll-type motion to ensure complete contact of the membrane with the growth medium, and to avoid the entrapment of air bubbles.

2.11. Replace the used filtration funnel with a sterile unit between the processing of each sample, and ethanol-sanitize the stainless steel manifold in order to prevent cross-contamination.

3.   Colony Enumeration

3.1. Following the incubation period, remove the growth plates from the incubator for enumeration.

3.2. Ideally, perform the colony counts under low power magnification using a cool, white light source.

3.3. Total coliforms

    3.3.1. Total coliform colonies are typically are pink to dark-red in color with a metallic surface sheen. The sheen itself may either partially or completely cover the colony. Atypical total coliform colony morphologies may be dark red, mucoid, or nucleated without sheen.

    3.3.2. Colonies that are pink, blue, white, or colorless while lacking sheen are considered non-coliforms.

3.4. Fecal coliforms

    3.4.1. Fecal coliform colonies appear as various shades of blue.

    3.4.2. Nonfecal coliform colonies are grey to cream in color.

3.5. Fecal streptococci

3.5.1. Fecal streptococci colonies range from light red to dark red in color.

4.   Colony Verification

4.1.   Total coliforms

4.1.1. For a presence-absence verification, swab the entire membrane using a sterile cotton-tipped applicator. For colonies, it is preferable to verify at least five each of typical and atypical morphologies.

4.1.2. Transfer the swab or individual colonies into a glass vessel containing lauryl tryptose broth with a Durham tube. Incubate the inoculated tubes at 35±0.5 °C for 48 hours. The presence of turbidity indicating growth in conjunction with gas production verifies the colony as a coliform.

4.2.   Fecal coliforms

4.2.1. Transfer colonies blue in color into glass vessels containing sterile EC medium with a Durham tube. Incubate the inoculated tubes at 44.5 ± 0.2 °C for 24 hours. The presence of turbidity indicating growth in conjunction with gas production verifies the colony as a fecal coliform.

4.3.   Fecal streptococci

4.3.1. Strike colonies typifying fecal streptococci morphology for isolation onto Brain-Heart Infusion Agar (BHIA), and incubate at 35 ± 0.5 °C for 24 to 48 hours.

4.3.2. Transfer growth from an isolated colony on BHIA into a Brain-Heart Infusion Broth (BHIB) tube, and onto two pre-cleaned glass slides. Incubate the broth tube at 35 ± 0.5 °C for 24 hours.

4.3.3. Add two to three drops of 3% hydrogen peroxide to prepared smears on the slides. The rapid appearance of bubbles indicates a "catalase positive" result, and the isolate <u>is not</u> a fecal streptococcus bacterium.

4.3.4. Perform a Gram stain for isolates testing as "catalase negative" (no bubbles observed). Fecal streptococci are Gram-positive, ovoid, and appear mostly in pairs or short chains.

4.3.5. Following incubation of the broth culture prepared per Section 4.3.2,

transfer a loopful of growth to bile esculin agar (BEA) and BHIB, then incubate at 35 ± 0.5 °C for 48 hours and 45 ± 0.5 °C for 48 hours, respectively.

4.3.6. Isolates demonstrating growth under the conditions specified in 4.3.5, and are also catalase-negative, Gram-positive cocci, are members of the fecal streptococcus group.

Applications:

Membrane filtration is used in virus capture and concentration from water. Human pathogenic viruses carry a net negative charge in aquatic solutions, and are often present at low levels in water sources. Therefore they must be concentrated prior to analysis. Membrane filtration is but one capture method for this purpose, and employs a negatively-charged filter. Water samples (e.g. 1-liter) of interest are amended with a salt solution (e.g. magnesium chloride) to impart a positive charge to the viruses, thereby facilitating their adsorption to the negatively-charged HA membrane filter as the water is filtered. A low concentration acid solution is used to rinse the membrane and rid it of excess salts. A low concentration and volume of sodium hydroxide is then used to release the viruses from the filter prior to further concentrations and analyses (e.g. cell culture infectivity assays or quantitative PCR).

Membrane filtration is also utilized in the production of high-purity process water for industrial use. Many industries require highly purified water for their operational processes. Membrane filtration (e.g. nano-filtration) serves to remove contaminants including dissolved metals and salts from water. Membrane filtration is also used in the desalination of salt water to produce potable water.

Legend:
Table 1. Commonly-used culture growth media for the detection of fecal bacterial indicators in environmental samples

Photo or Graphic File
Click here to download Photo or Graphic File: Pepper_membrane_filtration_tbl1.docx

Fecal Bacterial Indicator	Recommended Media (Incubation Temperature, Time)[a]
Total coliforms	LES Endo Agar (35 ± 5 °C, 24hr) M-Endo Medium (35 ± 5 °C, 24hr)
Fecal coliforms	m-FC Medium (44.5 ± 0.2 °C, 24hr)
Fecal streptococci	m *Enterococcus* Agar (35 ± 0.5 °C, 48hr)

[a] As recommended by the *Standard Methods for the Examination of Water and Wastewater* (American Public Health Asssociation and the American Water Works Association, 22nd Edition, 2012)