

*Implications and the Use of
Bacterial Indicators for the
Prediction of Microbial
Contamination of Water*

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Microorganisms

- ▶ single-celled
- ▶ generally microscopic
- ▶ include bacteria, protozoa, algae, fungi, viruses
- ▶ may or may not be disease causing



NUTRITION SOURCE

AUTOTROPHIC: live on inorganic matter

HETEROTROPHIC: live on organic matter

PARASITES: requires living organic matter, includes pathogens

SAPROPHYTES: dead organic matter



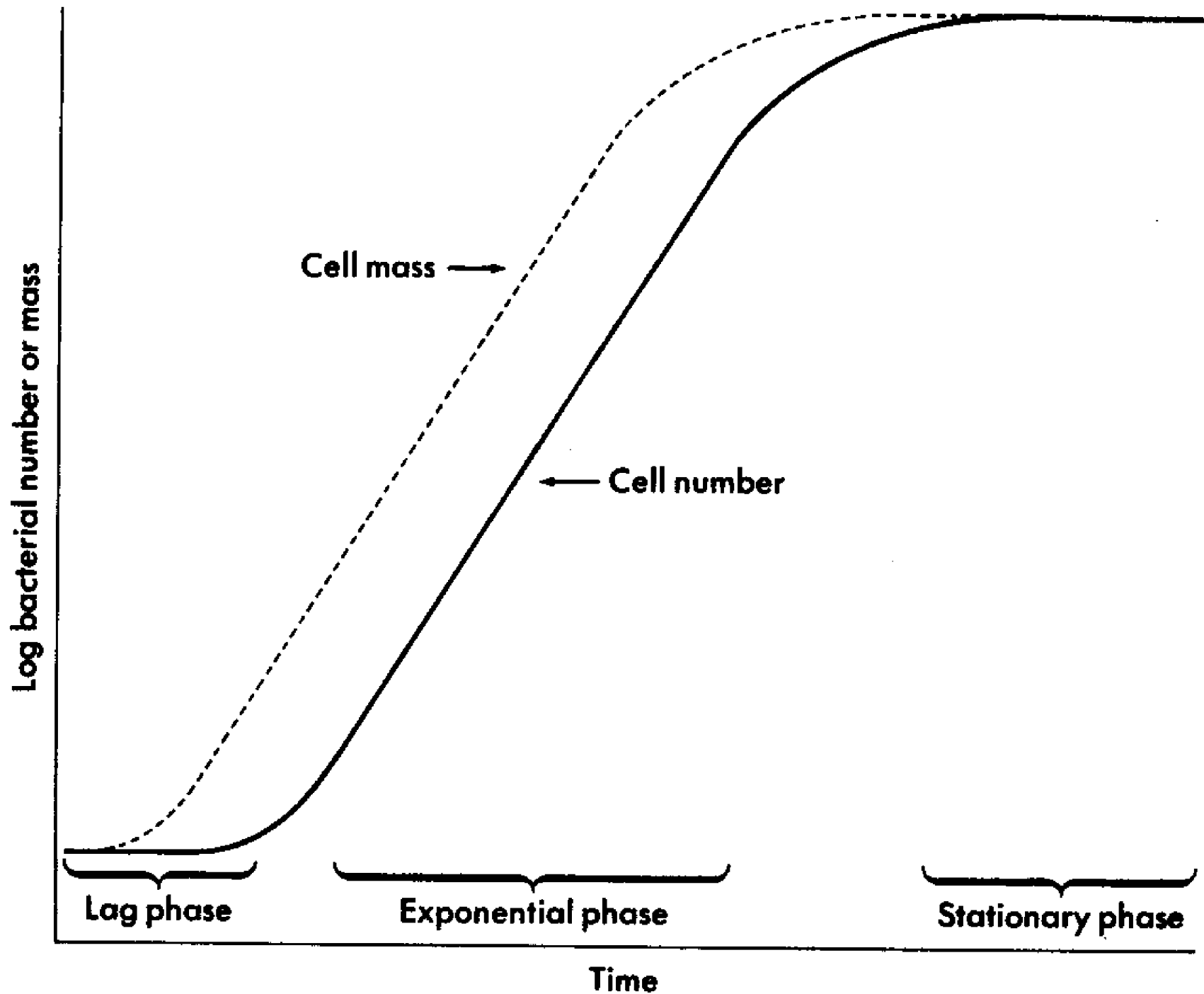
OXYGEN REQUIREMENTS

OBLIGATE AEROBES - Need oxygen to grow.

FACULTATIVE ANAEROBES -
Fundamentally anaerobes but can grow in absence of free oxygen

OBLIGATE ANAEROBES - Grow free of oxygen

FACULTATIVE AEROBES - Fundamentally aerobes but can grow in the presence of free oxygen

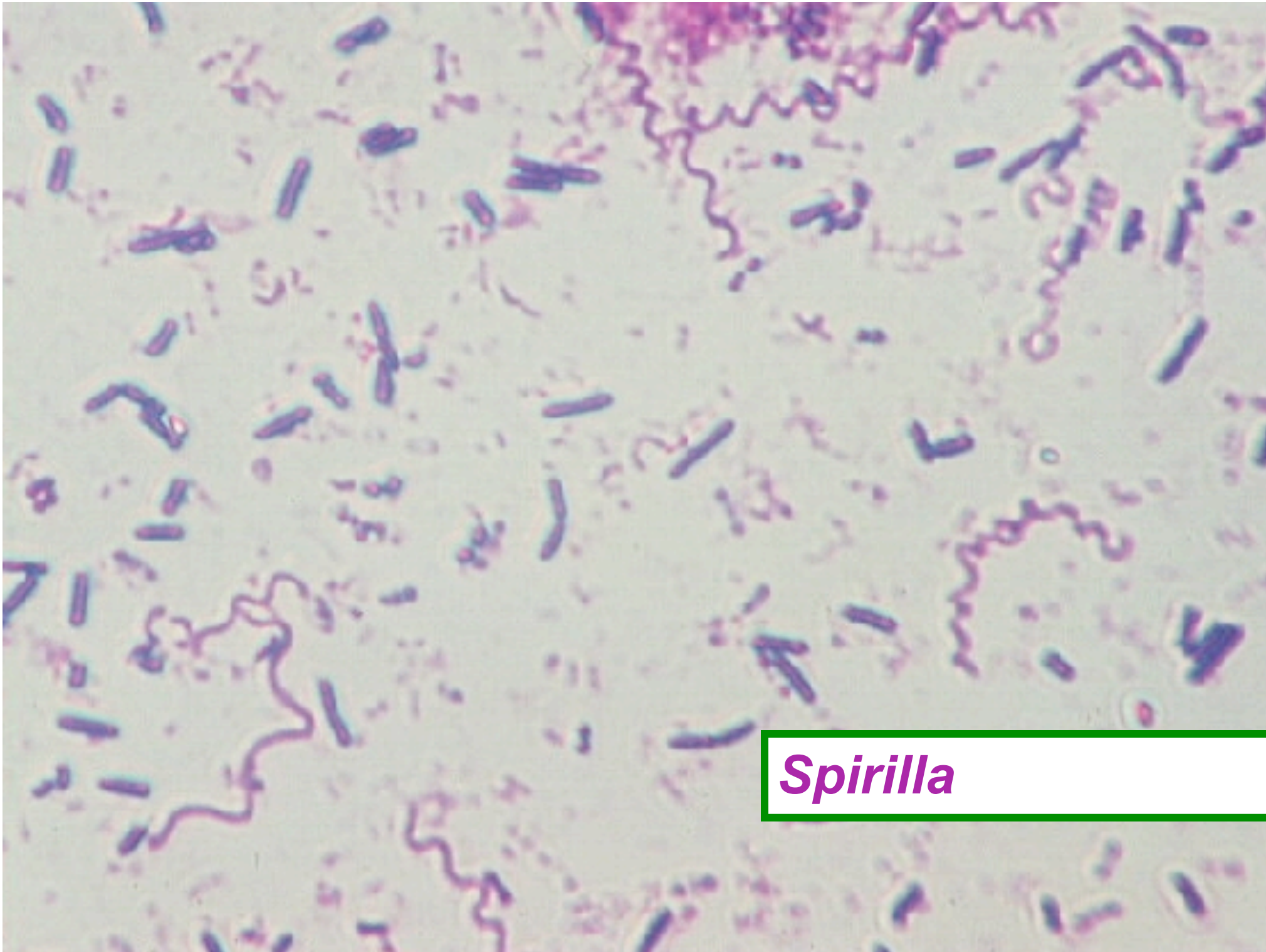




Bacillus

A black and white micrograph showing numerous small, dark, spherical bacteria (cocci) arranged in various patterns, including chains and clusters. The bacteria are densely packed in some areas and more sparse in others. A green rectangular box is overlaid on the right side of the image, containing the word 'Cocci' in purple text.

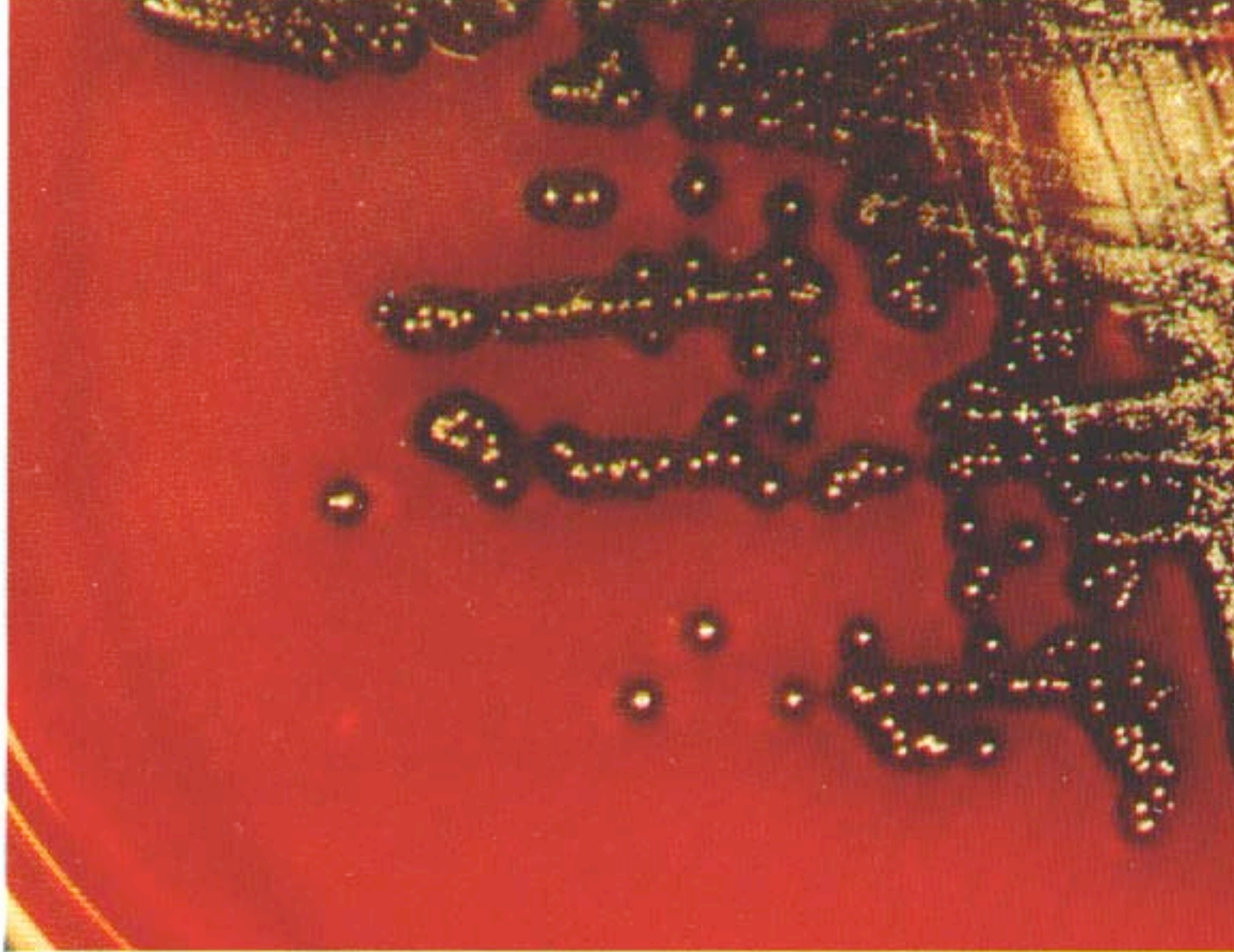
Cocci



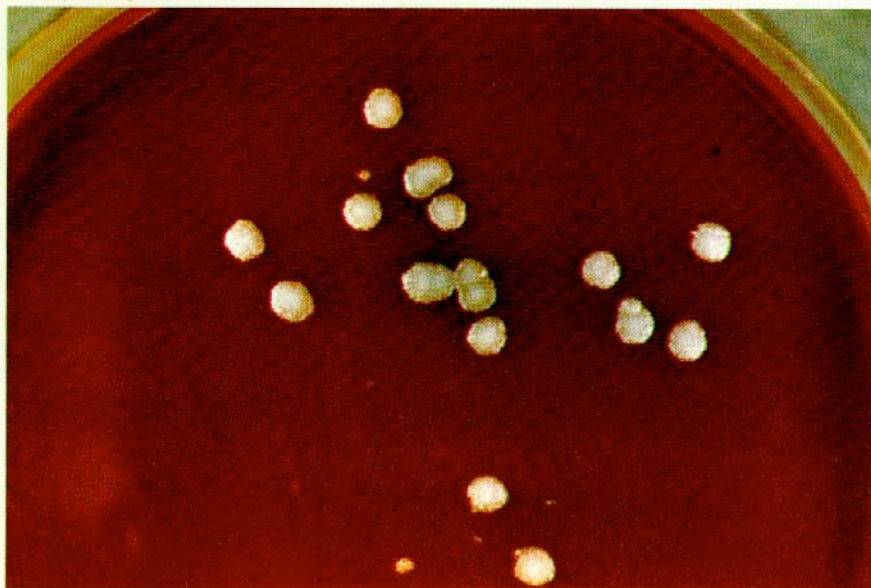
Spirilla



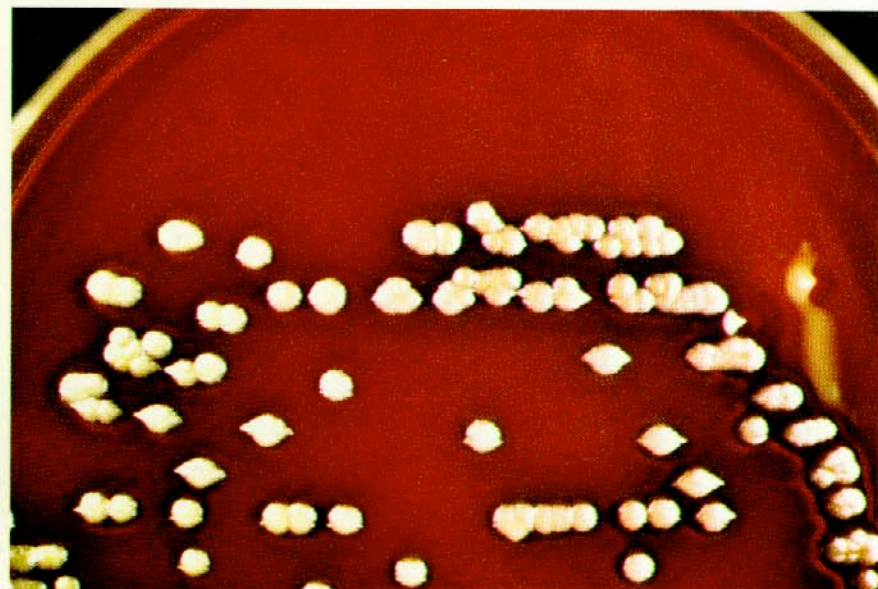
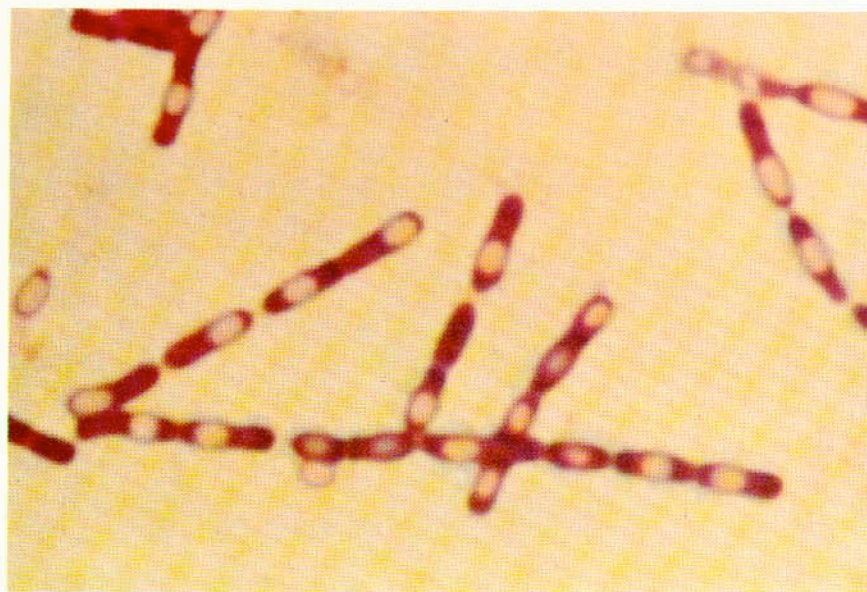




Bacillus anthracis. Culture on blood agar.



Bacillus anthracis. Gram stain of culture smear.



Bacillus subtilis. Blood agar culture. The large, abundant, irregular, spreading, rough and waxy colonies are usually surrounded by a large zone of beta hemolysis.



Bacillus subtilis. Gram stain of culture smear.



Implications of Microbial Contamination of Water

- ▶ potential for transmission of disease causing microorganisms (pathogens) via water contact - oral fecal route
- ▶ contact includes ingestion, primary contact, and secondary contact



Water Borne Diseases/Pathogens

• Bacteria

- *Vibrio cholera* - cholera
- *Yersinia enterocolitica* - gastroenteritis
- *Escherichia coli* - gastroenteritis
- *Shigella sp.*- gastroenteritis
- *Listeria monocytogenes* - flu-like symptoms
- *Salmonella sp.* - gastroenteritis, typhoid
- *Campylobacter jejuni* - gastroenteritis (animals sources)



Water Borne Diseases/Pathogens

• Viruses

- Polio Virus - paralytic poliomyelitis
- Rotaviruses - gastroenteritis
- Norwalk Viruses - gastroenteritis
- Hepatitis Type A and E - liver inflammation



Water Borne Diseases/Pathogens

• Protozoa

- *Entamoeba histolytica* - amoebic dysentery
- *Giardia lamblia* - intestinal disease
- *Cryptosporidium* - intestinal disease



Difficulties With Routine Testing Of Pathogens in Water

- ▶ present in low numbers
- ▶ limited survival time
- ▶ numerous pathogens to analyze
- ▶ time and cost prohibitive



Testing of Indicator Organisms

- need an indicator of potential pathogen contamination of water which is easy, reliable, inexpensive, quick, etc.



Requirements of Indicator Organisms

- ▶ present when pathogens present in water
- ▶ absent in uncontaminated water
- ▶ present in higher numbers than pathogens in contaminated water
- ▶ better survival in water than pathogens
- ▶ easy to analyze



Coliform Bacteria - Indicators of Fecal Contamination

- ▶ bacteria in the family Enterobacteriaceae
- ▶ generally include the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia*
- ▶ present in the intestinal tract of numerous organisms
- ▶ gram negative, non-spore forming rods
- ▶ ferment lactose with acid and gas production in 48 hours at 35 °C



Total Coliform Bacteria

- ▶ inhabit the intestinal tract of animals
- ▶ sources: fecal material, soil, water, grain
- ▶ some capable of reproduction in the environment
- ▶ non-fecal examples include: *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*



Fecal Coliform Bacteria

- ▶ subset of the total coliform group
- ▶ separated from non-fecal coliforms by growth at 44.5 °C
- ▶ sources: fecal material
- ▶ capable of limited survival and growth in the environment
- ▶ primary example is *Escherichia coli* (*E. coli*)



Source Water vs. Drinking Water

- ▶ Drinking water and source water have different requirements for coliform analysis methods and holding times



Coliform Sample Holding Times

- drinking water - 30 hours
[40 CFR 141.21(f)]
- surface/source water - 6 hours
[40 CFR 141.74 (a)(1)]



Effects of Holding Time on Samples

- coliform numbers may decline
- coliform numbers may increase
- heterotrophic (non-coliform) bacteria may increase and inhibit detection of coliforms in the sample



Heterotrophic Interference

- ▶ Non-coliform bacteria which are better than coliforms at surviving in the environment may be present in the sample. If these organisms are given a chance to grow, they may out-compete coliforms during analysis and may limit the analysts ability to detect coliforms. The effect of HI is an underestimation of coliform numbers or a false negative result.



Holding Times Are Critical

- greater chance of heterotrophic interference in untreated source water - hence, shorter allowable holding time



Membrane Filter Method

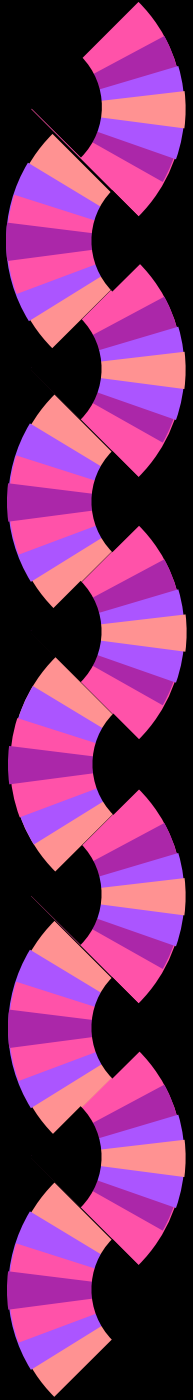
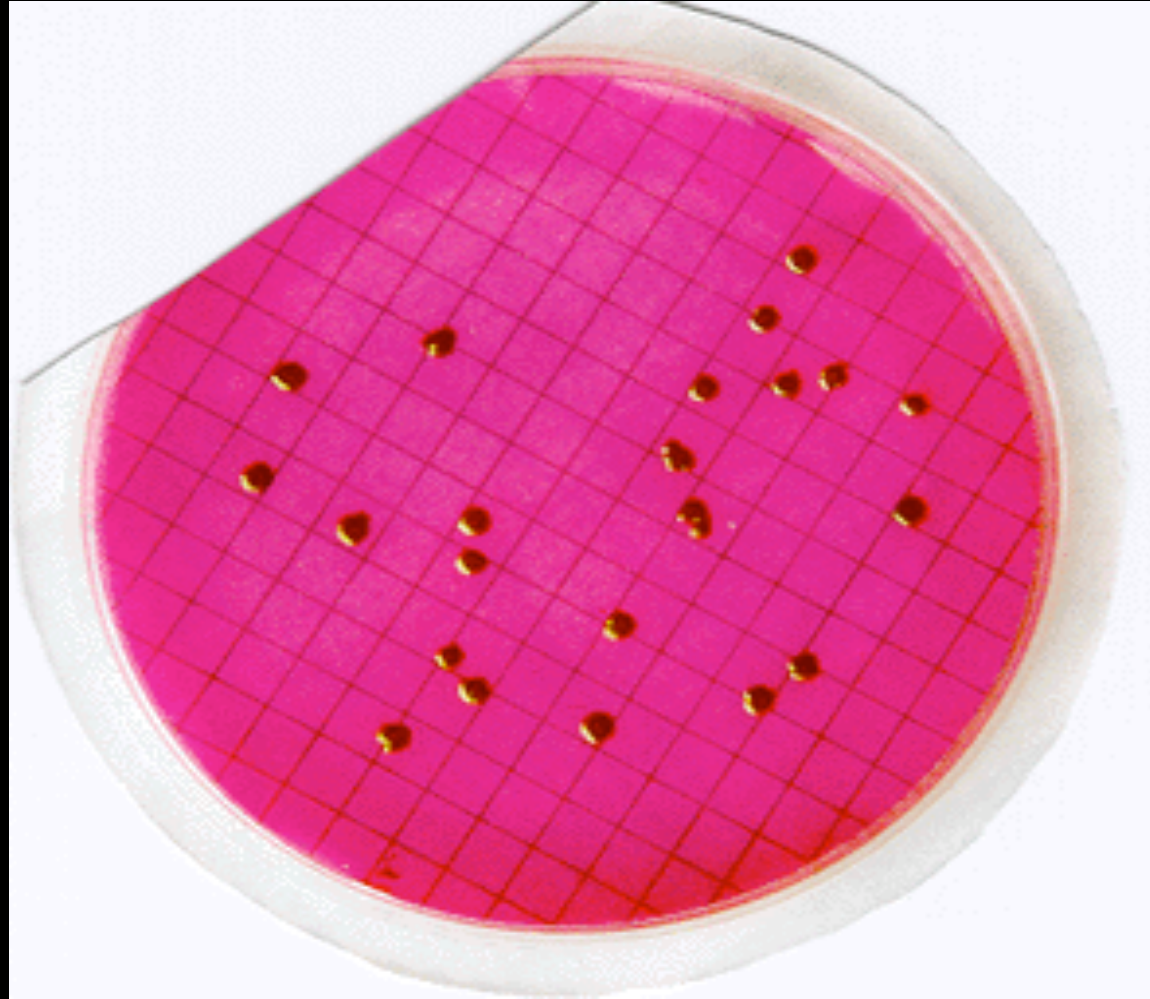
metallic green colonies on endo medium-
presumptive coliform positive

growth/gas in LTB and BGGBB - confirmed total
coliform positive

growth/gas in LTB and EC medium - confirmed
fecal coliform positive

growth and gas in LTB and growth and
fluorescence in EC + MUG - confirmed *E. coli*
positive

Membrane Filter Method (Endo agar with coliform growth)





Multiple Tube Method

5 day test

- growth/gas in LTB- presumptive coliform positive
- growth/gas in BGGBB - confirmed total coliform positive
- growth/gas in EC medium - confirmed fecal coliform positive
- growth and fluorescence in EC + MUG - confirmed *E. coli* positive



ADVANTAGES OF MF OVER MPN

Results in 24 hours as compared with 48-96

Much larger and more representative samples can be run

Numerical results from membrane filter have greater precision than MPN

The equipment and supplies are not bulky. More samples can be analyzed with less space and equipment



Enzyme Substrate or Chromogenic Substrate Method

- Used with the Presence-Absence, the Multiple Tube Methods, or Quanti-Trays™
- Total coliform have the enzyme
 - β -D-galactosidase which hydrolyses
 - ortho-nitrophenyl- β -D-galactopyranoside (ONPG)
 - Yellow when hydrolyzed
- *E. coli* has the enzyme
 - β -glucuronidase which hydrolyses
 - 4-methylumbelliferyl- β -glucuronide (MUG)
 - Fluoresces when hydrolyzed



INDICATOR REQUIREMENTS

- ▶ Ubiquitous in wastewater
- ▶ Survives or is detectable at least as long as pathogens
- ▶ Easy to isolate and identify



Streptococcus and Enterococcus

• Fecal Strep

- *S. faecalis*
- *S. faecium*
- *S. avium*
- *S. bovis*
- *S. equinus*
- *S. gallinarum*

• Enterococcus

- Fecal Streps that survive in 6.5% sodium chloride
 - *S. faecalis*
 - *S. faecium*
 - *S. avium*
 - *S. gallinarum*



Useful References

- *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, 1992, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C., 20005
- Total Coliform Rule (40 CFR 141.21)
- Surface Water Treatment Rule (40 CFR 141.74)