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Genetic Research in Tracking Water-Borne Pathogens

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Water studied to determine-

- the presence of virus and bacteria pathogenic to humans and animals,
- the presence of horizontally transferable genetic elements known to influence pathogenicity,
- the transfer and amplification of genetic elements involved in antibiotic resistance and virulence in aquatic environments and,
- the modulation of environmental bacteria involved in nitrogen cycling and organic transformation

Compucyte Laser Scanning Cytometer

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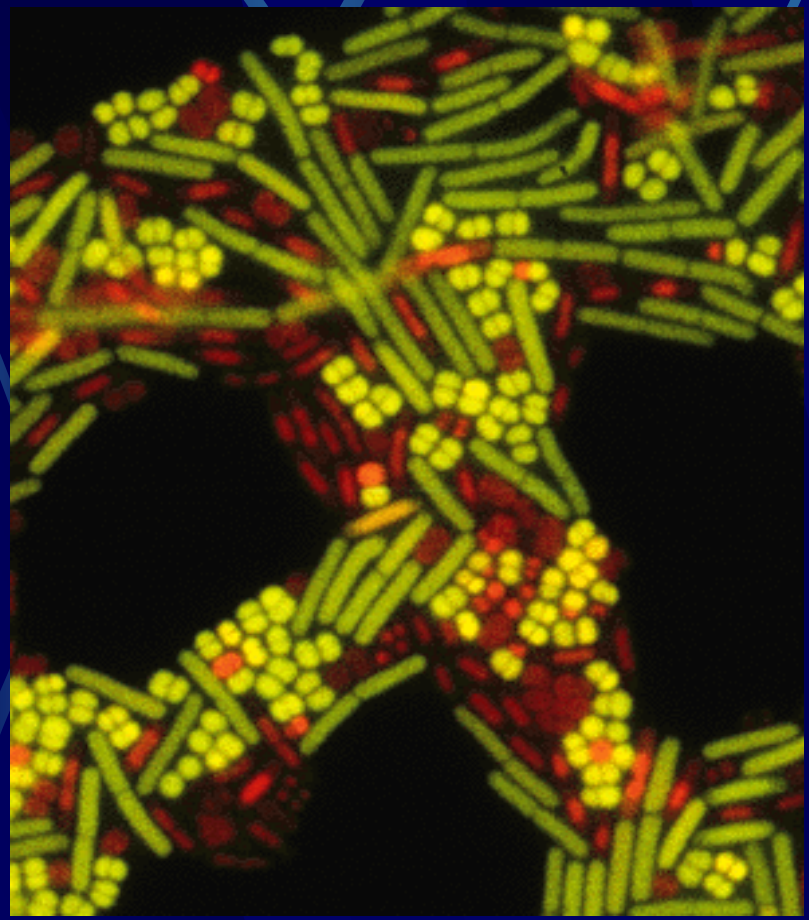
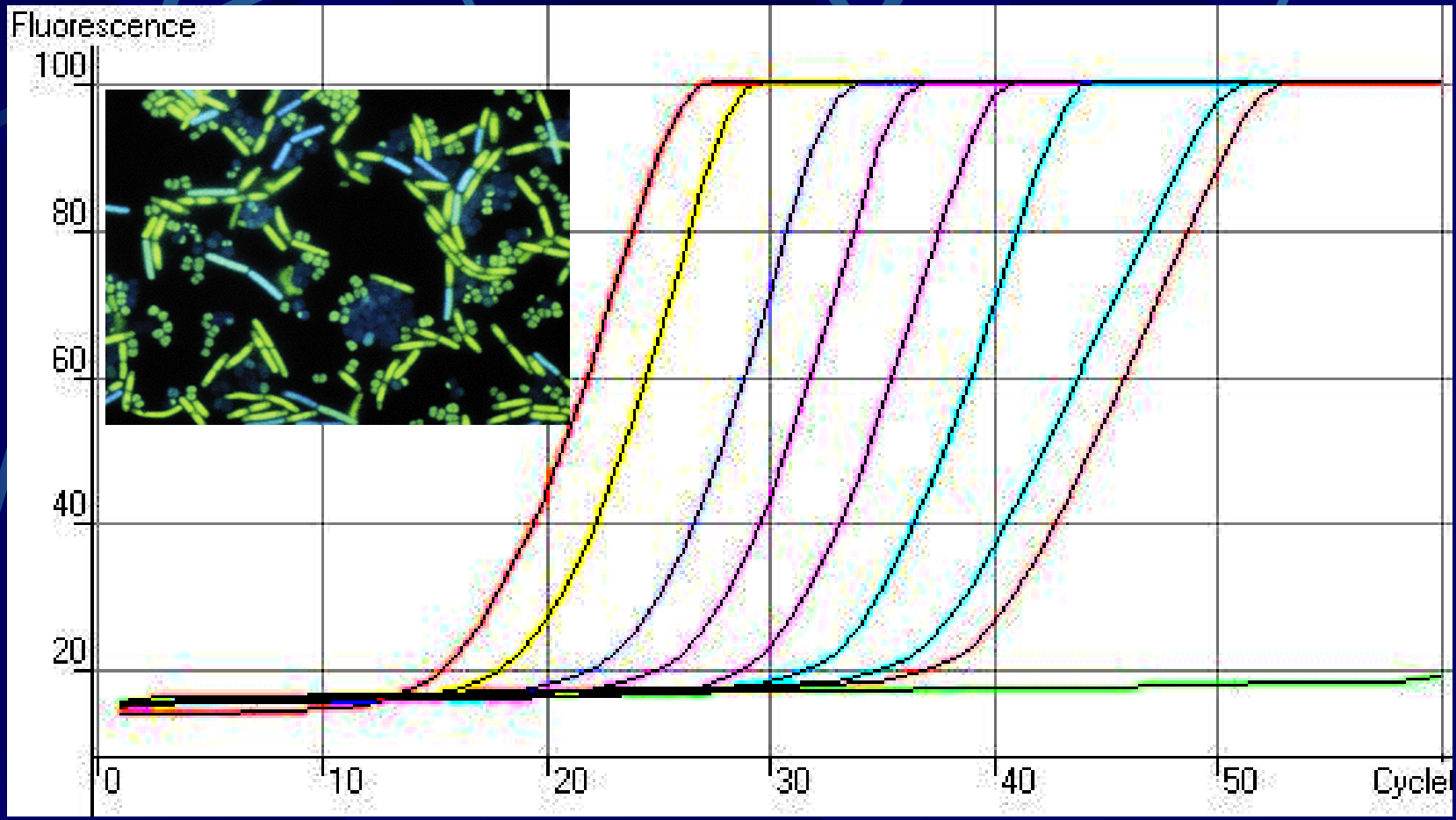
Laser Microscopy

Fluorescent antibody, hybridization, and nucleic acid staining for the examination of microbial assemblages



Quantitative Real-Time PCR



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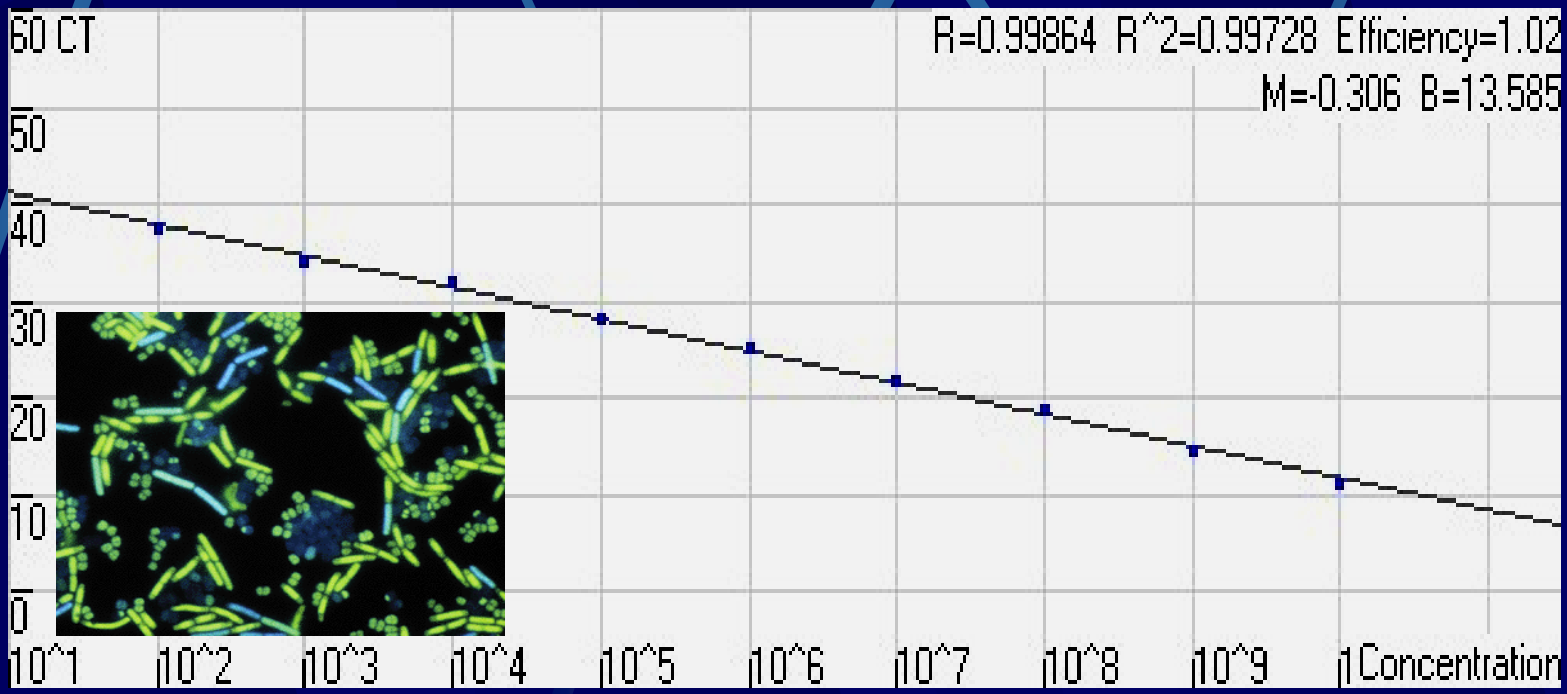
Quantitative Real-Time PCR



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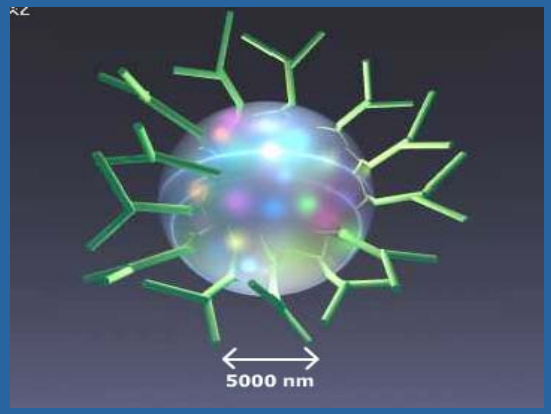
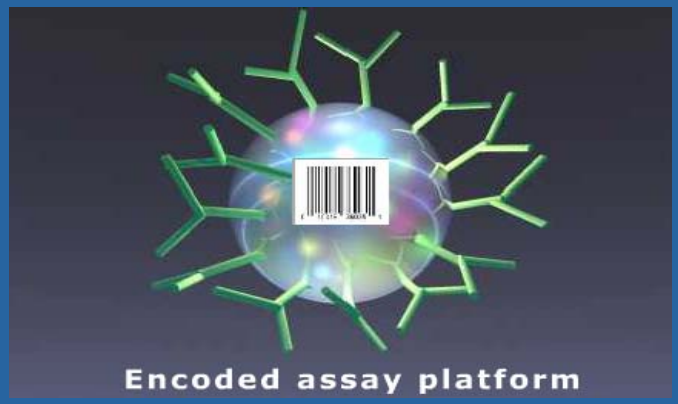
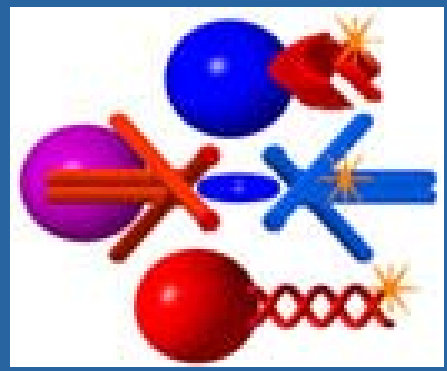
Luminex (LiquiChip™) Fluidized Microarray







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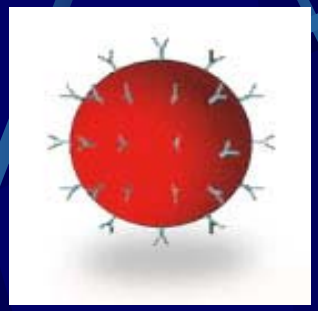
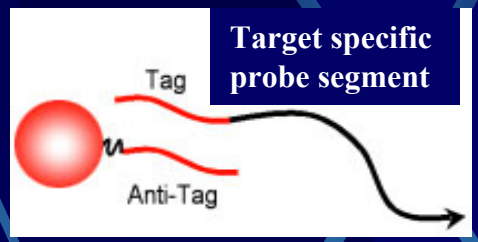
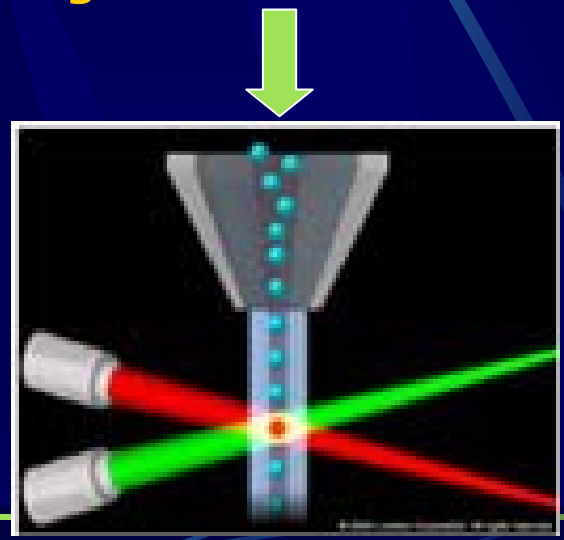




Quantification of 100 Analytes Simultaneously

- DNA Sequences
- RNA Sequences
- Proteins
- Others possible



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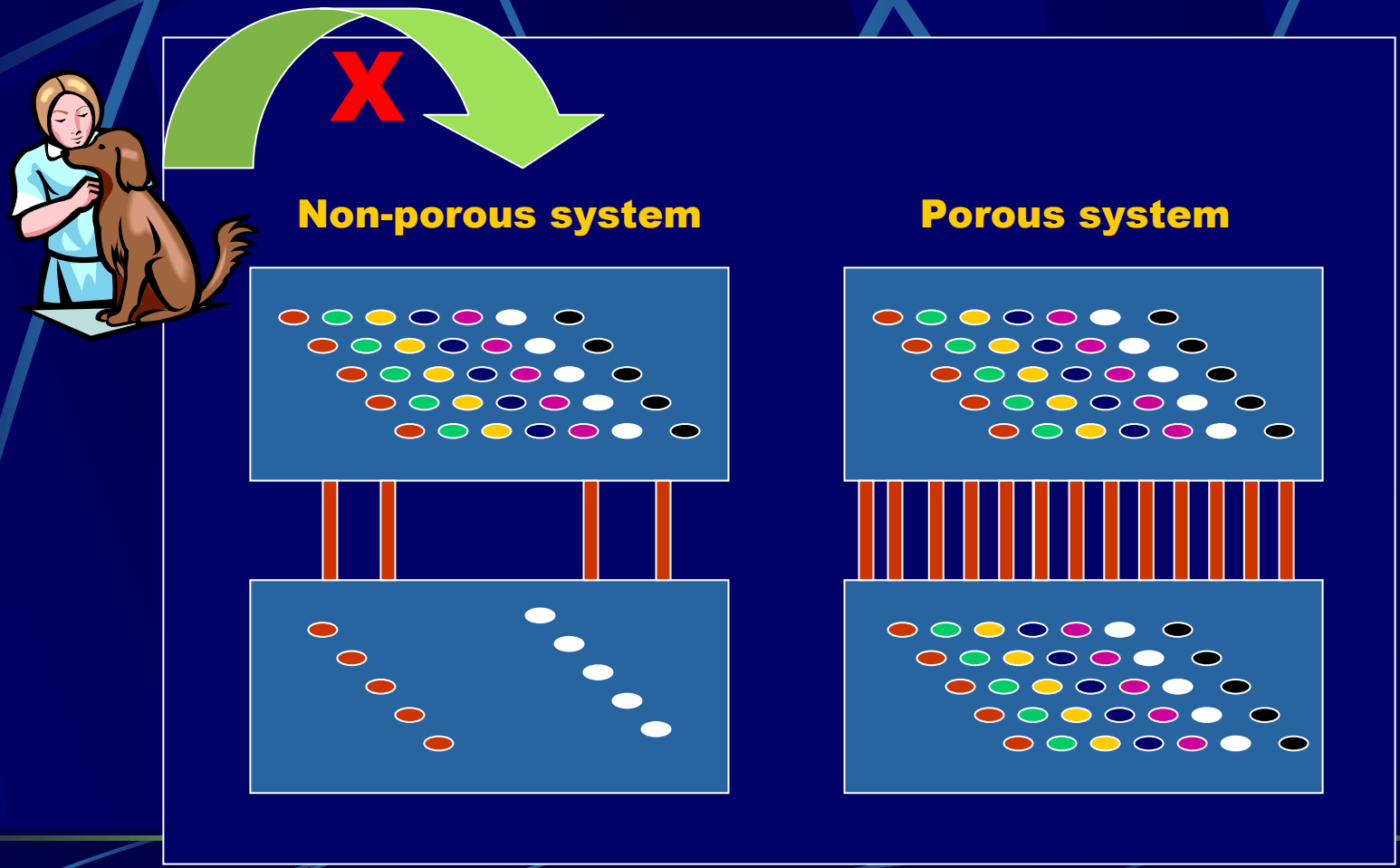
Research Overview

- Determine the degree to which surface and groundwater are distinct- are there few or many sources of contamination?
- Trial of alternative fecal microbial indicators
- Development of abiotic tracking methods

Relative Levels of Bacterial Genetic Diversity Reflect Surface-Groundwater Connectivity



What's with *E. coli*?

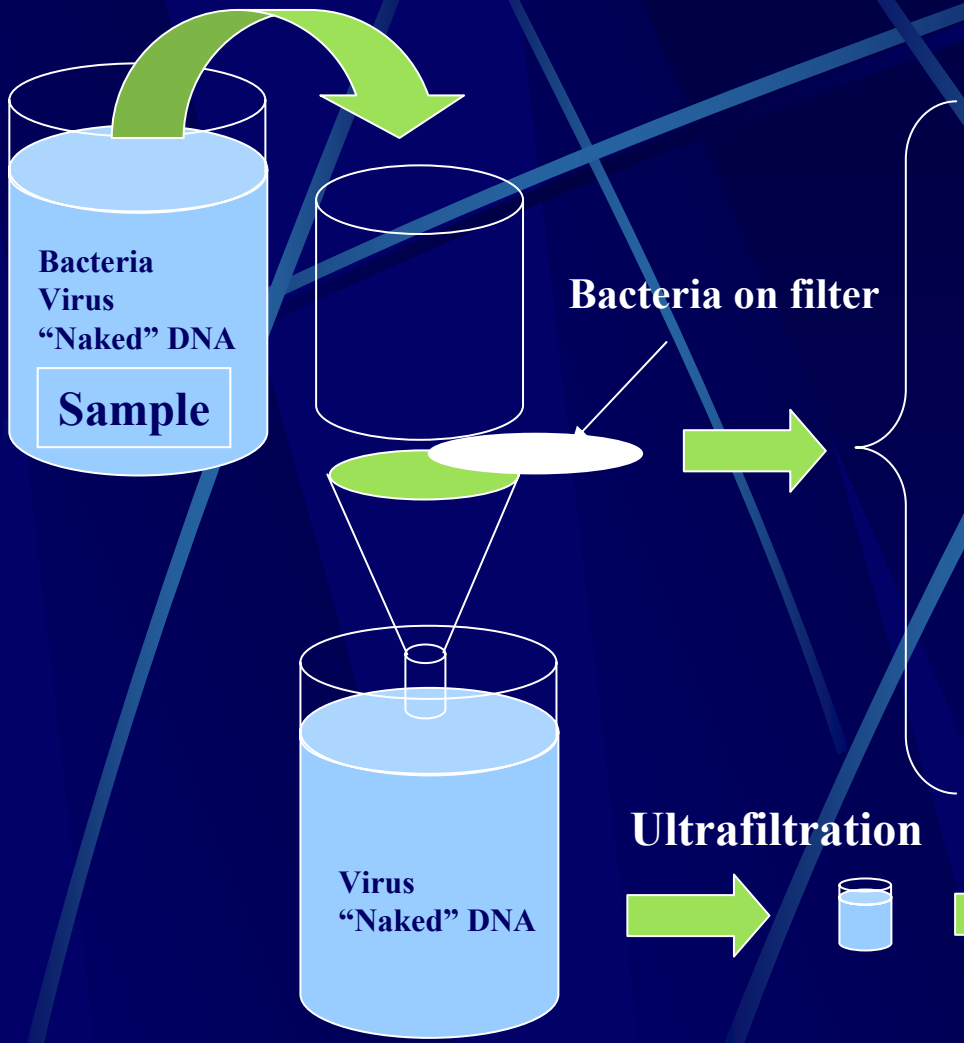
- Most widely used microbe for regulation of drinking water quality
- Well known genome- several strains completely sequenced
- Easy to culture and identify

BUT—

- **No evidence of true host specificity**

Fecal Anaerobes, Virus, and Virulence Determinants Generally Thought to Be More Host-Specific

- *Bifidobacterium dentium*
- *Bifidobacterium adolescentis*
- *Bacteroides-Prevotella*
- *Enterovirus*
- *Heat Labile Toxin IIa (LTIIa)*- Bovine marker
- *Heat Stable Toxin II (STII)*- Swine marker



Standard overnight culture

- Fecal coliforms
- *E. Coli*
- *Enterococcus*

Polymerase Chain Reaction

- *Bifidobacterium*
- *Bacteroides*
- Virulence determinants

Polymerase Chain Reaction

Enterovirus

"Naked" DNA

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Non-Microbial Tracking

- “Naked” (host) DNA
- Synthetic Oligonucleotide Tags
  - Free “armored” oligos
  - Oligos bound to fluorescent magnetic beads
- Raman infrared spectroscopy
  - Detection of laundry and agricultural chemicals