

Microbial Indicators and Onsite Wastewater Treatment Systems: What Do We Really Know?



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Outline

The **indicator paradigm**

Methods for identification and enumeration of microbial indicators

Case studies: methods are not fail-safe

Discussion



Why Microbial Indicators?

25% of U.S. homes using septic systems

More than 4 billion gallons of wastewater per day dispersed below the ground surface

Adequately managed systems can protect public health and the environment



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10% of onsite systems have stopped working – 3rd most common source of groundwater contamination



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Bacteria that are indicators of fecal contamination



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Criteria:

- Should be **present in the fecal material** of warm-blooded animals
- Should “**indicate**” the **presence of pathogens** but should have a longer survival time than pathogens
- Should **not grow** in environmental samples

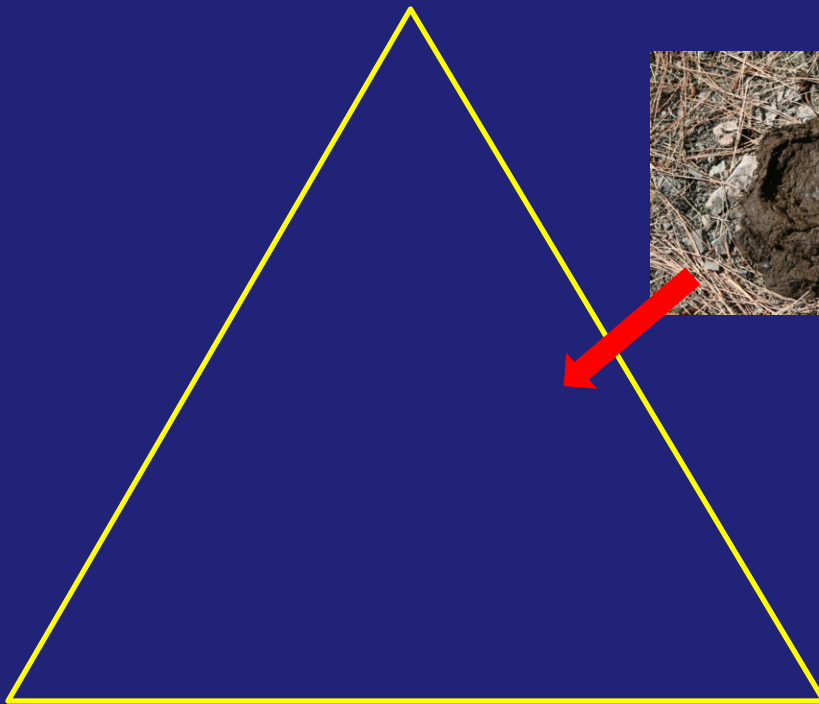


Why Microbial Indicators?

- Should be present in the fecal material of warm-blooded animals
- Should “indicate” the presence of pathogens but should have a longer survival time than pathogens
- Should not grow in environmental samples
- Relatively inexpensive to measure
- Measurements are accurate
- Total and/or Fecal coliforms;
Escherichia coli; *Enterococcus*

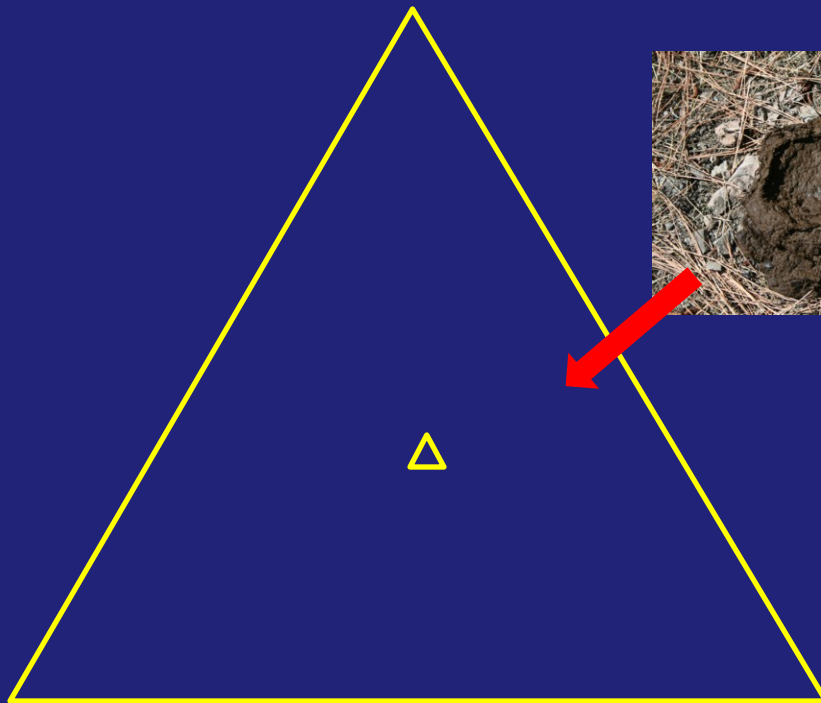


Why Microbial Indicators?



100 Billion bacteria per gram

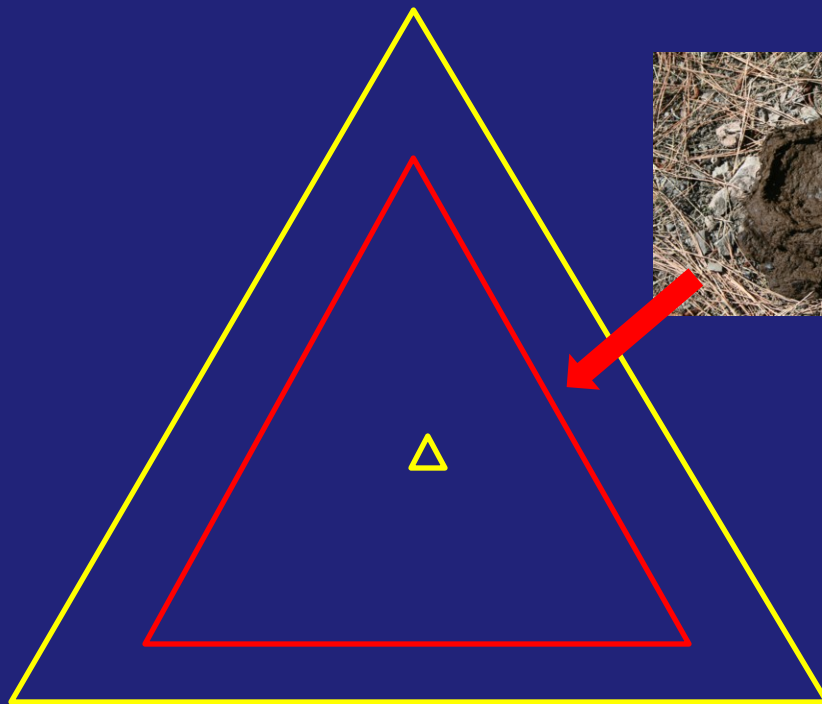
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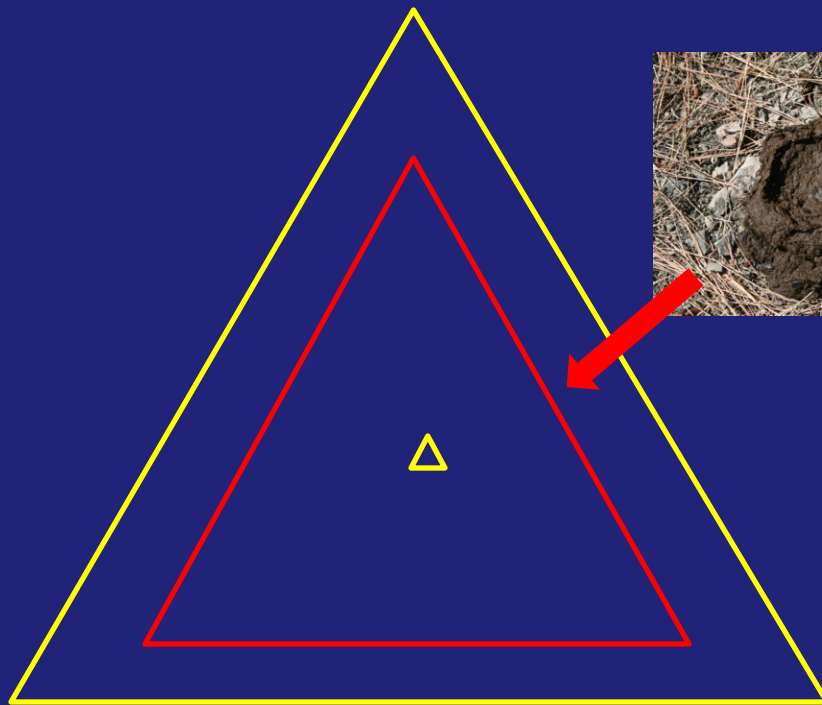


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Pathogens: have the ability to cause disease

Thus, use indicators

Why Microbial Indicators?



Statistical modeling has **estimated** predictive relationship between indicators and pathogens

Growth in environment = reduced utility as an indicator

Do Indicators Grow in the Environment?



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1999: Byanppanahalli and Fujioka – evidence for growth of *E. coli* in tropical soils

2004: Yamahara et al. – Growth of enterococci in sterile beach sands

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Total coliforms, fecal coliforms

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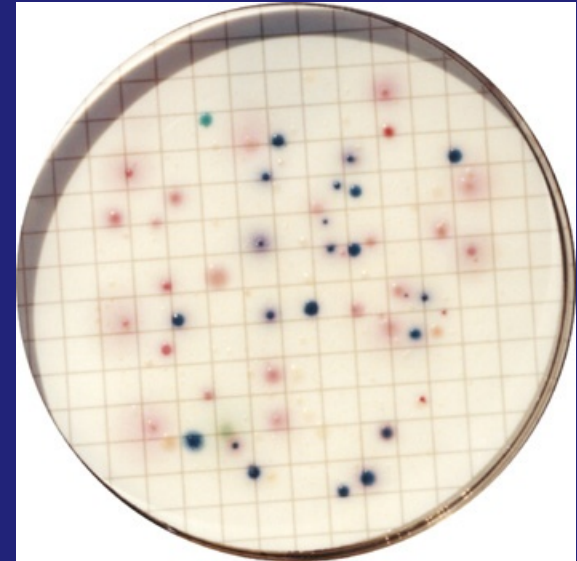
Defined Substrate Technology



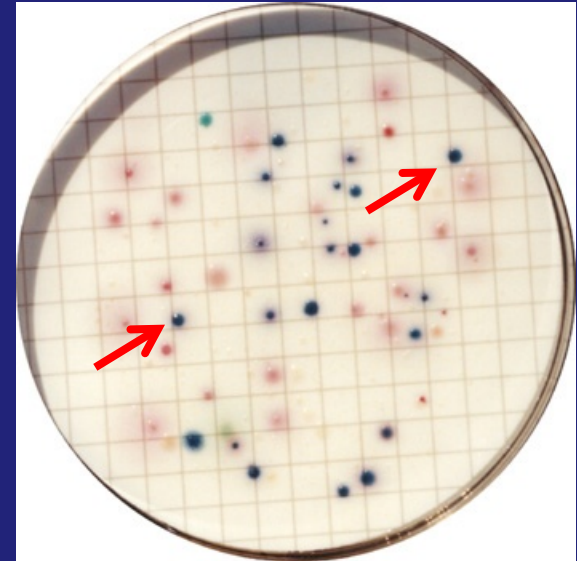
Membrane Filtration



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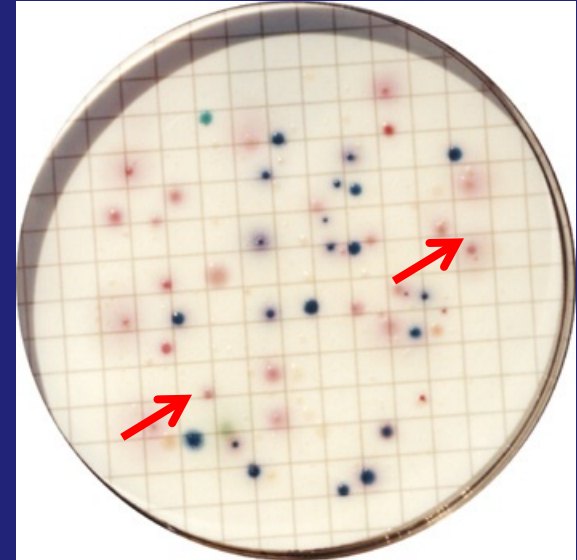


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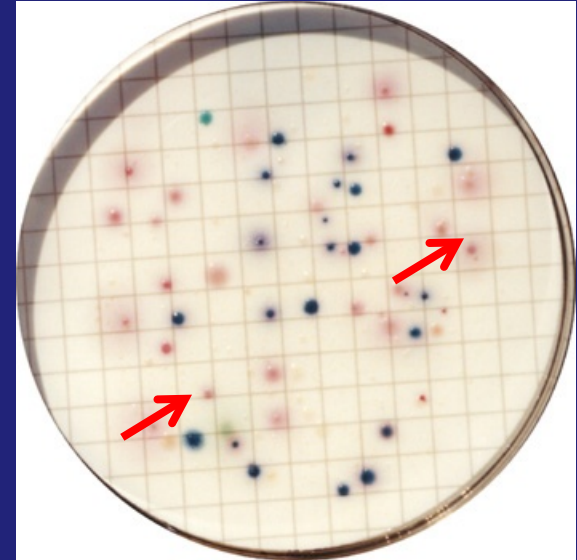
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- *E. coli*, Enterococcus, Total and Fecal Coliforms
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Constructed Wetland, Phoenix, AZ

- Tertiary-treated reclaimed municipal wastewater
- Water entering wetland: **no viable *E. coli***



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~ 2,500 CFU 100 mL⁻¹
- Guidelines for urban irrigation:
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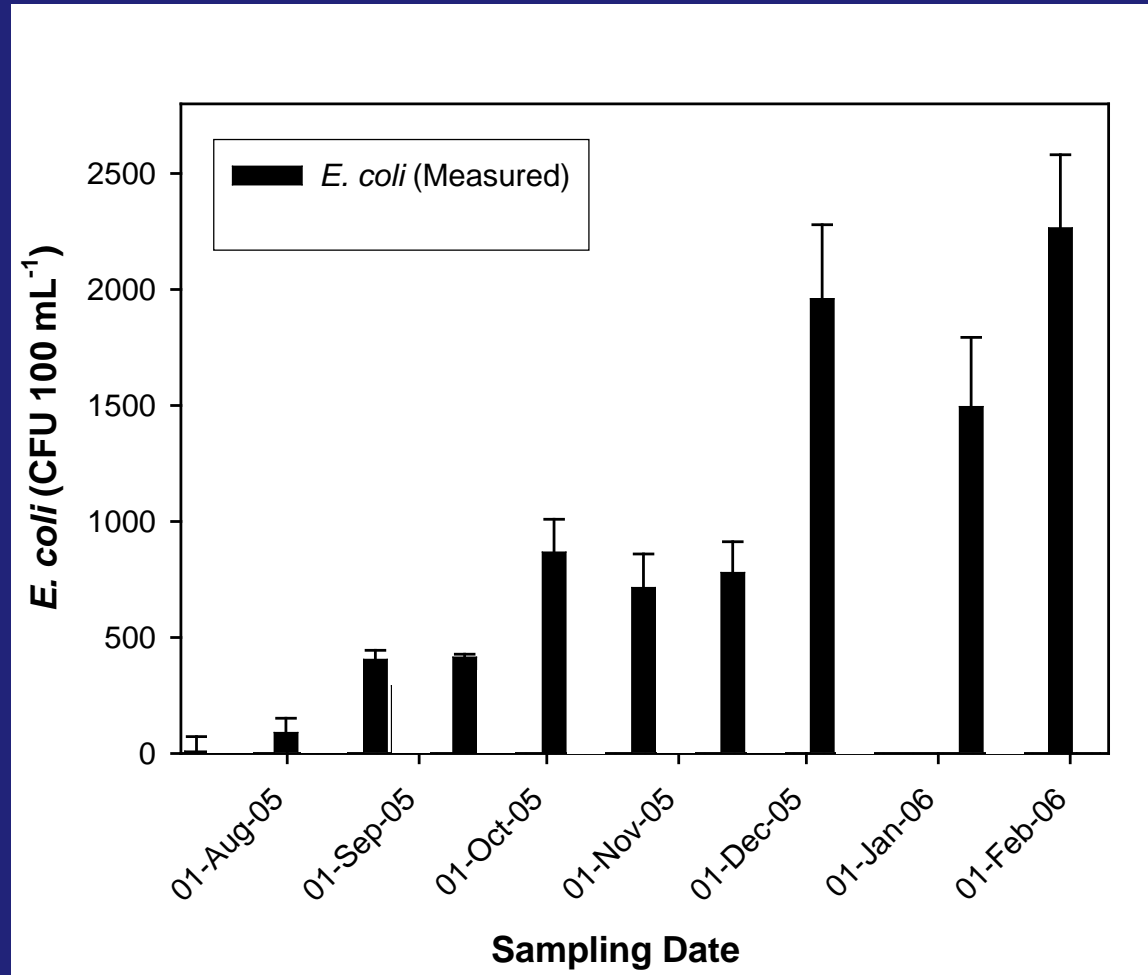


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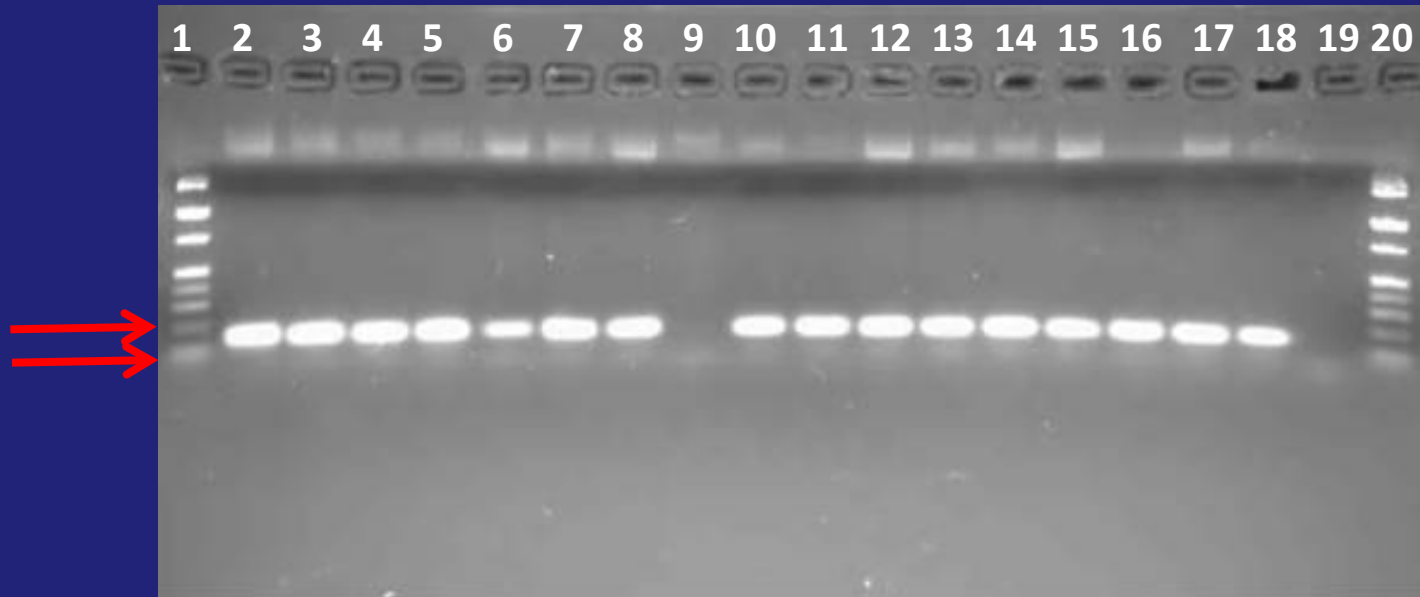
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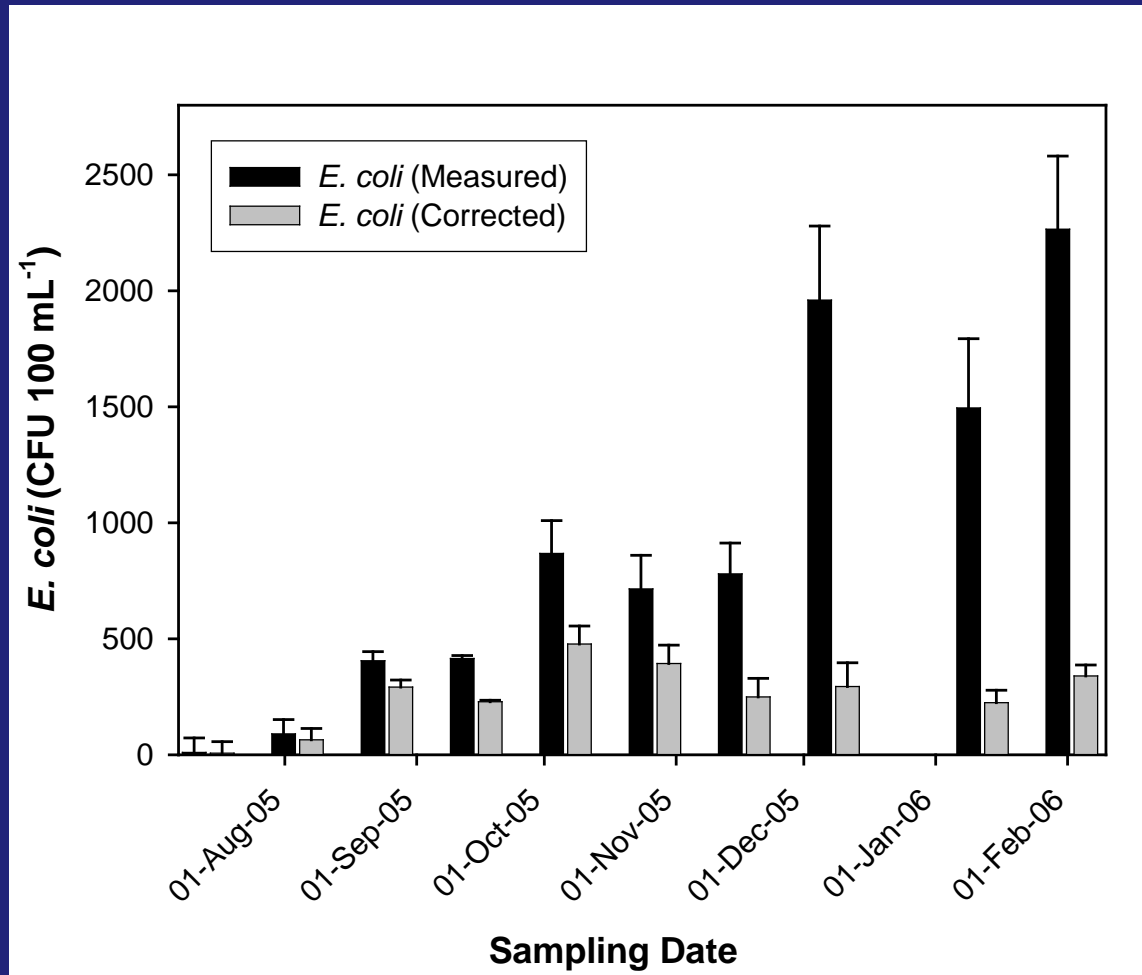


PCR Confirmation of Selected Isolates

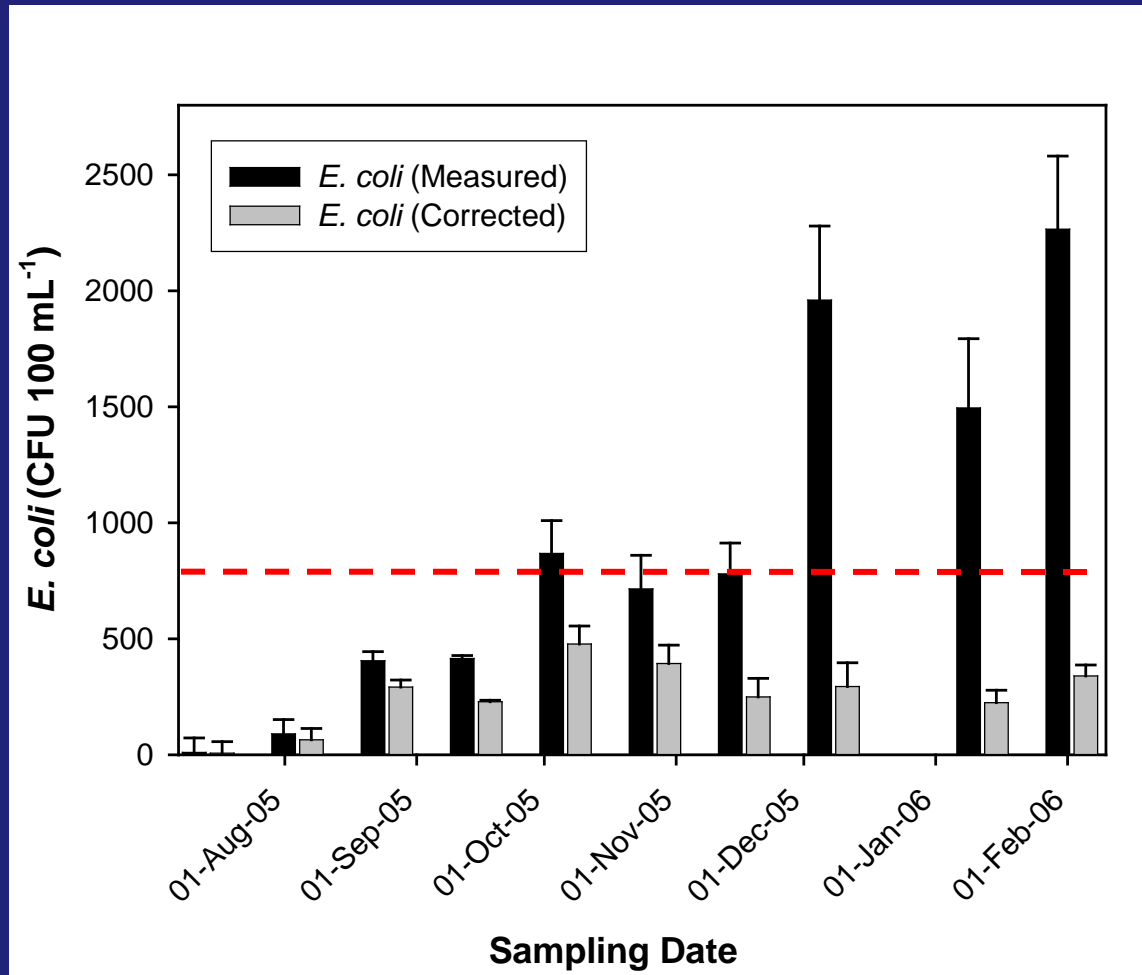


Gene target codes for outer membrane protein
common to all known *E. coli*: 116 bp

Corrected Data: *E. coli* in Wetland Outflow



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Water Quality: Pinal and Yuma Counties

- Colilert, Membrane Filtration



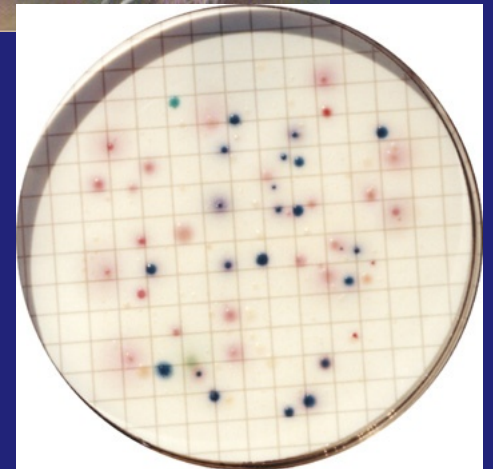
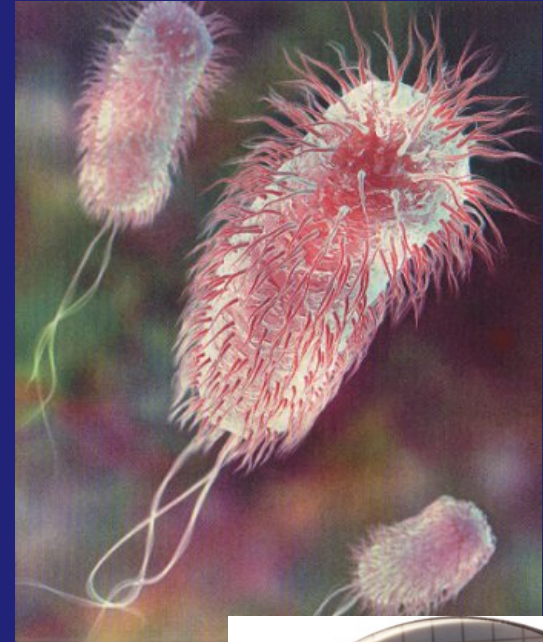
Water Quality: Pinal and Yuma Counties

- Colilert, Membrane Filtration
- Rate of “false positives” lowest in Tucson stormwater (9.1%)
- **More than 35%** in irrigation water and irrigated soils



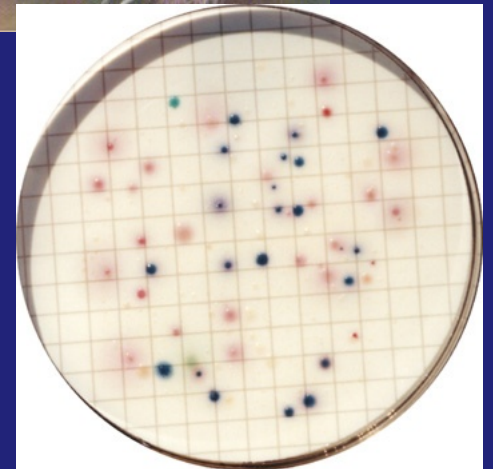
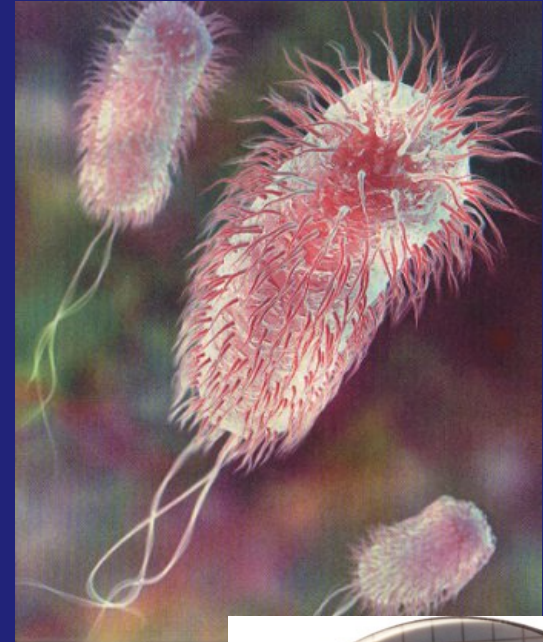
What Are The Implications?

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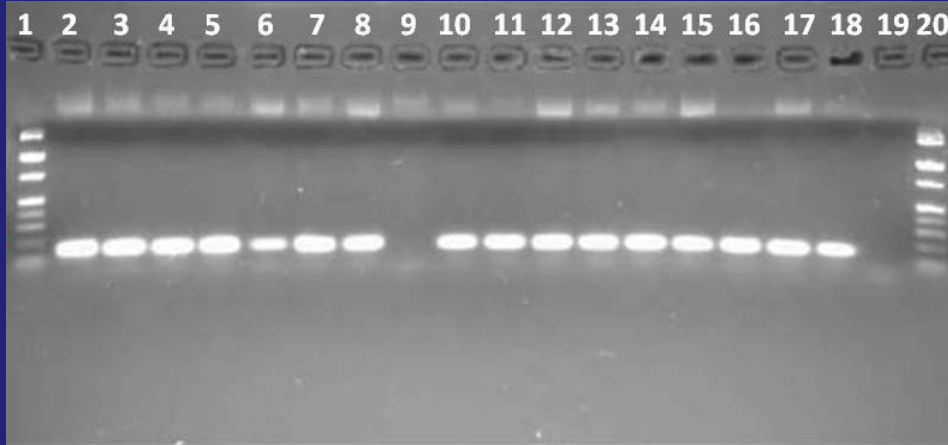


What Are The Implications?

- Sequencing confirms PCR results
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 - *Klebsiella*
 - *Brachy bacterium*,
Ochrobacterium, *Lysinibacillus*

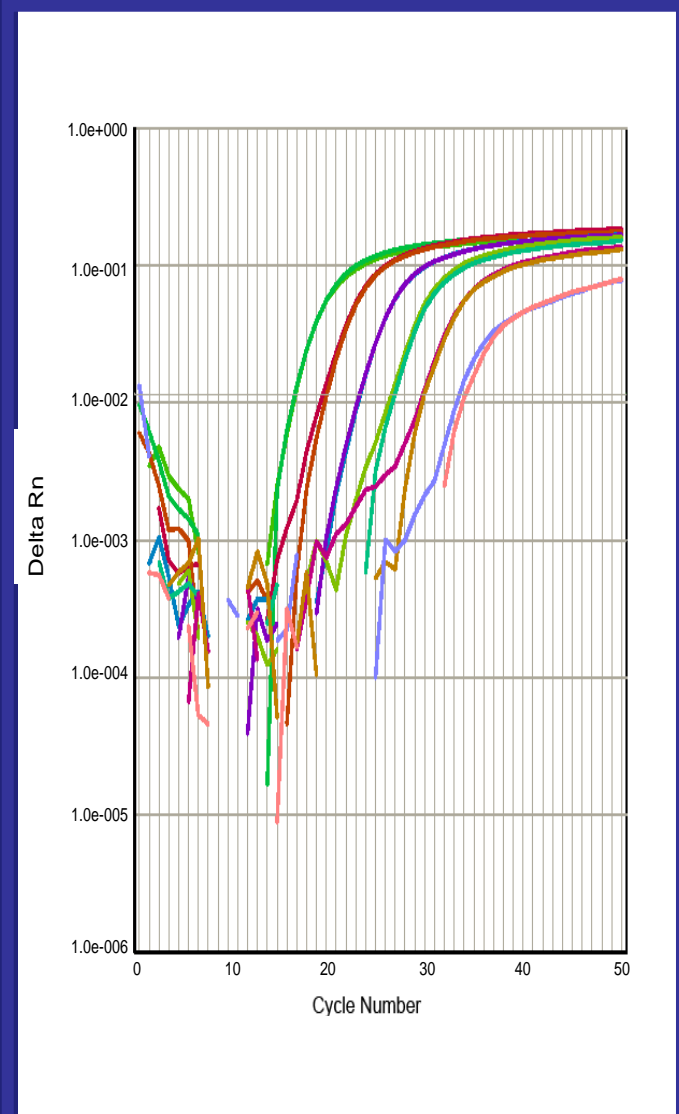


Molecular Methods: PCR, qPCR

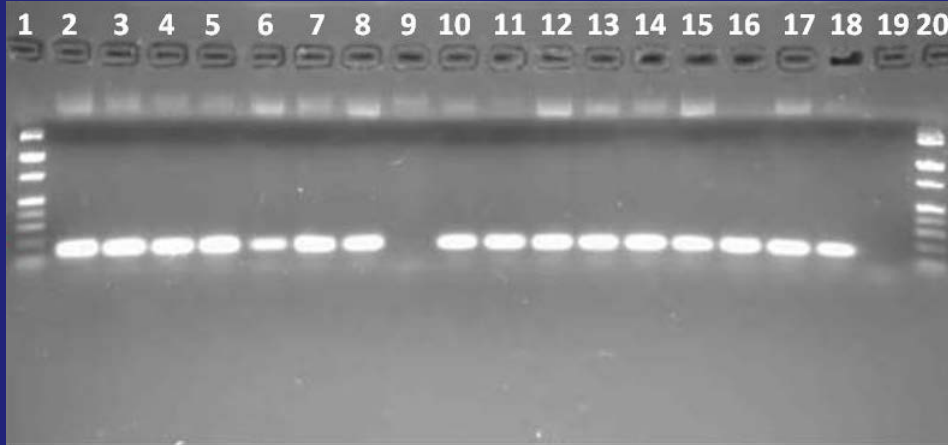


Amplifying a DNA region of interest that is unique to the target bacterial group

Inexpensive? Easy?



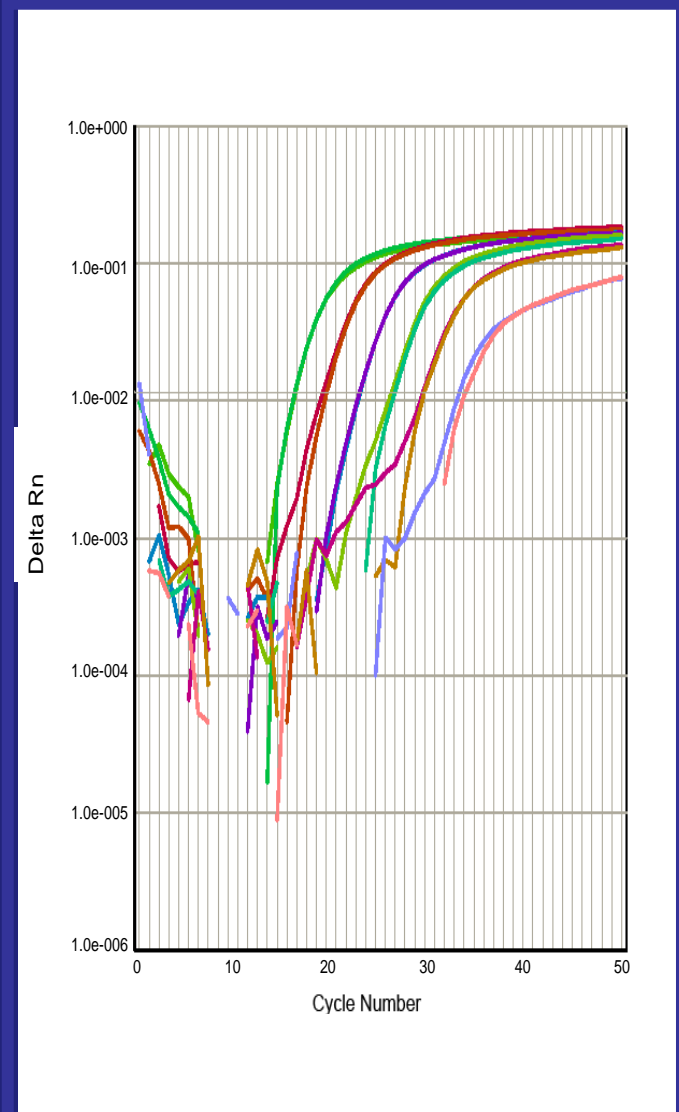
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Microbial and Chemical Source Tracking

Methodologies aimed at identifying dominant sources of contamination in environmental samples

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Phenotypic
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Molecular
markers

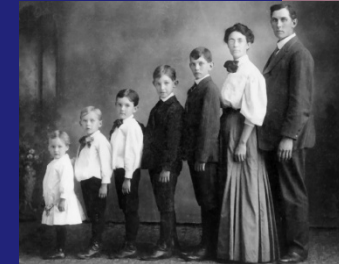
Microbial Source Tracking Using Host-Specific *Bacteroides* 16s rRNA Molecular Markers

Feces, rumens, and other cavities of humans and other animals

THE PROS

Strict anaerobes (limited potential for growth in the environment)

Host-specific genetic markers can be used to evaluate fecal pollution



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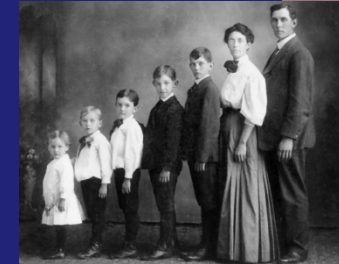
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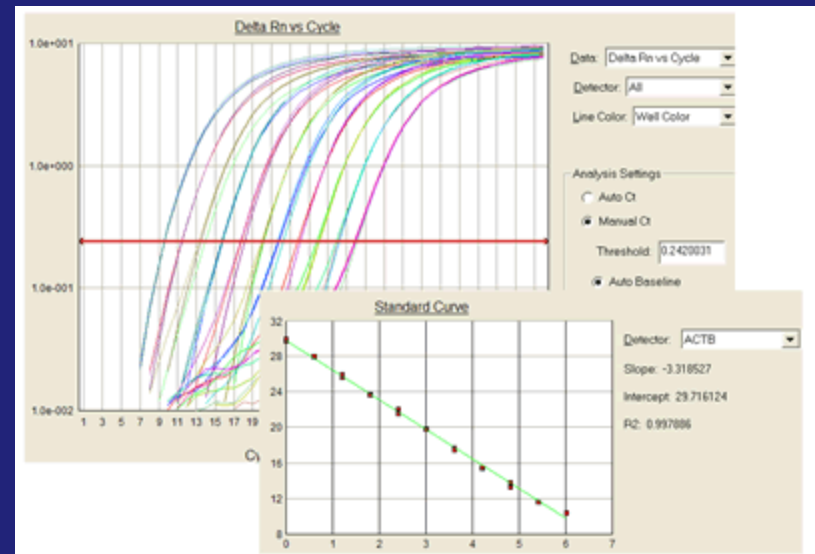
THE CONS

Misinformation abounds. “Exclusively in the guts of warm blooded-animals” (2000)



Source Tracking in Arizona

Recycled municipal wastewater pond

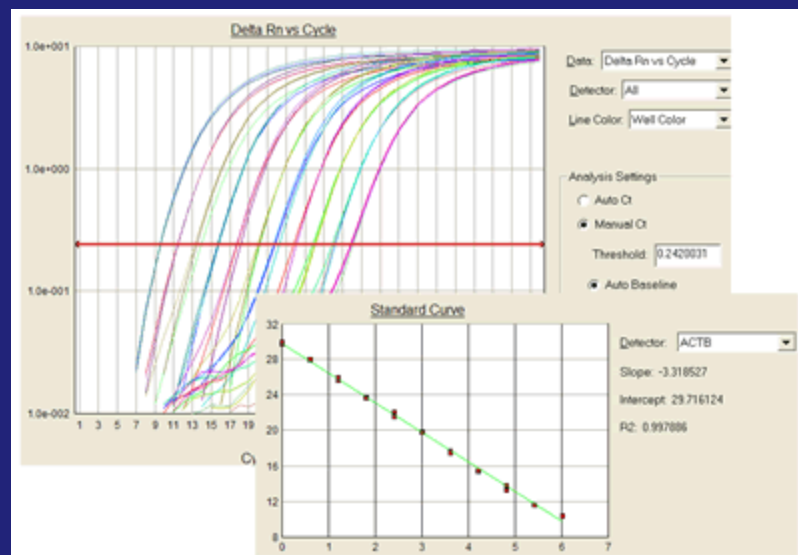


Source Tracking in Arizona

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Used real-time PCR to quantify human-specific *Bacteroides* molecular markers in pond and irrigation water

Over 6 months, human-specific markers averaged 4500 per 100 mL of water



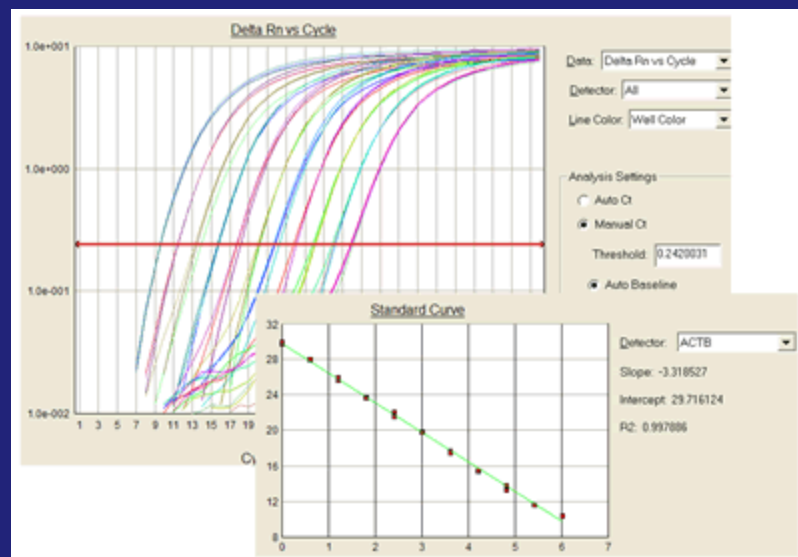
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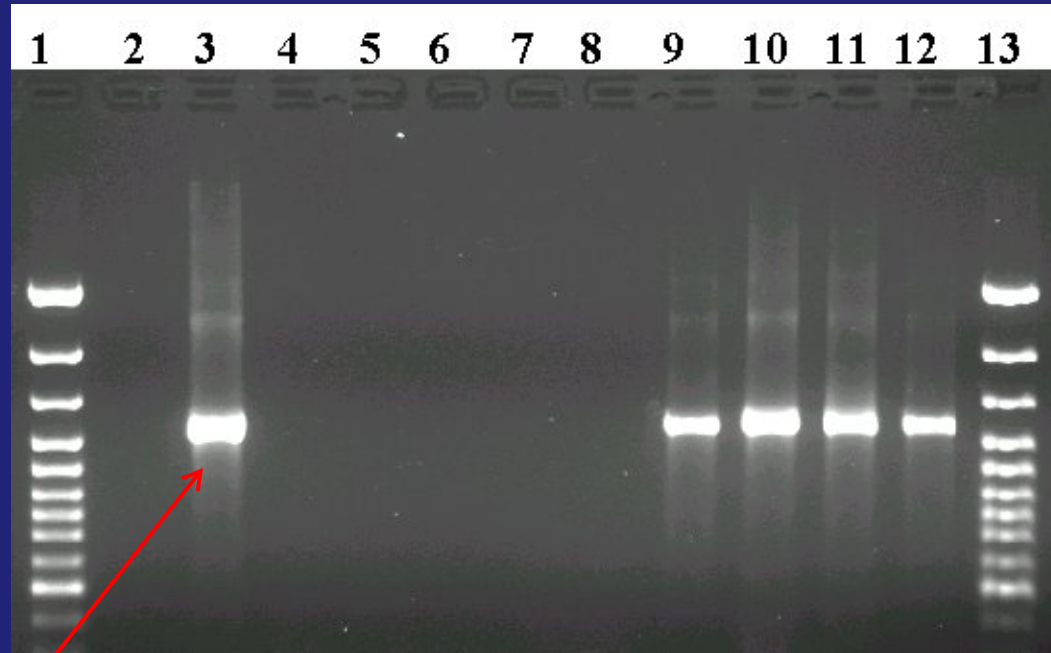
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Did we identify human fecal contamination?

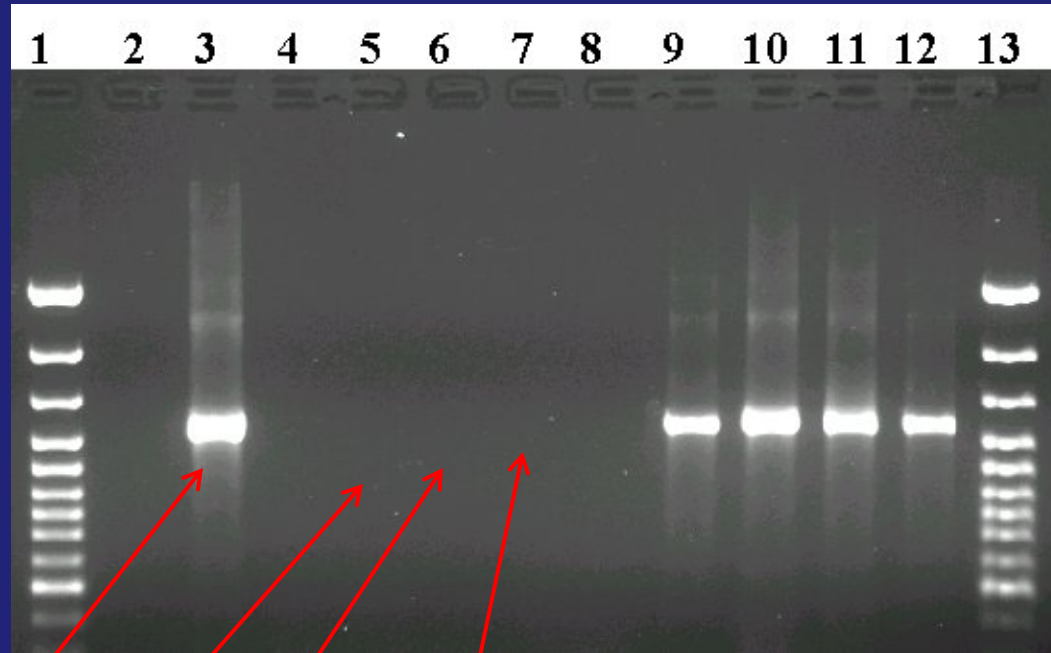


Validation of PCR Results with Known Fecal Sources



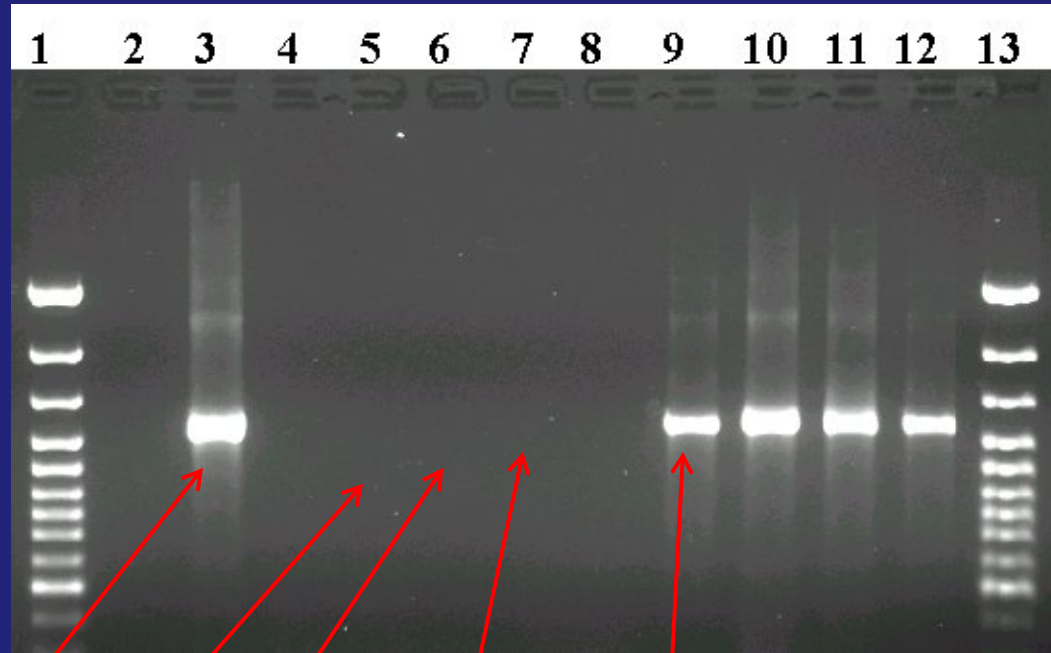
Human

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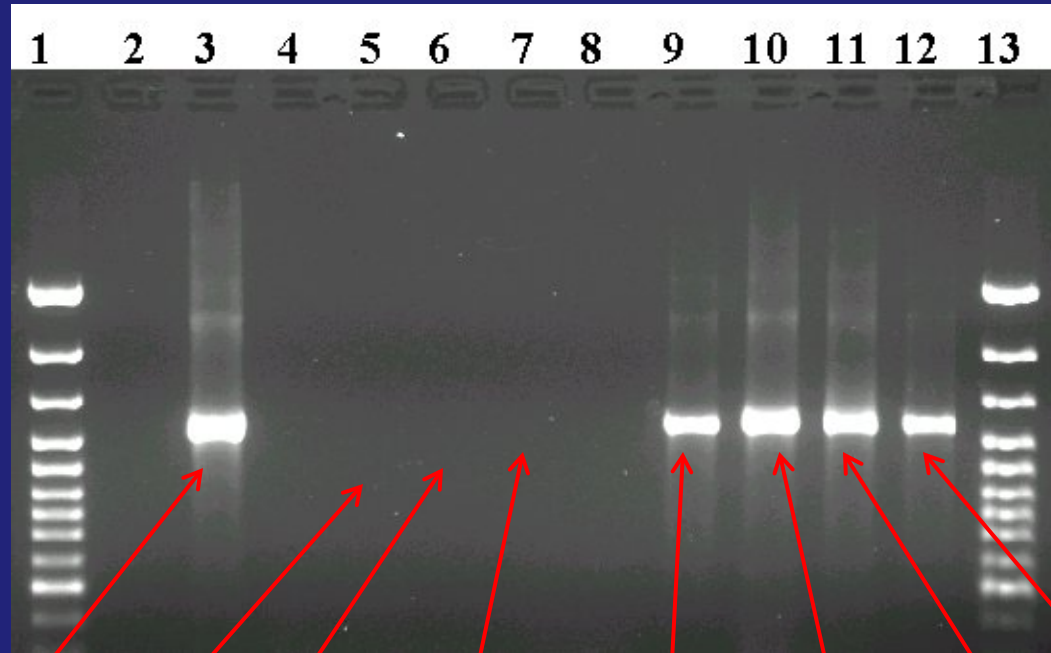
Human, Dog, Duck, Bovine

Validation of PCR Results with Known Fecal Sources



Human, Dog, Duck, Bovine, Tilapia

Validation of PCR Results with Known Fecal Sources



Human, Dog, Duck, Bovine, Tilapia, Catfish, Trout, Carp

Validation of PCR Results with Known Fecal Sources

4 of 5 “Human-specific” assays
cross-amplified with at least one
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Validation of PCR Results with Known Fecal Sources

4 of 5 “Human-specific” assays cross-amplified with at least one fish species

Affects conclusions of published source tracking studies performed in **water bodies containing fish.**



Benefits of Available Technology for Identification/Enumeration of Indicator Bacteria

A sound concept for predicting the presence of pathogenic bacteria



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A sound concept for predicting the presence of pathogenic bacteria

- **Total coliforms:** General sanitary conditions
- **Fecal coliforms:** Shellfish and shellfish harvest waters
- ***E. coli*:** Recent fecal contamination
- ***Enterococcus*:** beach/bathing waters



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Statistical methods for enumeration: an estimate, **not a “hard number”**



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Molecular methods: “where we are going”



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Statistical methods for enumeration: an estimate, not a “hard number”

Molecular methods: “where we are going”

Knowledge of limitations stimulates open dialogue and is very important in development of standards (my opinion)



Acknowledgements

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