# Microbial Source Tracking of Selected Sub-Watersheds in Howard County, Indiana

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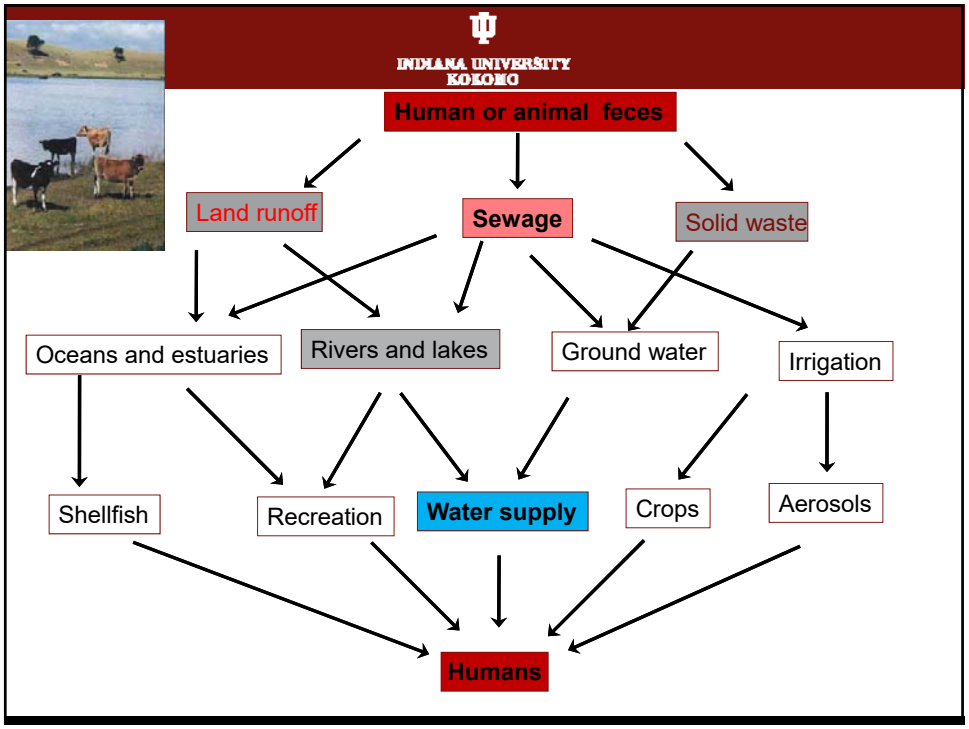
## Waterborne Diseases
## Some pathogens are often found in water

- $10^{12}$ bacteria / g feces
- Survival and/or growth in water
- Fecal contamination from:
  - ✓ Sewage discharges
  - ✓ Leaking septic tanks
  - ✓ Runoff from animal feedlots during rainfalls/snow melts
  - ✓ Birds and other wildlife/domestic animals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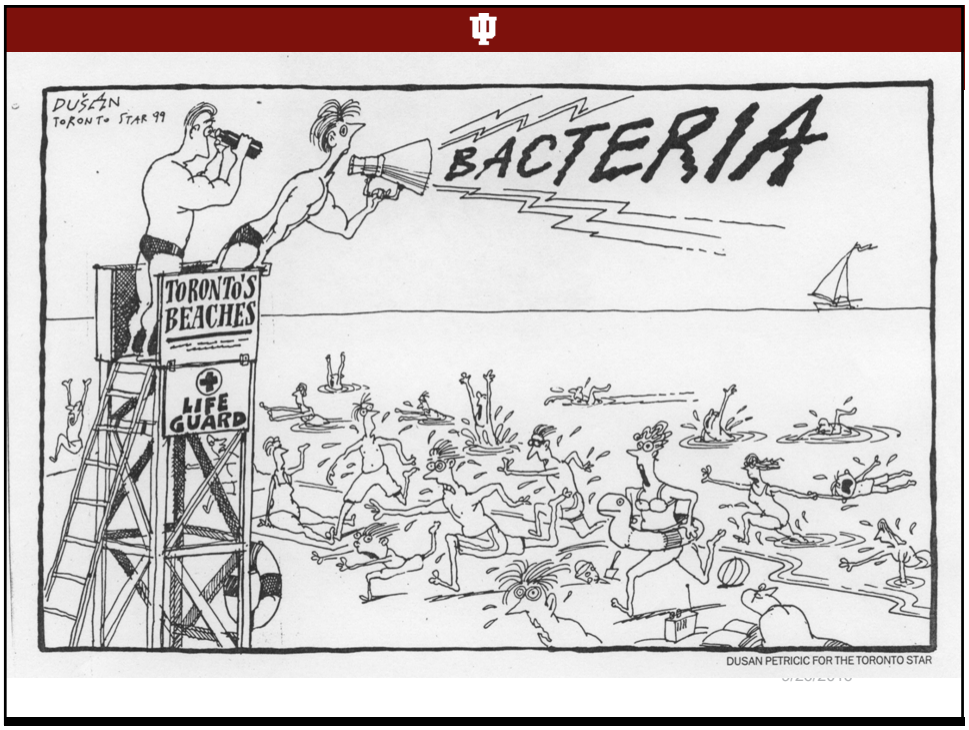
Human or animal feces
Land runoff
Sewage
Solid waste
Oceans and estuaries
Rivers and lakes
Ground water
Irrigation
Shellfish
Recreation
Water supply
Crops
Aerosols
Humans


DUŠAN
TORONTO STAR '99
BACTERIA
TORONTO'S BEACHES
LIFE GUARD
DUSAN PETRICIC FOR THE TORONTO STAR

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## Sources of fecal contamination









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## "Feces" production in the U.S. (estimates)

| | Feces or manure (kg/day/head) | U.S. Population Size (annual average) | Feces Production per Year (kg) |
|---|---|---|---|
| Swine | 4.5 | 66 millions | $1.08 \times 10^{11}$ |
| Dairy cows | 50 | 9.1 millions | $1.66 \times 10^{11}$ |
| Beef cattle | 25 | 89 millions | $8.12 \times 10^{11}$ |
| Chickens | 0.02 | 500 billions | $3.65 \times 10^{11}$ |
| Humans | 0.3 | 300 millions | $3.29 \times 10^{10}$ |
| Total | | | **$1.48 \times 10^{12}$** |

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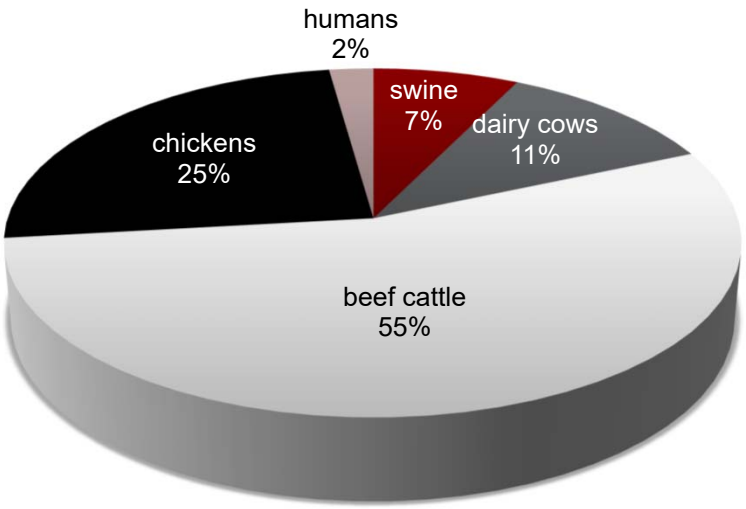


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## Major Waterborne Pathogens

| Microorganisms | Type of Microorganism | Main Sources | Disease Characteristics |
|---|---|---|---|
| *Giardia* | Protozoa | Wild animals, Humans | Long incubation, long duration (GI) |
| *Cryptosporidium* | Protozoa | Wild & farm animals, humans | Long incubation, long duration (GI) |
| *E. coli* (enterohemorrhagic and enterotoxigenic strains) | Bacteria | Wild & farm animals, humans | Watery and bloody diarrhea, can lead to hemolytic uremic syndrome |
| *Shigella* | Bacteria | Wild & farm animals, humans | Watery and bloody diarrhea |
| *Salmonella* | Bacteria | Poultry, birds | Cramps and diarrhea |
| *Campylobacter* | Bacteria | Wild & farm animals, humans, poultry | Watery and bloody diarrhea |
| Noroviruses | Viruses | Humans | Vomiting and diarrhea |
| Rotaviruses | Viruses | Humans | Vomiting and diarrhea in children |

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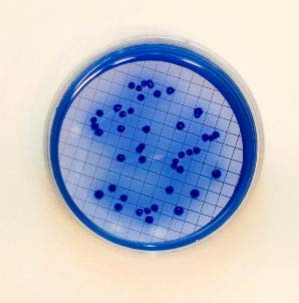
## True pathogens are often difficult to detect & identify in the lab - $$$ & time consuming methods

- Use "indicator microorganisms"
- Ideal characteristics:
  - Be associated with intestinal tract
  - Be present when pathogens are present
  - Be at least equally resistant as pathogens
  - Be present in greater numbers
  - Easy and cheap detection
  - Non pathogenic



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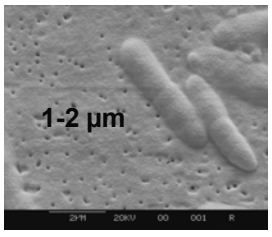
## *E. coli* and fecal coliform indicators

- Bacteria associated with human or animal wastes
- Coliforms: facultative anaerobic, gram-negative, nonspore forming, rod-shaped bacteria that ferment lactose with gas within 48 hrs at 35° C (at 44.5° C for "fecal coliforms")
  - Intestinal tracts (fecal coliforms)
- Their presence in water (or in food) is a strong indication of recent sewage or animal waste contamination.



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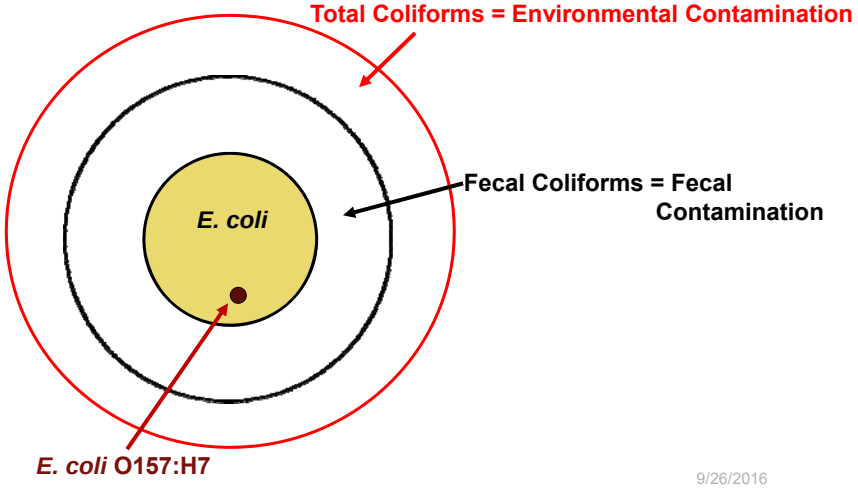
## Total Coliforms, Fecal Coliforms, *E. coli*, and *E. coli* O157:H7

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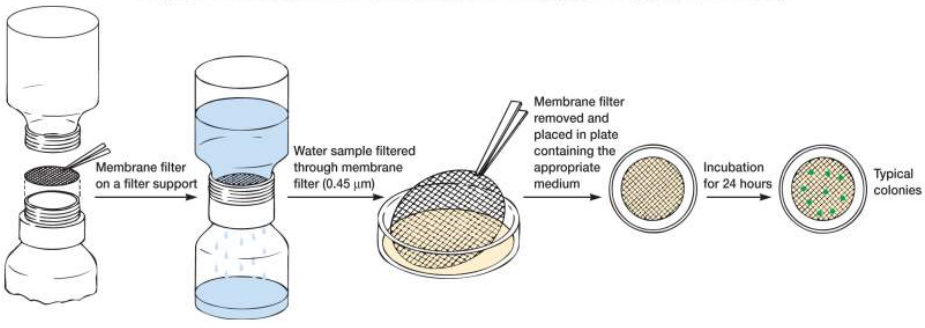
## "Traditional" Microbiological Tests



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## "Traditional" Microbiological Tests



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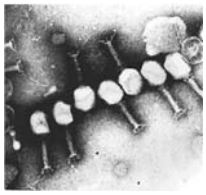
## Other Microbial Indicators

- *Bacteroides fragilis* (bacterium)



- Coliphages
- Bacterial spores


- Fecal streptococci

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## Sources of fecal contamination

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## What is Microbial Source Tracking

- Various methods used to match detected microorganisms with specific animal sources of fecal pollution
- Often done at the genetic level

- <u>Assumption:</u>

*Intestinal microorganisms of different animal groups are expected to be different.*

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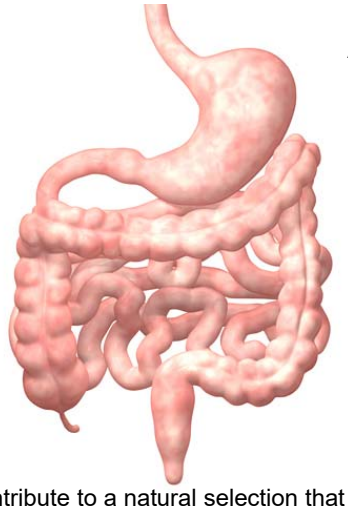
## Factors affecting microbial flora

**Temperature and pH**

**Diet**

**Antibiotic use**



**Anatomy and physiology**

**Microbe-microbe interactions**

**Host-microbe interaction**

These factors contribute to a natural selection that produces a unique microbial flora, even at the genetic level (genotypes)…

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## Microbial Source Tracking

- Used to supplement traditional methods
- Sources of pollution
  - ❑ Beach contamination
  - ❑ TMDL issues, source assessment & prevention
- Risks
  - ❑ Human vs. non-human

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# Total Maximum Daily Loads (TMDLs)

- Estimate significant sources of pollution
- Used to prioritize impaired waters
- **Involved source assessment and prevention**

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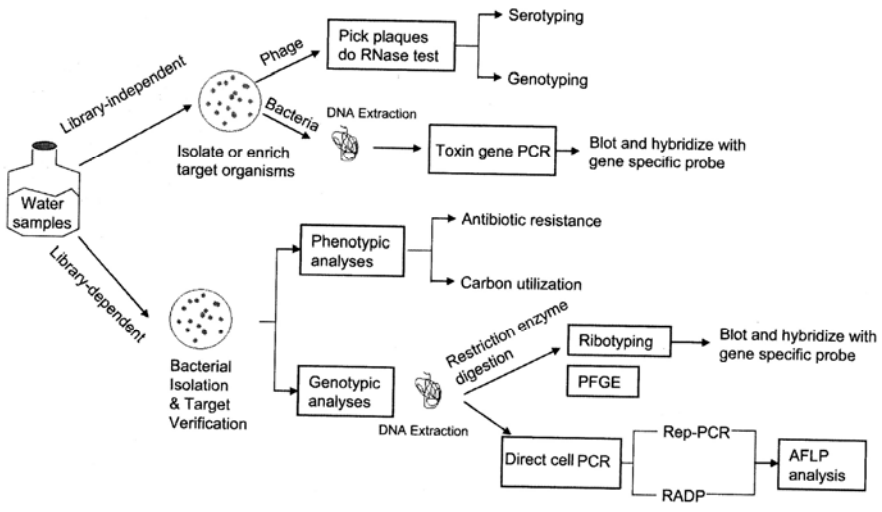


**Overview of microbial source tracking methods (source: Harwood *et al.* 2011)**

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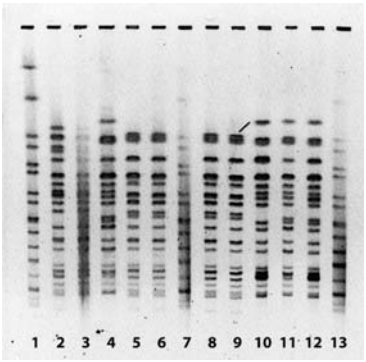
## Library-Dependent Methods

- Large local databases ("libraries") of bacterial genetic "fingerprinting" data
- Hundreds/thousands of indigenous local bacterial strains from known sources
- PFGE ("pulsed field gel electrophoresis") or ribotyping (restriction digest of specific genomic DNA)
- Compare unknowns to database
- Time and labor intensive
- Very specific for a given site

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## Library-Independent Methods

- Identification and characterization of specific genetic markers (i.e. specific genes or DNA sequences) that have been shown to be host specific
  - E.g.: Detection of host-specific bacterial genes by PCR (polymerase chain reaction)
- Not site specific and do not require a local library
- But do not provide the same level of discrimination as library-dependent methods

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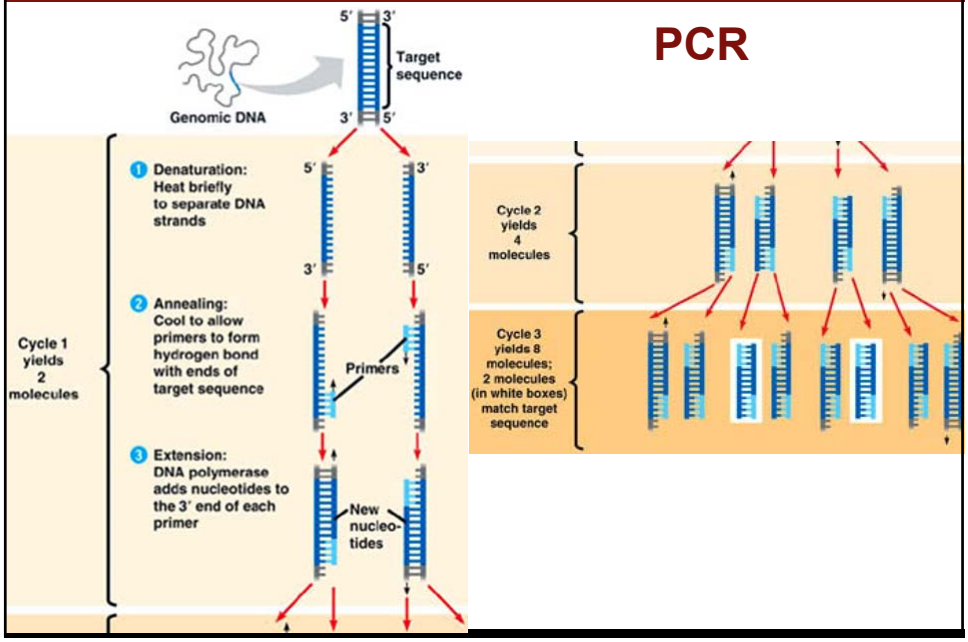
# PCR

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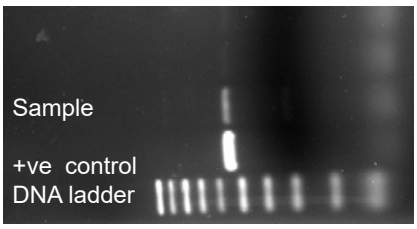
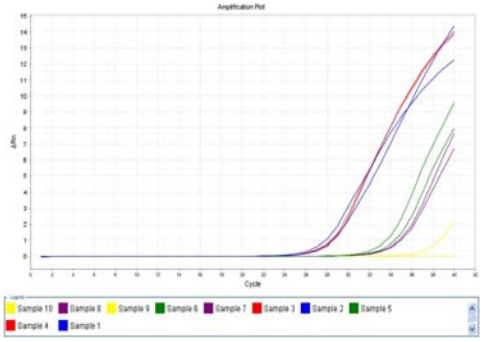
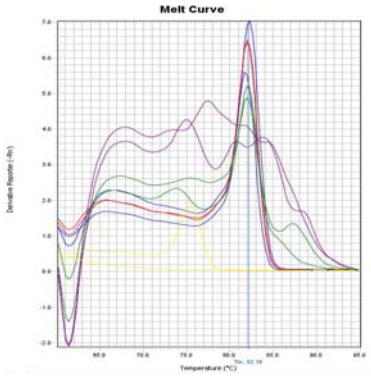
# PCR Products (results)





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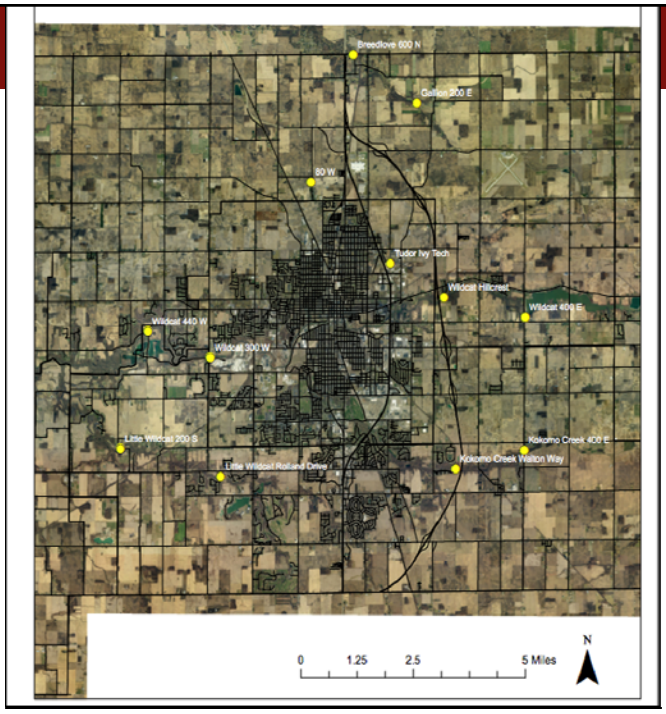
## Study Goals and Design

- IU Kokomo collaborated with the Howard County Stormwater District to pilot a few selected library-independent MST methods.
- Another goal was to test genetic markers of bacterial virulence.
- Twelve samples per week were collected over a period of 8 weeks from several sub-watershed sites (summer 2015).
- The samples were analyzed for the presence of *E. coli* as well as for host-specific genetic markers and other non-host specific markers.



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| Site Description | Type of sample |
|---|---|
| 1 McKay 80W | McKay Dredge: open ditch with tile drainage from cropland and minor urban areas |
| 2 Breedlove | Breedlove Drain: natural stream with tile and surface drainage from rural residential and cropland |
| 3 Galion | Galion Drain: natural stream with tile and surface drainage from rural residential and cropland |
| 4 Tudor | Tudor Drain: open ditch with tile and surface drainage from cropland and urban areas |
| 5 WCE Hillcrest | Wildcat Creek: natural stream with tile and surface drainage from mixed rural residential and cropland |
| 6 WCE 400E | Wildcat Creek: natural stream below spillway outfall of 484 acres reservoir |
| 7 KC 400E | Kokomo Creek: natural stream with tile and surface drainage from rural residential and cropland |
| 8 KC Walton | Kokomo Creek: open ditch with tile and surface drainage from cropland rural and residential |
| 9 LWC Rolland | Little Wildcat Creek: Natural stream with tile and surface drainage from mixed urban residential and cropland |
| 10 LWC 200S | Little Wildcat Creek: Natural stream with tile and surface drainage from rural residential and cropland |
| 11 WCW 440W | Wildcat Creek: natural stream with tile and surface drainage from urban, residential and cropland approx. 4.8 miles downstream of WWTP outfall |
| 12 WCW 300W | Wildcat Creek: natural stream with tile and surface drainage from urban, residential and cropland approx. 2.7 miles downstream of WWTP outfall |

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## Gene markers and DNA targets

| Gene/DNA target | Sources | Growth medium | Medium purpose | Organism | Reference |
|---|---|---|---|---|---|
| ***eae*** | Pig, bovine, and humans | EC Broth | Enrichment | *E.coli* (virulence marker) | Wang, 2002 |
| ***ltII*** | Bovine | EC Broth | Enrichment | *E.coli* | Chern et al, 2004 |
| ***stx2c*** | Bovine, human | EC Broth | Enrichment | *E.coli* (virulence marker) | Wang, 2002 |
| ***rfbO81*+B2 subgroup** | Human | EC Broth | Enrichment | *E.coli* | Clermont, 2002 |
| ***trpB*** | *E. coli* positive control | EC Broth | Enrichment | *E.coli* | Clermont, 2002 |
| **HF183** | Human | none | Direct DNA extraction | *Bacteroides fragilis* | U.S. EPA |

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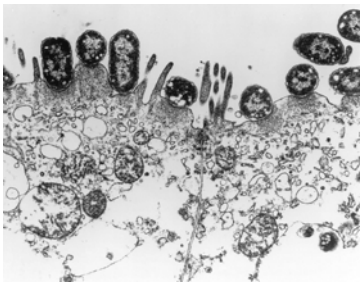
# Example of a marker – *eae* gene

- Intimin adherence protein (*eae* gene)
- Present in several pathogenic strains of *E. coli* originating from various animals including humans
- These strains are said to be enterohemorrhagic, attaching and effacing microvilli in the small intestines



(From ASM)

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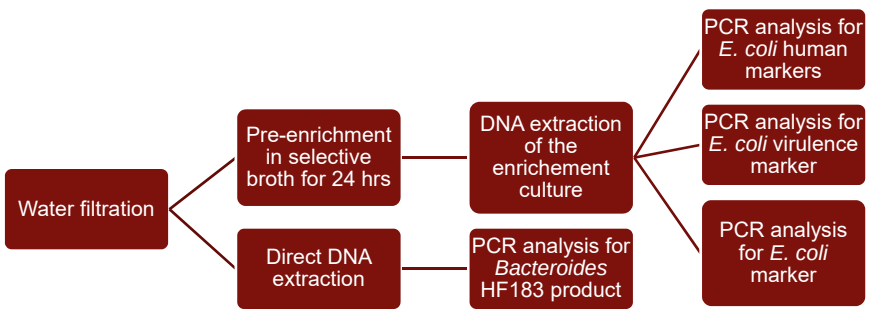

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The US EPA ("Using Microbial Source Tracking to Support TMDL Development and Implementation", 2011) recommends host specific *Bacteroides* genetic markers as one of the library-independent MST methods.

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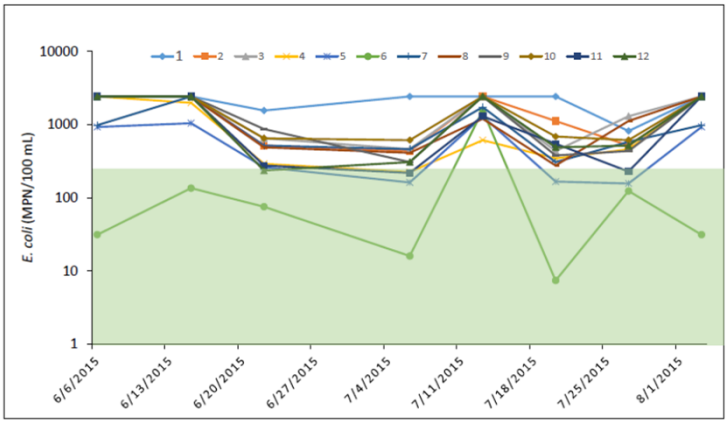
***E. coli*** **levels (MPN/100 mL) measured over the duration the study. The shaded area represents counts that are less than 235 CFU/100 mL, which is the standard for full body contact from recreational water in Indiana.**




In the vast majority of samples, the *E. coli* counts were greater than 235 MPN/100 mL indicating that these water sources are unsafe for recreational activities. A notable exception was site 6 (Wildcat Creek) in which 7 or the 8 *E. coli* counts were less than 235 MPN/100 mL.

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## Overall detection of specific genetic markers from all water samples

| Indicator/Marker | Number of samples | Number of positive samples | Percent of positive samples |
|---|---|---|---|
| ***E. coli*** **marker (trpB)** | 96 | 96 | 100.0% |
| **Virulent *E. coli* marker (eae)** | 96 | 49 | 51.04% |
| **Human *E. coli* markers (rfbO81+ B2)** | 96 | 26 | 27.08% |
| **Human *Bacteroides* marker (HF183)** | 96 | 10 | 10.41% |

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- *Escherichia coli* was detected from all 12 sites (100% of samples) using a variety of methods (genetic marker, MPN, plate counts)
- Virulent strains of *E. coli* were detected at least twice from each site
- Overall, about 51% of the samples were positive for virulence (intimin adherence protein - *eae* gene)
- Human strains of *E. coli* were detected at every site and in about 27% of samples
- The combination of two markers for detecting human strains of *E. coli* is still somewhat controversial in the literature and not fully accepted.

| | No. of samples positive by PCR/total number of samples | |
|---|---|---|
| **Site Description** | Type of sample | Human *Bacteroides* marker (HF183) |
| **1 McKay Dredge** | Open ditch with tile drainage from cropland and minor urban areas | 4/8 |
| **2 Breedlove Drain** | Natural stream with tile and surface drainage from rural residential and cropland | 0/8 |
| **3 Galion Drain** | Natural stream with tile and surface drainage from rural residential and cropland | 0/8 |
| **4 Tudor Drain** | Open ditch with tile and surface drainage from cropland and urban areas | 0/8 |
| **5 WCE Hillcrest** | Wildcat Creek: natural stream with tile and surface drainage from mixed rural residential and cropland | 0/8 |
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| **12 WCW 300W** | Wildcat Creek: natural stream with tile and surface drainage from urban, residential and cropland approx. 2.7 miles downstream of WWTP outfall | 1/8 |

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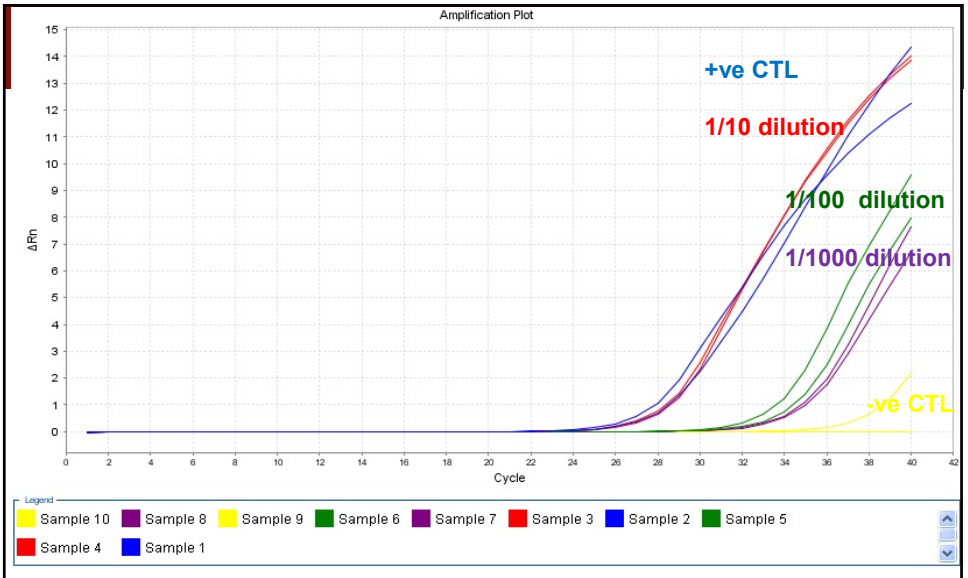
- Human strains of *Bacteroides* were not detected at every site, but were present in a variety of source waters and in about 10% of all samples
- The *Bacteroides* marker was tested using a conventional PCR method.
  - Recent literature shows that the use a real-time PCR is desirable.
  - More quantitative and presumably more sensitive, but requires more work to set up and optimize.
- Desirable to put more efforts towards the continuous improvement of the HF183 method for human *Bacteroides*.




Detection of the *Bacteroides* marker in sewage samples diluted in natural stream water by using a Real-Time PCR technique.

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## Challenges with *Bacteroides* marker

- Low numbers in water
- Limited growth in the lab (no pre-enrichment)
- Direct DNA extractions are required
  - ➢Large water volumes and low DNA yields

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## Conclusion

- MST was successfully used and demonstrated presence of human fecal contamination at some sites.
- The detection of the *Bacteroides* marker (HF183) is a promising technology
- Real time PCR is being optimized
- Future technologies?
  - ➢ Better DNA extraction methods from water samples are needed
  - ➢ Metagenomics

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## Acknowledgments


- Funding form the Howard County Stormwater District supported this study.