# Indicator and Pathogen Monitoring for Assessing Risk and Source Tracking

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# SAFE DRINKING WATER ACT ISSUES

- MCL and Treatment Technology
- New Rules: Ground water Rule,
- Long-Term Enhanced Surface Water Treatment Rule
- Watershed Protection.
- Sensitive Populations.
- Contaminant Candidate List.

# CLEAN WATER ACT

- Fishable/ Swimmable
- Biological/chemical/Physical Integrity
- NPDES Discharge permitting system (wastewater and stormwater)
- BEACH ACT
- Total Maximum Daily Load (TMDL)
- Concentrated Animal Feeding Operations (CAFO)
- Water Quality Protection Plans

# Attributes of an Indicator System

- Correlation with Health Risk
- Similar Survival to Pathogens
- Similar Transport to Pathogens
- Specific to Fecal Source or Source of Origin.

# Indicators

- *Fecal Coliforms*
- *E.coli*
- *Enterococci*
- *Clostridium*
- *Coliphage*
- *Direct Pathogen*
- *Cryptosporidium/Giardia*
- *Enteric viruses*

# Current and Alternative Indicators

Indicator Standards for Recreational and Marine Water

**Fecal coliforms** 200 cfu/100mL Ave density

***E.coli*** 126 cfu/100mL Ave density

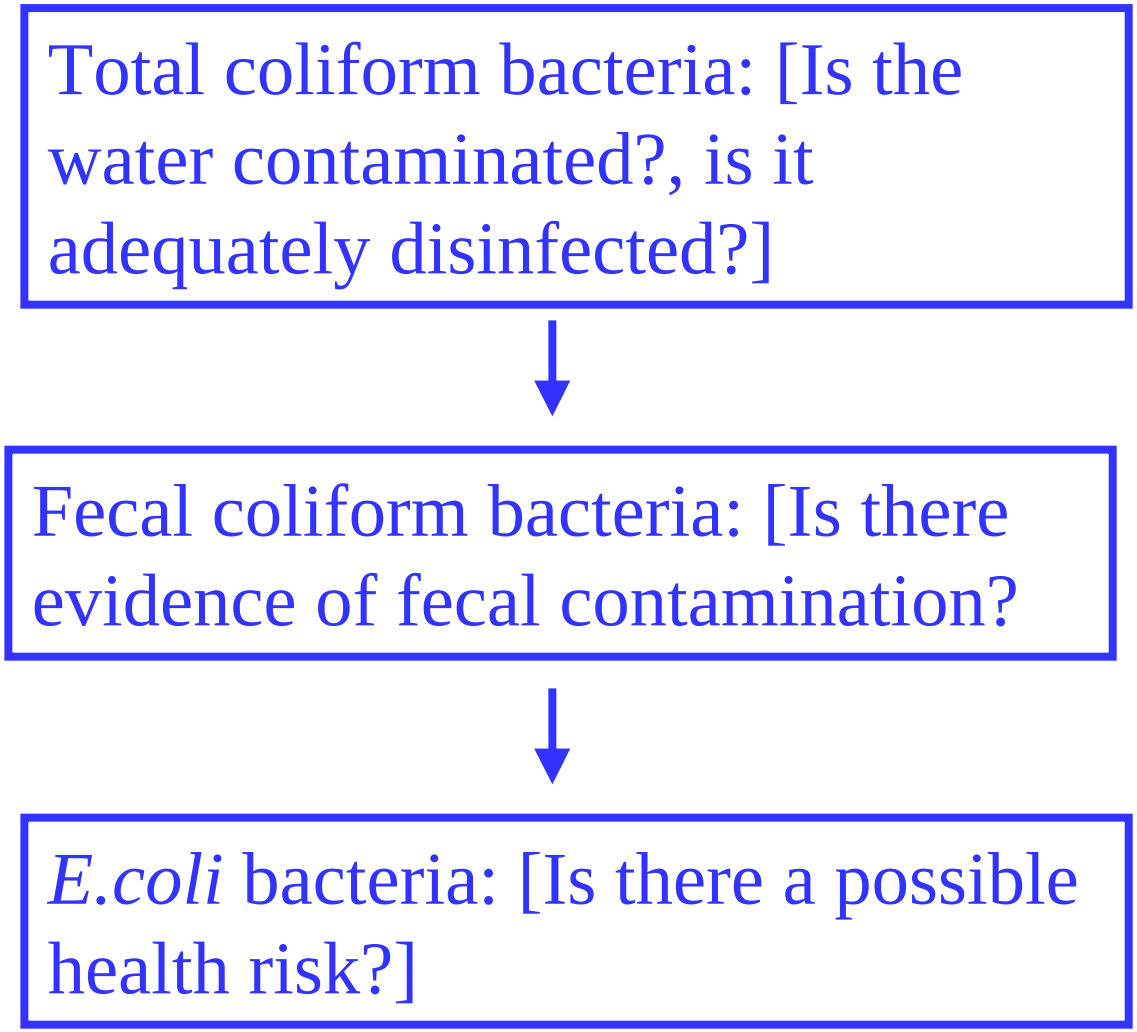
**Enterococci** 33 to 35 cfu/100ml Ave density

***Clostridium perfringens*** 50 or 5 cfu/100ml (Fujioka et al 1985)

**Coliphage** No current suggested standards 100 pfu relates to enteric virus presence.


TIME
WILL CLINTON TESTIFY?
THE CAPITOL HILL GUNMAN
THE KILLER GERM
It's turning up everywhere: in your water, your food, the pool. How to protect yourself from
E.COLI

# Evolution of the Indicator Concept



# Evolution of the Indicator Concept cont.

*E. coli* present

↓ ↓

Type the 0157 H7???: [Is it a pathogen]

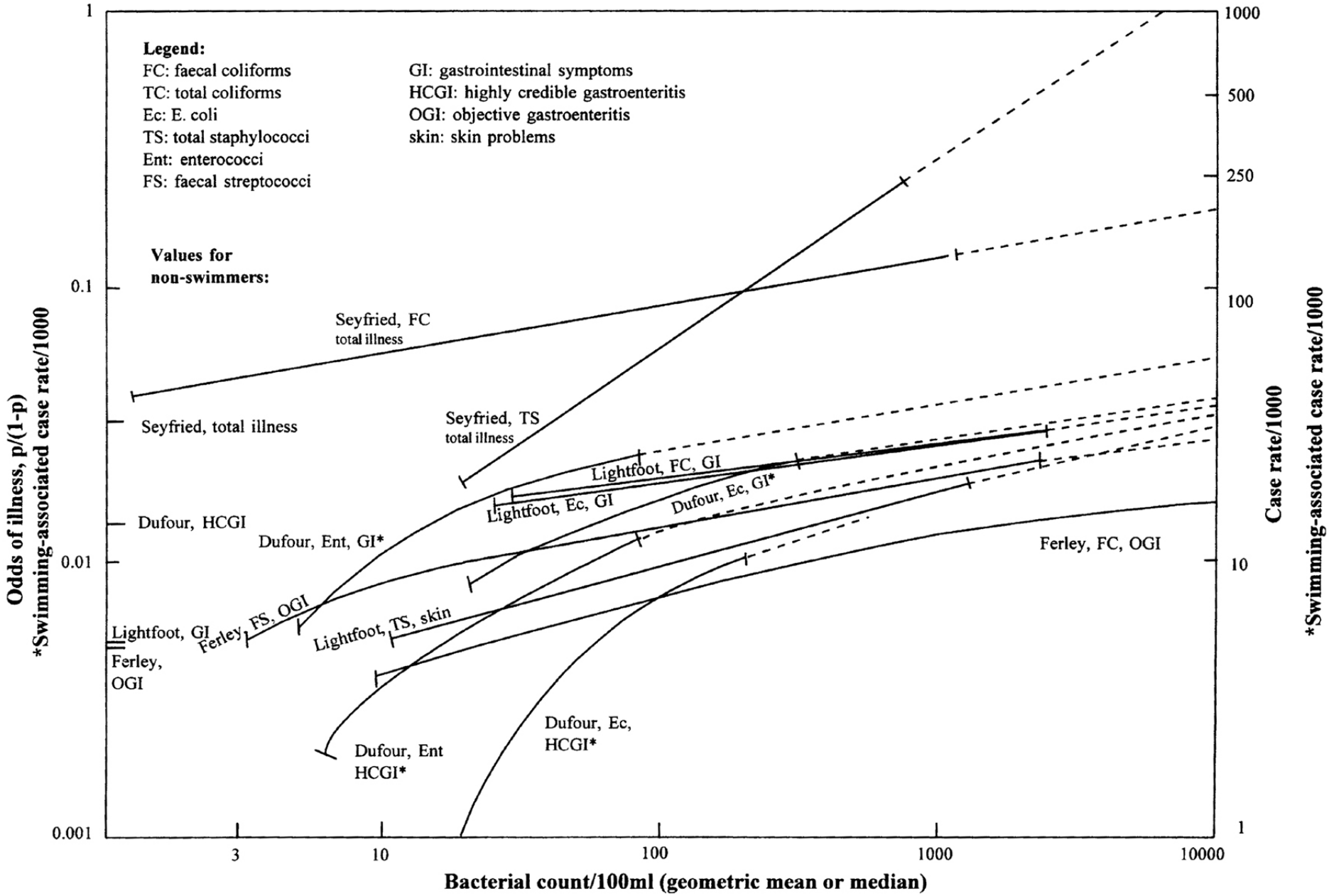
Source of the bacteria: [Animal or human?]

# Health Risks
# Epidemiological Methods
# Quantitative Risk Assessment

- Tools used to estimate adverse health effects associated with specific outcomes, exposures and or specific hazards.
- Statistical relationships and estimate or probability of harm.
- Used for setting water quality criteria or risk management decisions.

# Health Data Associated with Swimming in Polluted Waters

- Disease endpoints: Respiratory, gastrointestinal, ear, eye, nose and skin infections.
- England: respiratory most common, 24% had one symptom.
- Santa Monica Bay, CA: gastrointestinal, 3.7%, associated with storm drains.
- Key West, FL: gastrointestinal most common, 33% associated with leaky sewer, liveaboards.


Legend:
FC: faecal coliforms
TC: total coliforms
Ec: E. coli
TS: total staphylococci
Ent: enterococci
FS: faecal streptococci
GI: gastrointestinal symptoms
HCGI: highly credible gastroenteritis
OGI: objective gastroenteritis
skin: skin problems
Values for non-swimmers:
Seyfried, FC total illness
Seyfried, total illness
Seyfried, TS total illness
Lightfoot, FC, GI
Lightfoot, Ec, GI
Dufour, Ec, GI*
Dufour, HCGI
Dufour, Ent, GI*
Ferley, FC, OGI
Ferley, FS, OGI
Lightfoot, TS, skin
Lightfoot, GI
Ferley, OGI
Dufour, Ent HCGI*
Dufour, Ec, HCGI*
Odds of illness, p/(1-p)
*Swimming-associated case rate/1000
Case rate/1000
*Swimming-associated case rate/1000
1
0.1
0.01
0.001
1000
500
250
100
10
1
3
10
100
1000
10000
Bacterial count/100ml (geometric mean or median)

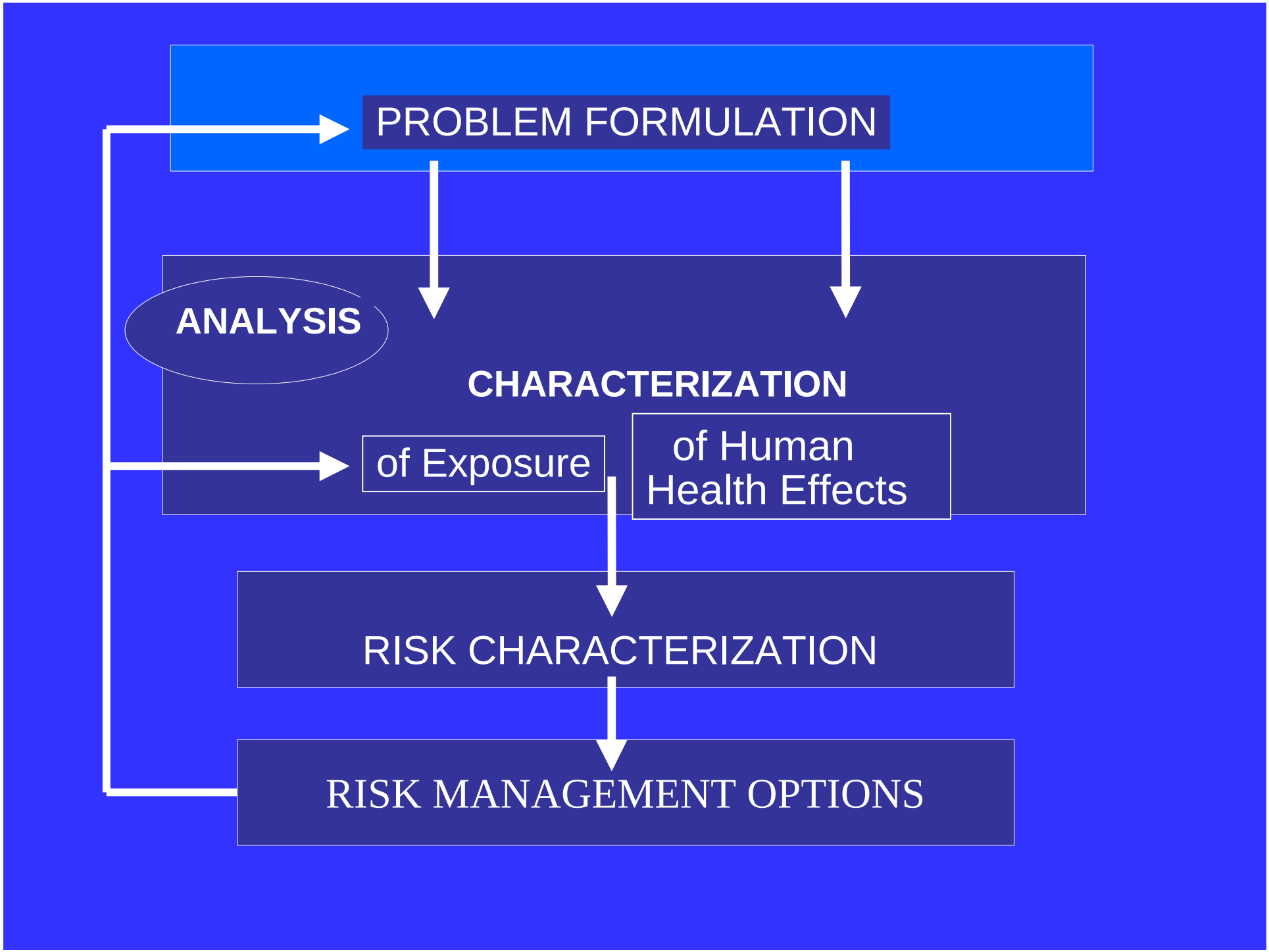
# Meta Analysis
# Jack Colford

- Recreational Epidemiological Studies
- Enterococci demonstrated the best statistical association in Marine Waters and was a good statistical fit in Fresh Waters
- E.coli was the best indicator of risk in Fresh Waters
- Coliphage and Enteric virus showed some relationship, however only a few studies.

Studies in Wisconsin have shown statistical Association between diarrhea in children and Septic tank density.

- No evidence of well contamination based on coliforms.
- Virus infections associated in a 640-acre section.
- Bacterial infections in a 40-acre section.
- Unknown infections associated with Enterococci contamination.

Borchardt et al. May 2003 Envir. Hlth Perspec vol 111


PROBLEM FORMULATION
ANALYSIS
CHARACTERIZATION
of Exposure
of Human
Health Effects
RISK CHARACTERIZATION
RISK MANAGEMENT OPTIONS

# MICROBIAL CONTAMINANTS IN SEWAGE DISCHARGES

**Levels in the sewage.**

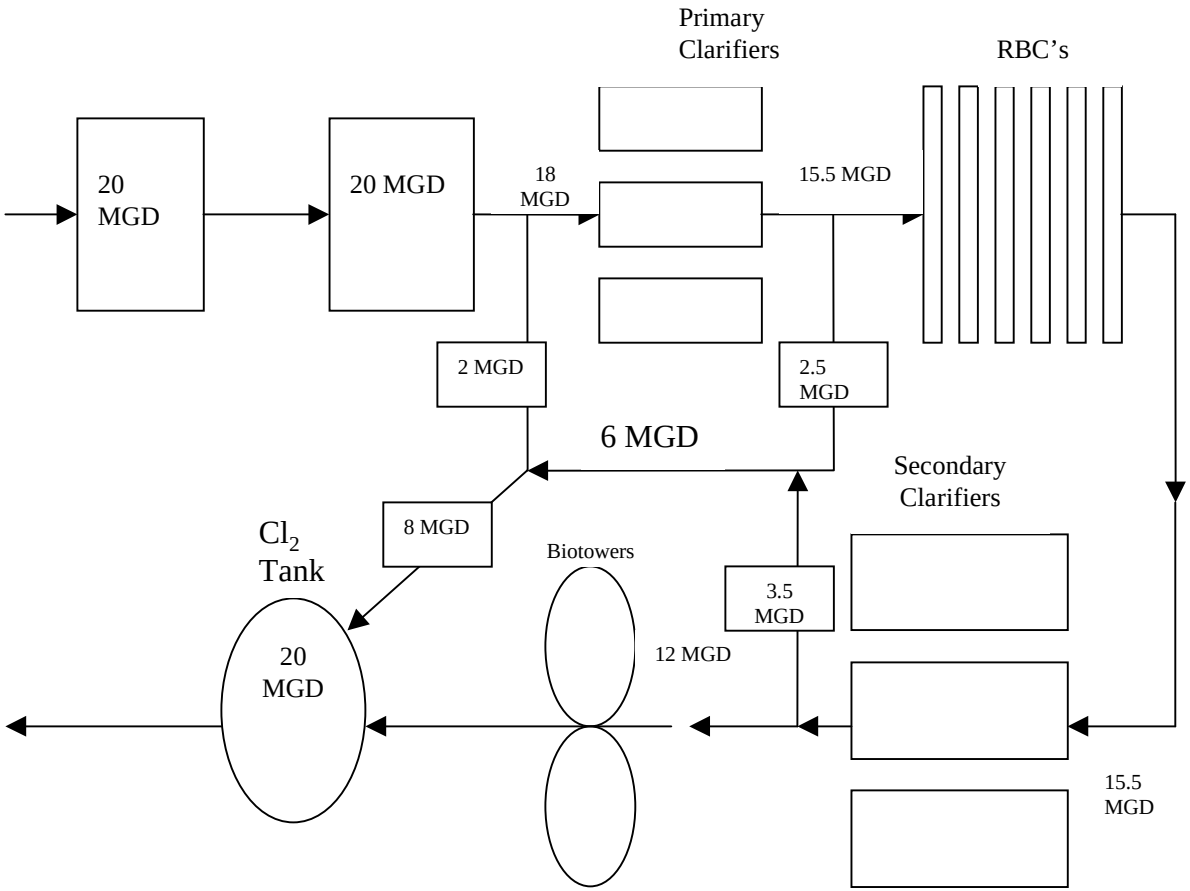
| | | | |
|---|---|---|---|
| ***Cryptosporidium* oocysts** | **600 (untreated)** | **400 (treated)** | **/100L** |
| ***Giardia* cysts** | **155,200 (untreated)** | **34,900 (treated)** | **/100L** |
| **Enteric viruses** | **96,200 (untreated)** | **97,200 (treated)** | **/100L** |

**Levels in the river.**

| | |
|---|---|
| ***Cryptosporidium* oocysts** | **range 6-1560/100L** |
| ***Giardia* cysts** | **range 7-3830/100L** |
| **Enteric viruses** | **range 100-2950/100L** |

**Drinking Water Risk**
**45 drinking water treatment facilities studied along the Saint Lawrence.**
**Risk 0.75 to 0.0001. Goal is 0.00001**

Payment et al. 2000 & 2001, Can J. Microbiol. 46: 565-576 & 47:188-193.




Max. Flow 20 MGD
Average Daily Flow 8.5 MGD

# Risk Estimates Associated with Blending

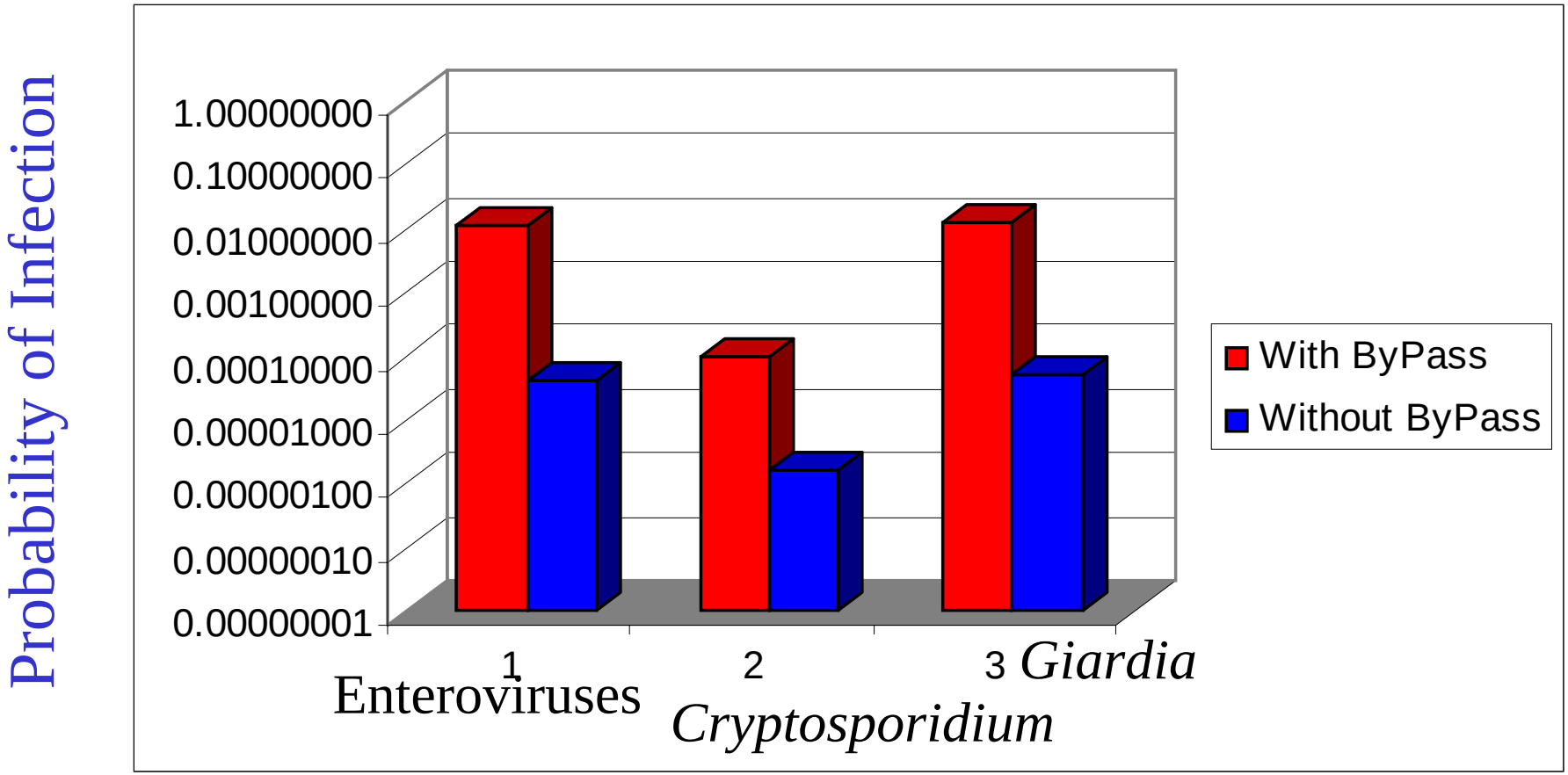
Based on monitoring data in wastewater for *Cryptosporidium, Giardia* and Enteric viruses.

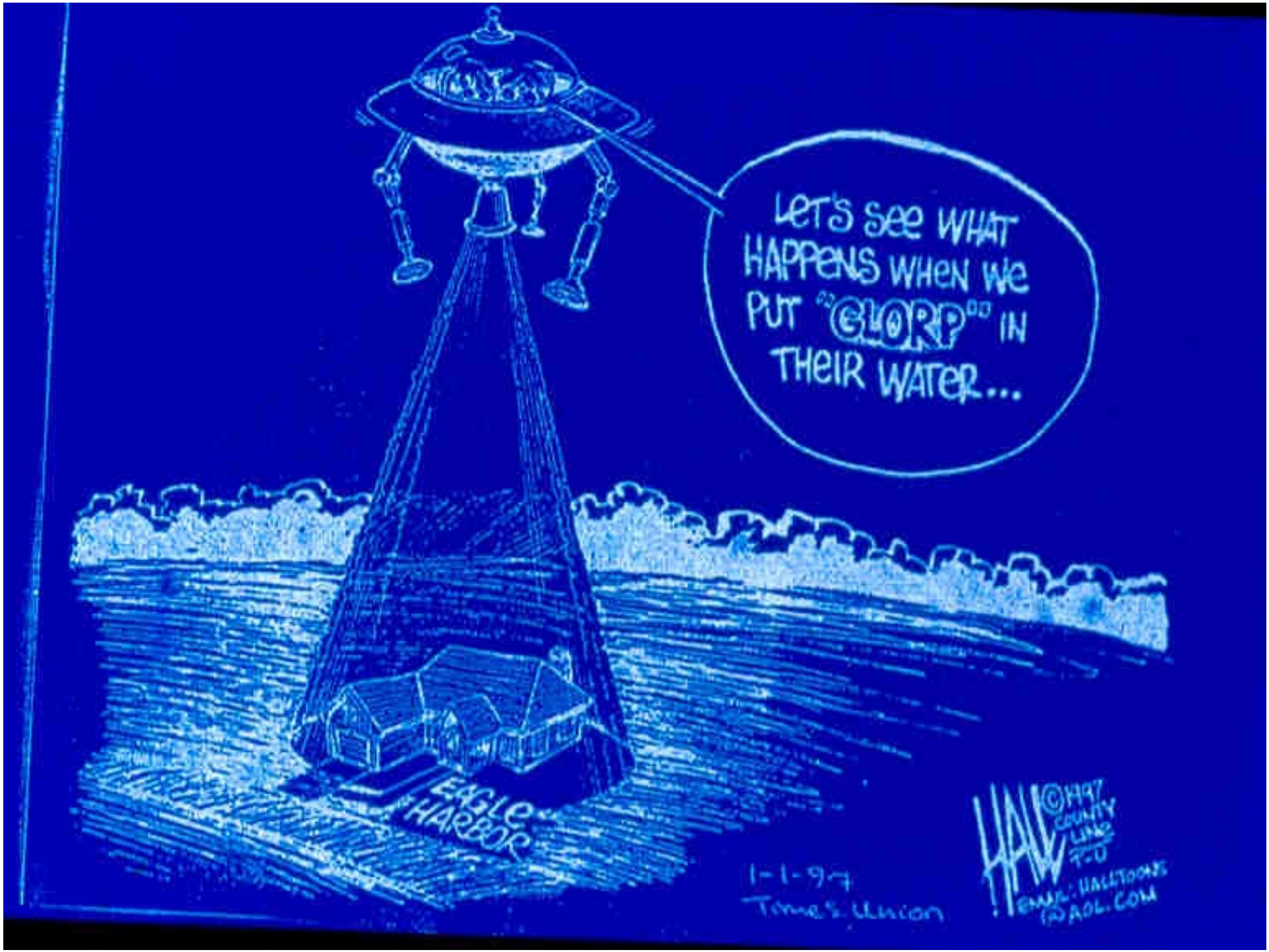
Assumed all viruses and protozoan cysts and oocysts are viable.

Assumed 1/10 dilution in environment.

Assumed 50 mL ingestion during a single swim event.

# Risk Estimates



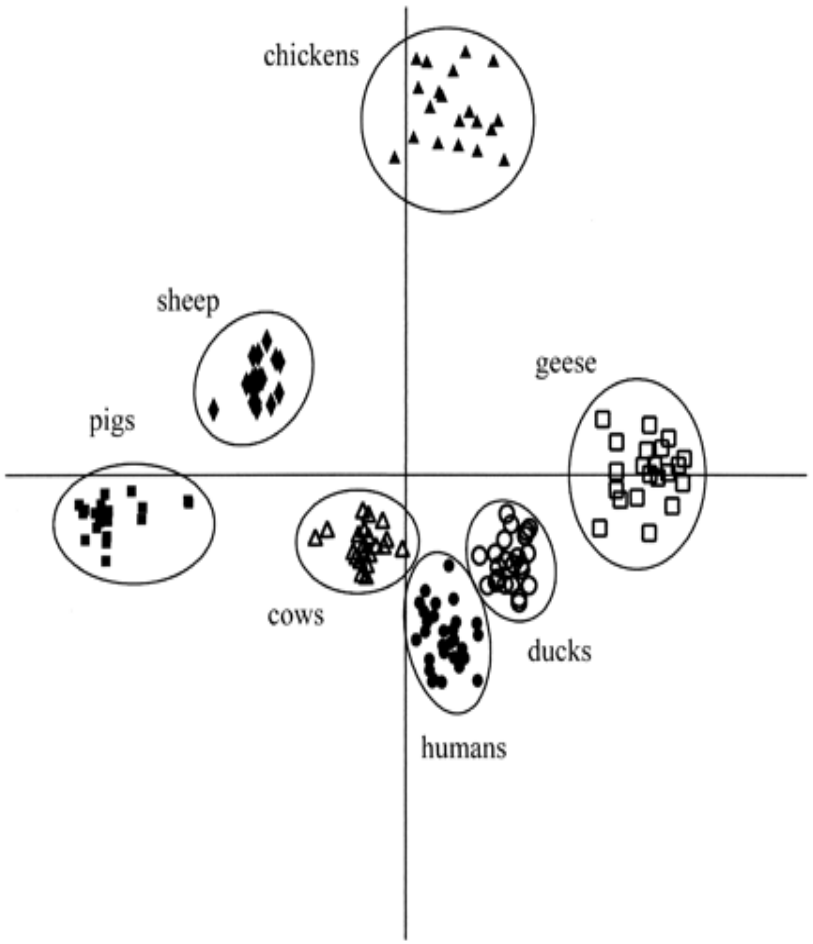

LET'S SEE WHAT HAPPENS WHEN WE PUT "GLORP" IN THEIR WATER...
EAGLE HARBOR
1-1-97
Times-Union
HALL ©1997 COUNTY LINE PUB
EMAIL: HALLTOONS@AOL.COM

# Source Tracking

- Recently developed techniques can be used to identify the source of a contaminant. Without source identification, contamination may not be contained. Molecular fingerprints, host-specific genes, and chemical constituents provide direct evidence of source origin.
- Library-based methods Ribotyping; Box PCR, Antibiotic Resistance most often used.

# REP PCR

- The DNA fingerprints were analyzed and assigned to a source
- The source assignments were from 89.5 to a 100% correct
- *Dombek et al., 2000; Applied and environmental microbiology, 66, no.6.*



# Source Tracking Round Robin Study

J. Water and Health Vol 1, No. 4 Dec. 2003

- 22 researchers 12 methods.
- 12 prepared samples
- 4 contained untreated sewage
- 4 contained human feces
- 5 contained cow feces
- 4 contained dog feces
- 4 contained gull feces

# Results

- *F+ Specific Coliphage were isolated at high concentrations from all samples that had sewage.*
- *Typing did not address source*
- *Human feces were negative*

- *Antibiotic Resistance gave 39-66% False positives*
- Ribotyping , Rep PCR and PFGE gave 14-33% False positives
- Small library
- Fecal Streptococci performed better

# Host Specific Markers

- *Bacteroides (PCR)*
- *4/4 sewage; 4/4 human; 4/5 cow (lowest concentration missed) 4/4 dogs however no marker for Birds: Missed 2 samples with dog and 2 with cow that were mixed.*

- *E.coli Toxin genes able to detect sewage (4/4).*

- Enteroviruses and Adenoviruses found in 3 of 4 sewage samples.

# Source Tracking at MSU

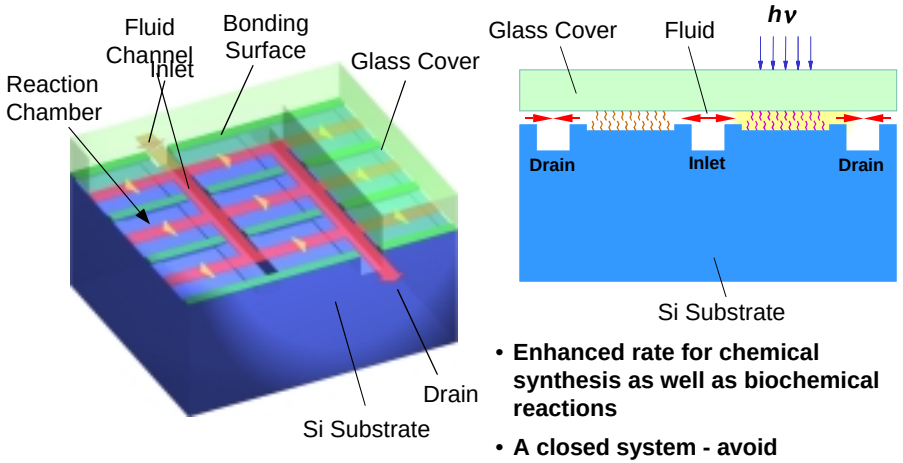
- Recently developed method to track a Human marker in Enterococci.
- Water samples are concentrated via membrane filtration onto mEI agar according to US EPA Method 1600. Filter membranes are then lifted and suspended in TSB followed by a 3 hour incubation period at 41°C. DNA extraction is performed on the resulting culture of bacteria using a QiaAmp DNA extraction kit according to manufacturer's instructions (Qiagen, Inc.). PCR is then performed using primers specific for *esp*

# Source Tracking at MSU

- 68/70 samples from human sewage and septic tanks were positive.
- 0/80 samples from cattle, swine, bird, fecal samples and lagoons were positive.
- Can be used with Enterolert or membrane filtration methods.
- Sensitive at 100 colonies

# High Throughput DNA Chip Platform

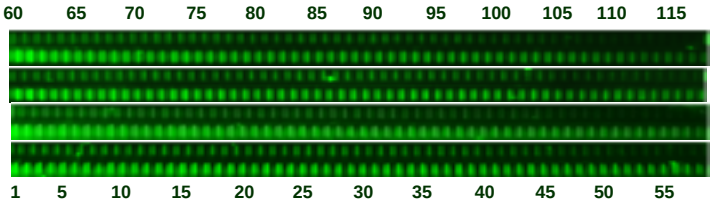
## Microfluidic Reactor Array



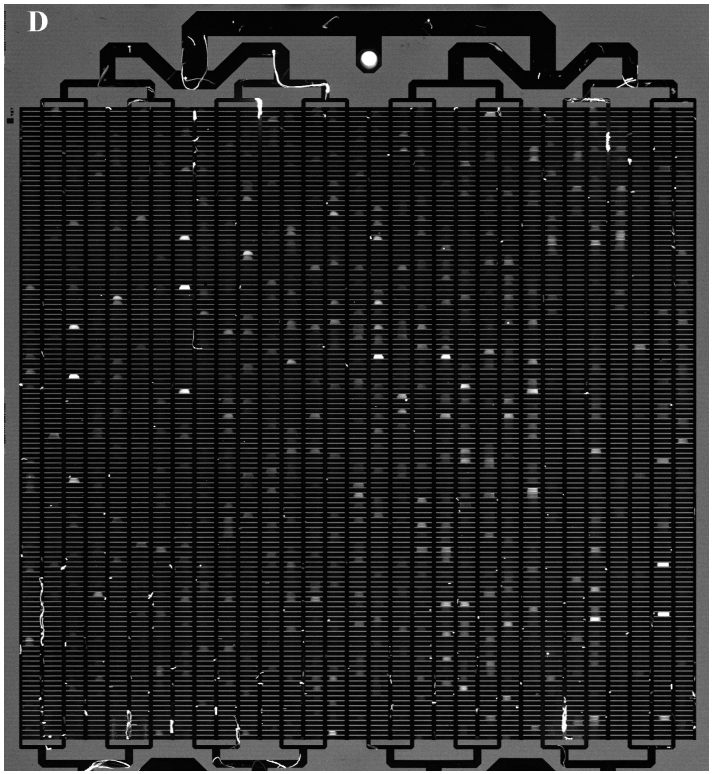

- **Enhanced rate for chemical synthesis as well as biochemical reactions**
- **A closed system - avoid contamination**

## High Stepwise Yield

DNA Sequences of more than 100-mers were synthesized and an average stepwise yield ~ 98% was obtained




Fluorescein is coupled at the last the synthesis step

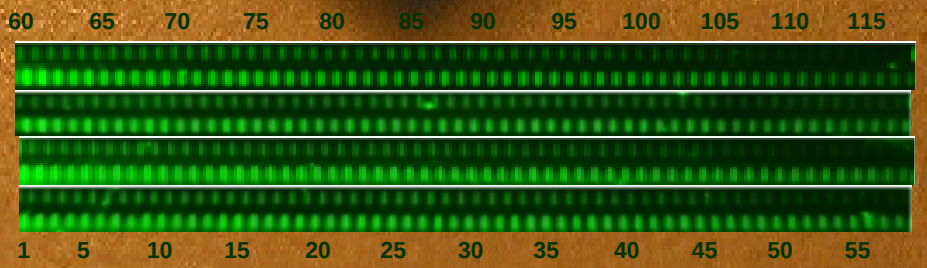



**COST AND EFFICIENCY**

Thousands of gene targets tested simultaneously

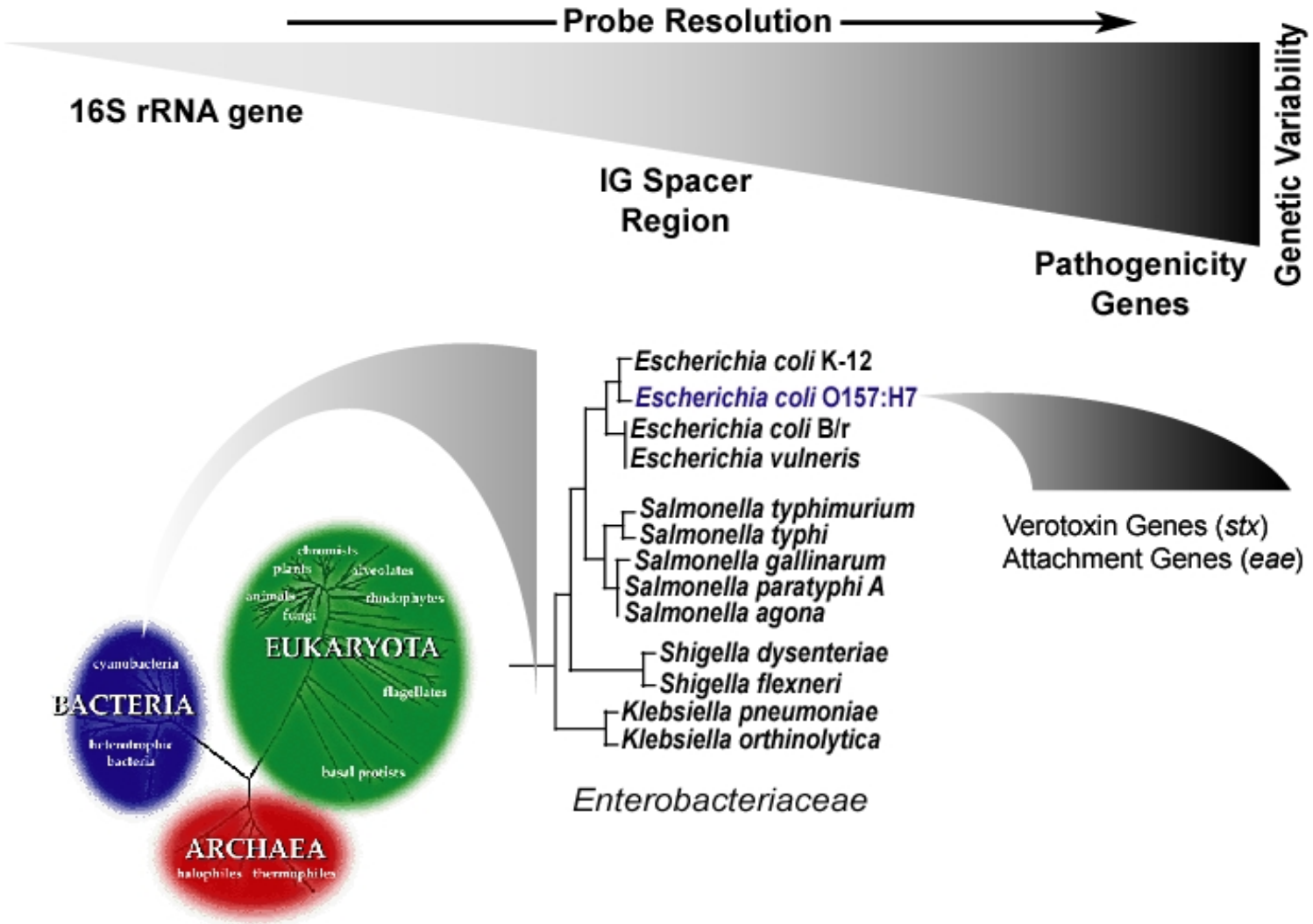
# The Xeotron BioChip Platform

- Patented chemistry
- Unique 3-D nano-reactors
- Size: 2 cm x 1.8 cm
- Current capacity: 8,000
- Future capacity: 30,000


- 100-mer probes possible!
- High Stepwise yield > 98%

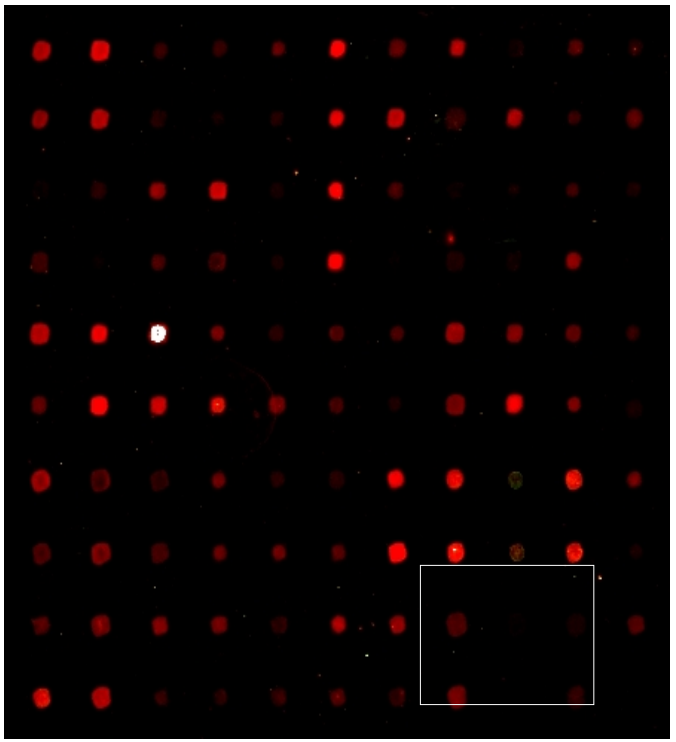
# *Probe Design Approach*



# *Indicator Biochip: E.coli, Enterococci, Coliphage, Bacteriodes*

## ***stx*** (Shiga-like toxin)

| *Stx group* | GenBank | Probes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | V 1 | V 2 | V 3 | V 4 | V 5 | V 6 | V 7 | V 8 | V 9 | V 10 | V 11 | V 12 |
| 2 | AJ271139 | | | | | | | | | ■ | | | |
| 2 | AJ270998 | | | | | | | | | ■ | | | |
| 2 | AJ010730 | | | | | | | | | ■ | | | |
| 2 | AJ271063 | | ■ | | | | | | | | | ■ | ■ |
| 2 | AJ251520 | | ■ | | | | | | | | | ■ | ■ |
| 2 | AJ250954 | | ■ | | | | | | | | | ■ | ■ |
| 2 | AJ251483 | | ■ | | | | | | | | | ■ | ■ |
| 2 | AJ251452 | | | | | | | | | | | | ■ |
| 2 | AB028899 | | ■ | | | | | | | | | ■ | |
| 2 | AB028900 | | ■ | ■ | | | | | | | | ■ | |
| 2 | AB028901 | | ■ | | ■ | | ■ | | | | | ■ | |
| 2 | AB028902 | | ■ | | | | | | | | | ■ | |
| 2 | AB028903 | | ■ | | | ■ | ■ | | | | | ■ | |
| 2 | AB028904 | | ■ | | | | | | | | | ■ | |
| 2 | AB028905 | | ■ | | | ■ | ■ | | | | | ■ | |
| 2 | AB028906 | | | | | | ■ | ■ | | | | | |
| 2 | AB028907 | ■ | | | | | | | | | | | |
| 2 | AB028908 | | | | | | | | ■ | | | | |
| 2 | AB028909 | | ■ | | | | ■ | | | | | ■ | |
| 2 | AB028910 | | ■ | | | | ■ | | | | | ■ | |
| 2 | U41250 | | ■ | | | | | | | | ■ | ■ | ■ |
| 2 | U41252 | | ■ | | | | | | | | ■ | ■ | |
| 2 | U41255 | | ■ | | | | | | | | ■ | ■ | ■ |
| 2 | U41258 | | ■ | | | | | | | | | ■ | ■ |
| 2 | U41260 | | ■ | | | | | | | | ■ | ■ | ■ |
| 2 | X81418 | | | | | | | | | ■ | | | |
| 2 | X81415 | | | | | | | | | ■ | | | |
| 2 | X81416 | | | | | | | | | ■ | | | |
| 2 | X81417 | | | | | | | | | ■ | | | |
| 2 | M21534 | | | | | | | | | ■ | | | |
| | AF175707 | | ■ | | | | | | | | | ■ | |
| 2 | AP000422 | | ■ | | | | ■ | | | | | ■ | |
| 2 | M36727 | | | | | | | | | ■ | | | |
| 1 | AJ251754 | | | | | | ■ | | | | | | |
| 2 | M29153 | | | | | | | | | ■ | | | |
| 2 | U41246 | | | | | | | | | | ■ | | |



20-mer oligonucleotide probes:
C6 aminolinker on
SuperAldehyde™ substrate

# Water Quality & Health

## Evaluating Indicators

- **The use of two indicators is warranted, *E.* coli, and Enterococci should both be used.**
- **Coliphage should be added as a third indicator in areas with sewage inputs and study of storm events.**
- **Pathogen monitoring should be included in specialized studies.**

## Building Data bases

- **Larger Data bases, more information on spatial and temporal changes, accumulation in sediments.**

## Monitoring

- **Rainfall linked to water quality, but other environmental factors, wind and temperature associated with contamination.**
- **Studies from source to exposure.**

# The FUTURE

- Insufficient studies are on-going
- Political Will
- Longer term strategy for studying microorganisms and water quality
- Funding for surveys
- Application of new technology; better and more specific information.
- BETTER PROTECTION OF WATERS
- ABILITY TO PRIORITIZE
- LONG TERM SUSTAINABILITY