

A NEW TOOL TO QUANTIFY SOURCES OF
MICROBIAL CONTAMINATION AND DEVELOP
PATHOGEN REDUCTION STRATEGIES

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Myth: “Pathogens are easy to measure and well defined”

- Why measure pathogens directly?
- Why distinguish between different animal sources of fecal contamination?
- Technology developed to date: hollow fiber ultrafiltration, quantitative detection of nucleic acids of pathogens and source indicators
- Small Case study: Sonoma Creek Watershed
- Large Case study: Calleguas Creek Watershed (Second talk)

Top Water Quality Problems

(Source: Craig J. Wilson, State Water Resources Control Board)

| Problem | Percent of Listed Waters |
|------------|--------------------------|
| Pathogens | 41 |
| Nutrients | 28 |
| Pesticides | 27 |
| Metals | 27 |
| Sediment | 20 |



Main idea

- Provide reliable methodology for a science-based urban-drain discharge management program
- Address current and future Bacteria TMDLs
- Provide new technology to calculate (real) pathogen loads
- Identify and quantify sources of fecal pollution from storm drains

Technical Objectives

- A Develop large-volume filtration and molecular methodology to reliably quantify microbial pathogens in natural and waste waters
- Bacteria: e.g., *Salmonella*, *E. coli*,
Francisella tularensis
 - Human viruses: Adenovirus, Enterovirus
 - Protozoa: *Cryptosporidium*, *Toxoplasma gondii*
- B Use same methodology for microbial source tracking (MST)
- *Bacteroidales*



Method Overview - Pathogen Quantification

50-100 L water sample

Water quality parameters

- Physicochemical
- Microbiological

Concentration
1st - To 2 L
2nd - To 100 mL

- Recovery of PP7 using plaque assay and TaqMan (molecular method)

Extraction of nucleic acids

- RNA/DNA from 10 mL sample
- Remove inhibitors

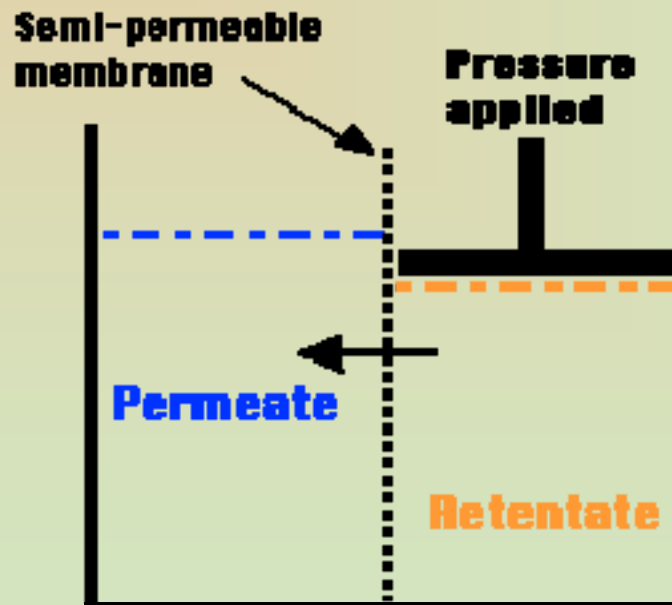
Quantification by TaqMan

- Adenovirus: DNA
- Enteroviruses: RNA

Hollow Fiber Ultrafiltration (HFF)

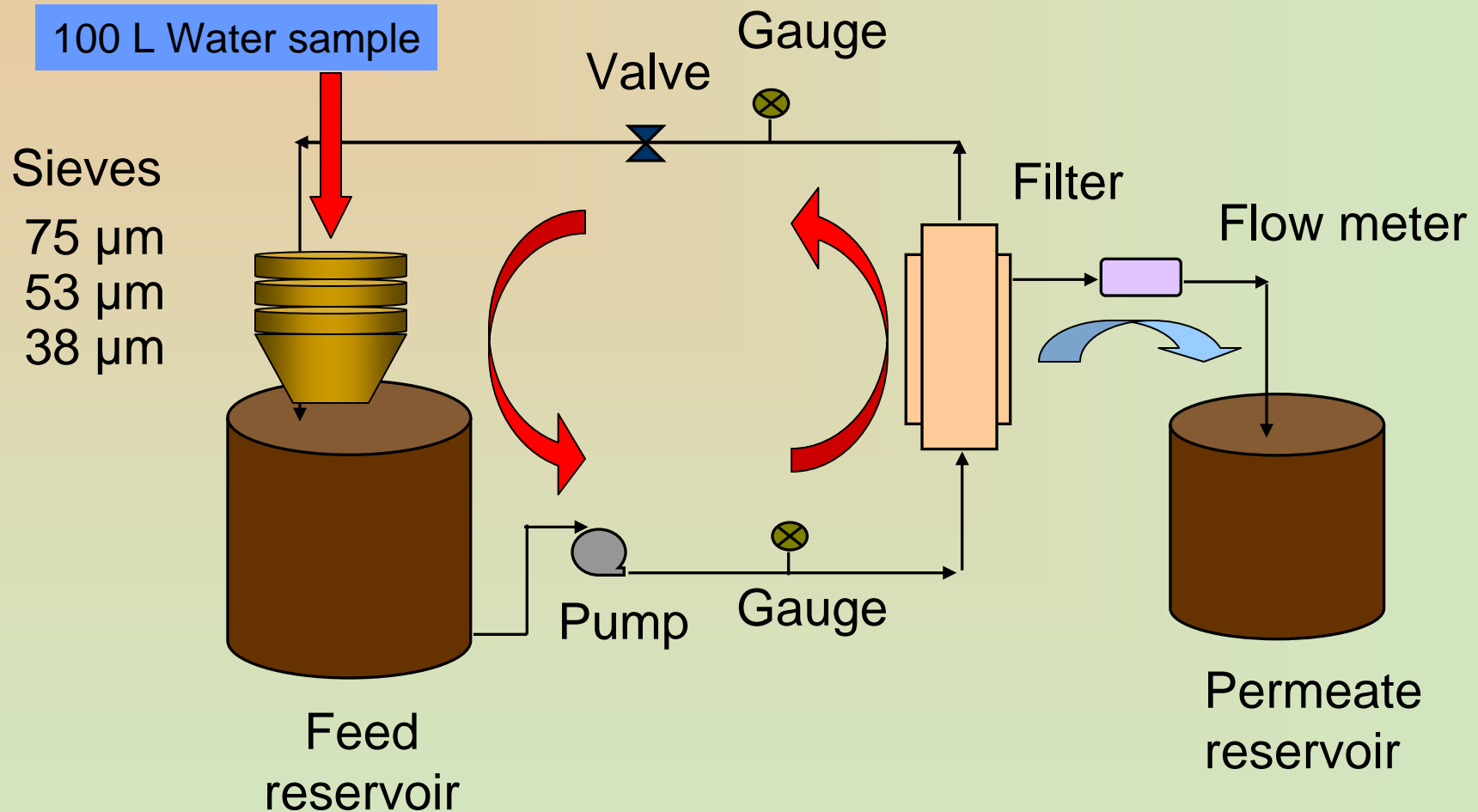
- HFF is a membrane separation process that is driven by a pressure gradient.
- The membrane fractionates components of a liquid as a function of their solvated size and structure, e.g. a 50,000 MW cutoff.

Membrane Processing



<http://www.energymanagertraining.com/dairy/Membrane%20Processing.htm>

Ultrafiltration scheme – Large System (BS)



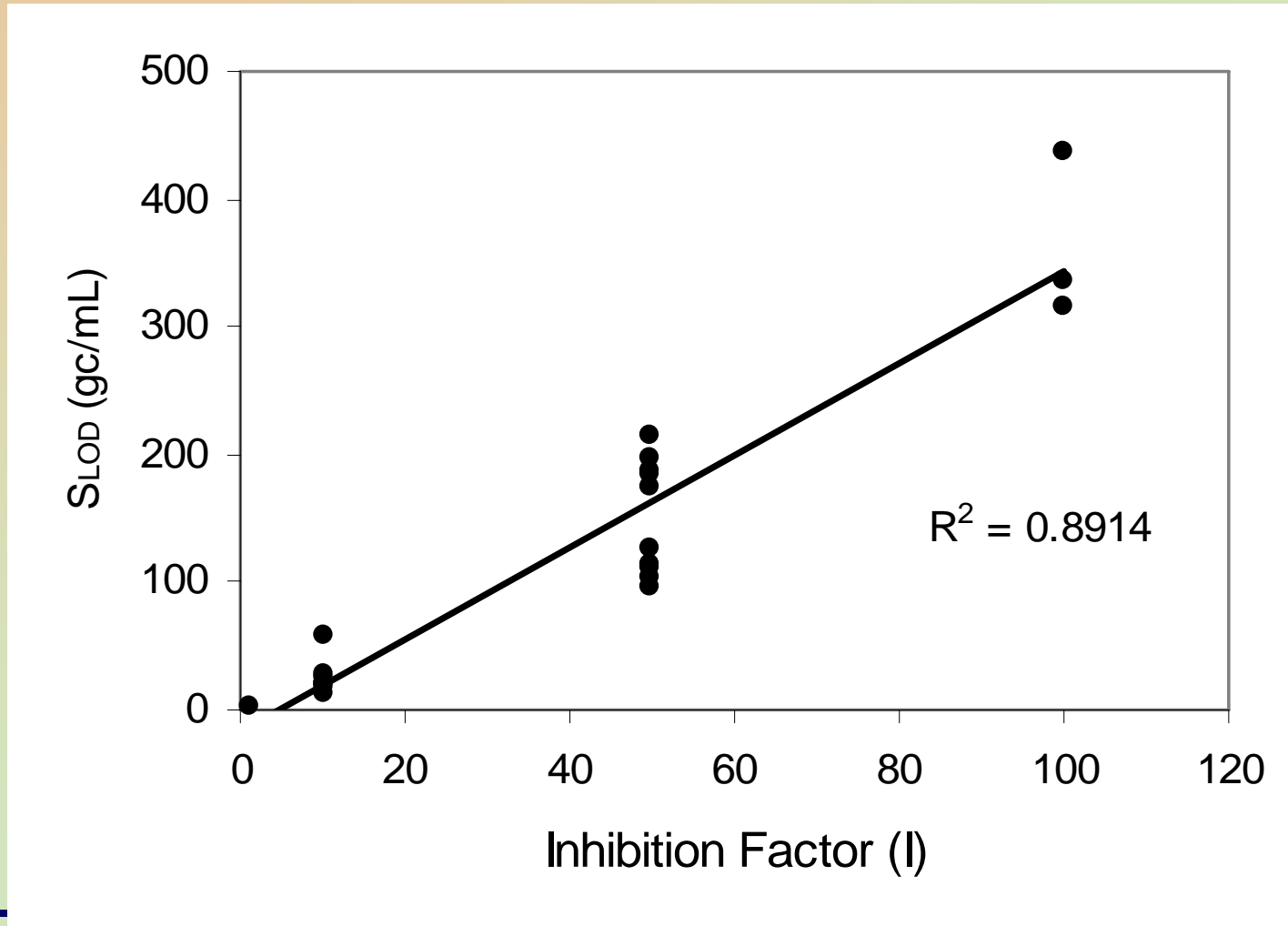
Oshima (2001)



5. 2. 2004

Inhibition is a Controlling Factor of Sample Limits of Detection

$$S_{LOD} = \frac{A_{LOD} \times I \times V_{el} \times V_{RF}}{V_T \times V_{RF,ex} \times V_S \times R_{filtration} \times E_{ex}}$$



2.- Microbial Source Tracking (MST)

Objective

Genetic marker sequences of *Bacteroidales* for host-specific bacteria associated with feces

The Bacteria Conundrum

- Responsibility to Protect Human Health
- 3rd Most Common in California (2002)
- 298 Waterbodies 303(d) Listed
- Some relatively undeveloped
- Others are subject to very little or no recreational use
- 300 TMDL's = \$\$\$\$\$\$\$\$\$\$\$\$\$



Source Identification

- Usual suspects:
 - Leaking sanitary sewer lines
 - Poorly maintained septic systems
 - Renegade RVs
 - Homeless persons
 - Household pets
 - Livestock
 - Wildlife
 - Regrowth

Microbial Source Tracking Methods

- Chemical (caffeine, fecal sterols, etc.)
- Non-molecular methods (antibiotic resistance, carbon utilization profiles, etc.)
- Molecular methods (ribotyping, pulsed-field gel electrophoresis [PFGE], and host-specific PCR markers)



Host-specific PCR Markers

- Library-dependent methods scrutinized
- PCR markers performed very well in SCCWRP comparison studies
- Geographically-independent
- No need to build library for each study
- No need to culture bacteria
- Analysis is relatively fast
- Methods are precise (field and lab duplicates)
- Relatively inexpensive



**Percentage of Samples Targeted by
Listed Assay (no of samples in
parentheses)**

| Fecal Host | No of Fecal Samples | Universal (U520F) | Mixed Human (H160F) | Cow/Horse (C128FB) | Dog (D142F) |
|---------------------|----------------------------|--------------------------|----------------------------|---------------------------|--------------------|
| Human | 18 | 100% (18) | 67% (12) | 0% (0) | 22% (4) |
| Cow | 11 | 100% (11) | 0% (0) | 100% (11) | 0% (0) |
| Horse | 8 | 100% (8) | 0% (0) | 38% (3) | 0% (0) |
| Dog | 8 | 100% (8) | 13% (1) | 0% (0) | 63% (5) |
| Cat | 7 | 100% (7) | 0% (0) | 0% (0) | 14% (1) |
| Seagull | 7 | 100% (7) | 0% (0) | 0% (0) | 0% (0) |
| Wastewater Influent | 14 | 100% (14) | 100% (14) | 0% (0) | 29% (4) |

Bayes' Theorem: Human Fecal Contamination

$$P(H \setminus T) = \frac{P(T \setminus H) \cdot P(H)}{P(T \setminus H) \cdot P(H) + P(T \setminus H') \cdot P(H')}$$

$P(T \setminus H)$ is the probability of a positive signal with the mixed human-specific assay in a fecal sample that is human derived. This value was obtained from the laboratory validation study (Table 1) as 1.00 due to the 100% detection of mixed human samples screened with this assay.

$P(T \setminus H')$ is the probability of a positive signal with the mixed human-specific assay in a fecal sample that is not human derived. This value was obtained from the laboratory validation study (Table 1) as 0.13 due to the 13% detection of dog-derived fecal sources by this assay.

$P(H)$ is the background probability of detecting the H160F marker in a watershed. This value is 0.89 since the marker was detected in 65 of 73 samples.

$P(H')$ is the background probability that the H160F marker is absent in a watershed. This value is $1 - P(H)$, or 0.11.

Bayes' Theorem contd.\...

$$P(H \setminus T) = \frac{(1.00) \cdot (0.89)}{(1.00) \cdot (0.89) + (0.13) \cdot (0.11)} = 0.98$$

Sensitivity is the ratio of those samples that correctly tested positive to all those samples that actually experienced fecal contamination of mixed human origin.

$$\text{Sensitivity} = \frac{\text{TPC}}{(\text{TPC} + \text{TNI})} = \frac{0.89}{(0.89 + 0)} = 1.00$$

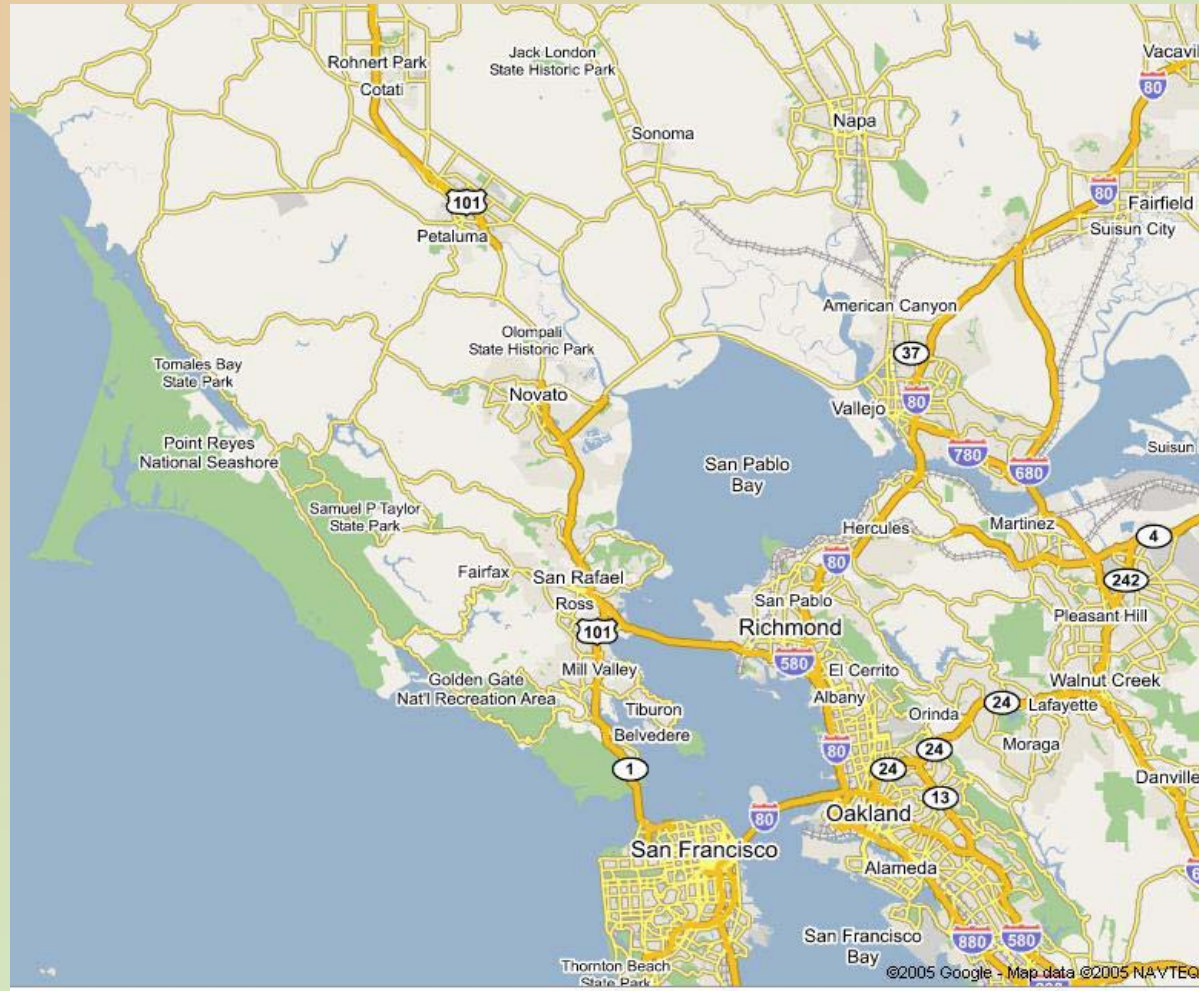
Specificity is the ratio of those samples that correctly tested negative to all those samples that actually did not experience fecal contamination of mixed-human origin.

$$\text{Specificity} = \frac{\text{TNC}}{(\text{TNC} + \text{TPI})} = \frac{0.0957}{(0.0143 + 0.0957)} = 0.87$$

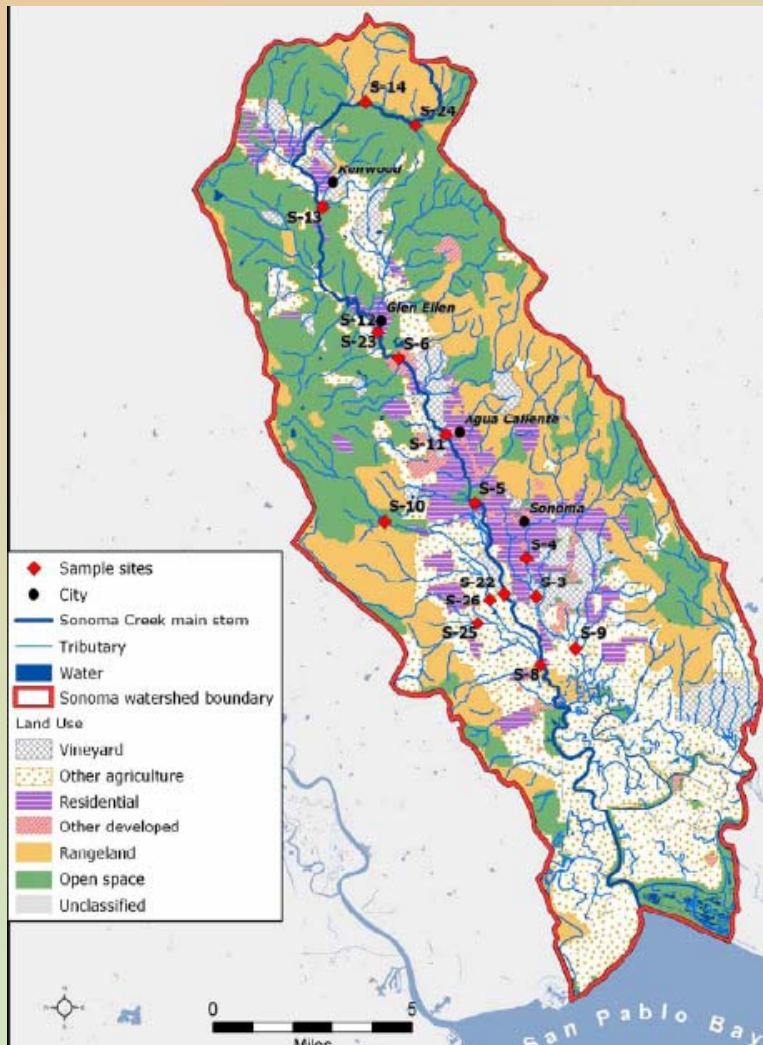
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TMDL Development – Sonoma Creek



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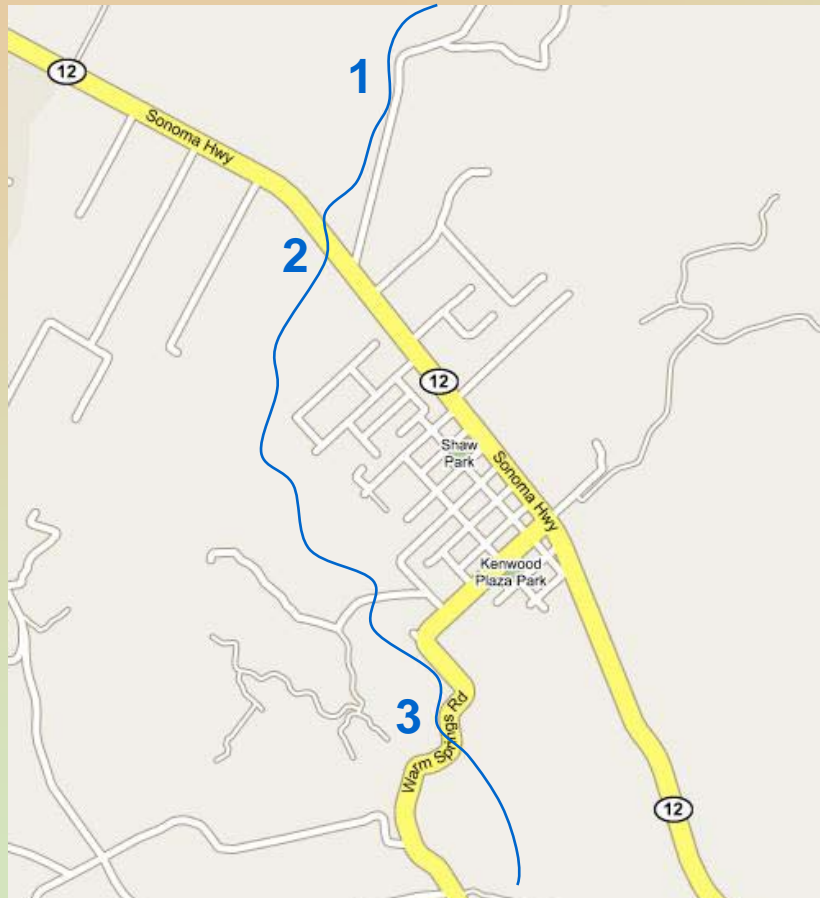


Major Land Uses:

- Rangeland
- Agriculture
- Residential

Hot Zones and Water Board Source Identification

Upper Watershed - Kenwood



E.coli
(CFU/100mL, geometric mean) ¹

Site 1: 19
Site 2: 38
Site 3: 147

*** Assumption: Septic tanks in Kenwood are the likely source of fecal contamination**

Hot Zones and Water Board Source Identification

Lower Watershed – Town of Sonoma



E. coli
(CFU/100mL, geometric mean)

Range from 94 to 483

***Assumption: the likely source of fecal contamination include**

- Sanitary sewer system failure
- Municipal runoff
- Cattle grazing
- Dairies
- Wildlife

Table 2: Sampling locations for this study and corresponding water board names and data

| Sample Site Location | Site Name (CA Water Board) | Site Name (This Study) | <i>E. coli</i> (CFU/100 mL) |
|-----------------------------------|-----------------------------------|-------------------------------|------------------------------------|
| Sonoma Creek @ Hwy 12 | S-05 | KU | 38 |
| Sonoma Creek below Kenwood | S-04 (S-13) ^b | KL | 147 |
| Sonoma Creek @ Maxwell Park | S-03 (S-5) | SU | 132 |
| Sonoma Creek @ Hwy 121 | (S-8) | SL | 323 |
| Nathanson Creek @ Nathanson Park | NA-02 (S-4) | NU | 483 |
| Schell Creek @ 8 th St | SC-01 | NL | 193 |



Indicators at Hot Zones

E.coli (CFU/100 mL)

Dry Event

Wet Event

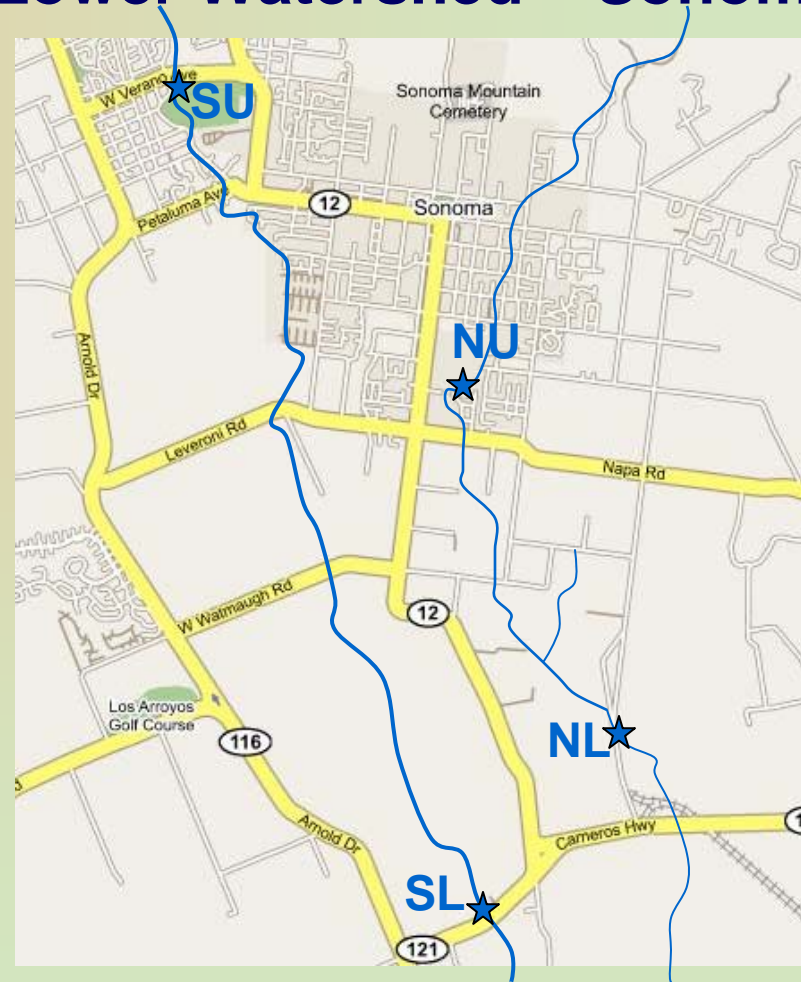
| | Dry Event | Wet Event |
|----|-----------|-----------|
| KU | 58 | 200 |
| KL | 160 | 610 |
| SU | 81 | 1100 |
| SL | 96 | >2400 |
| NU | 690 | 330 |
| NL | 130 | 1400 |

Highlighted #s exceed the 90th percentile criteria for *E.coli*

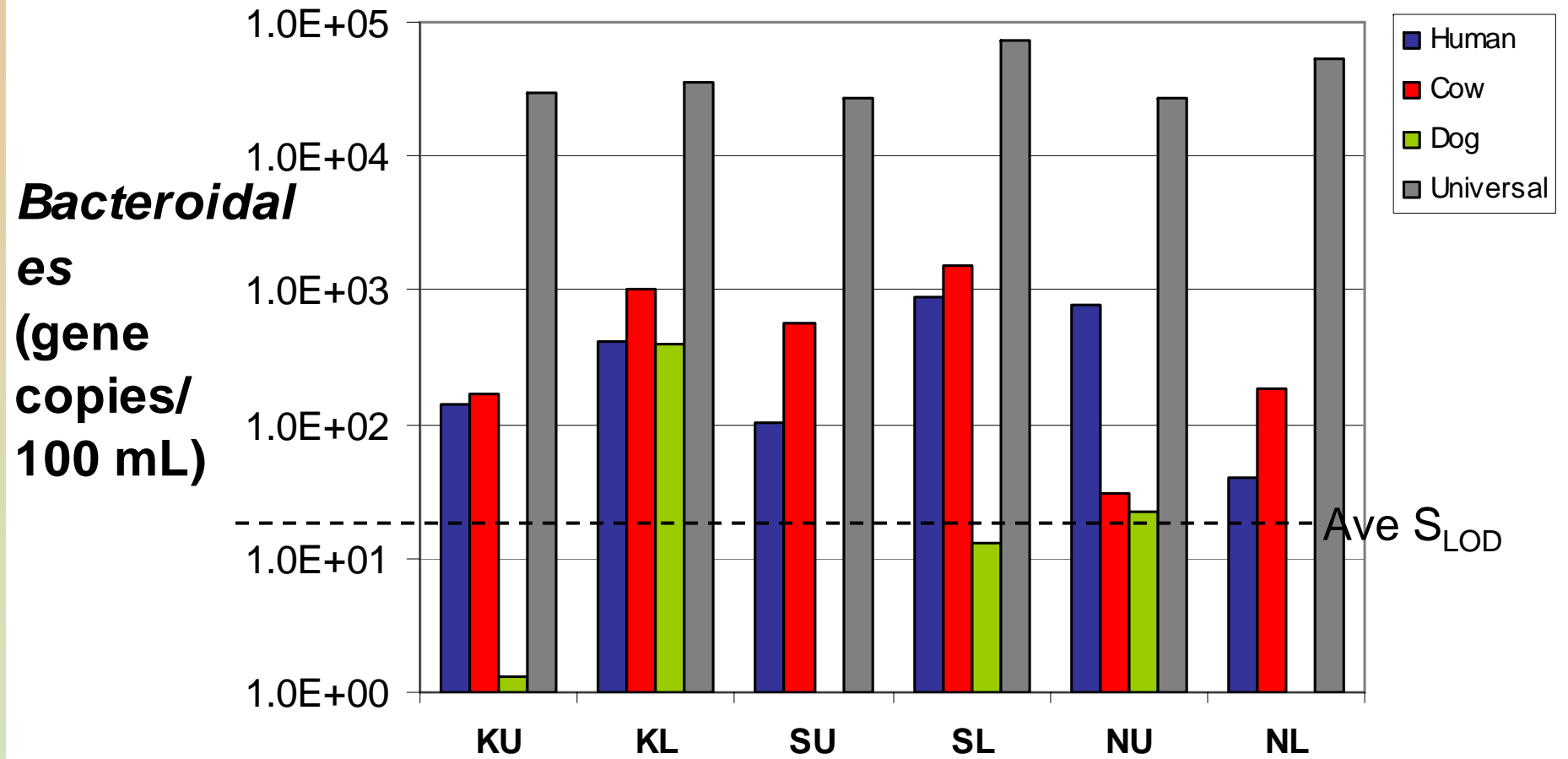


Microbial Source Tracking at Hot Zones

Upper Watershed - Kenwood Lower Watershed – Sonoma



Microbial Source Tracking at Hot Zones: Dry Event



Microbial Source Tracking at Hot Zones: Wet Event

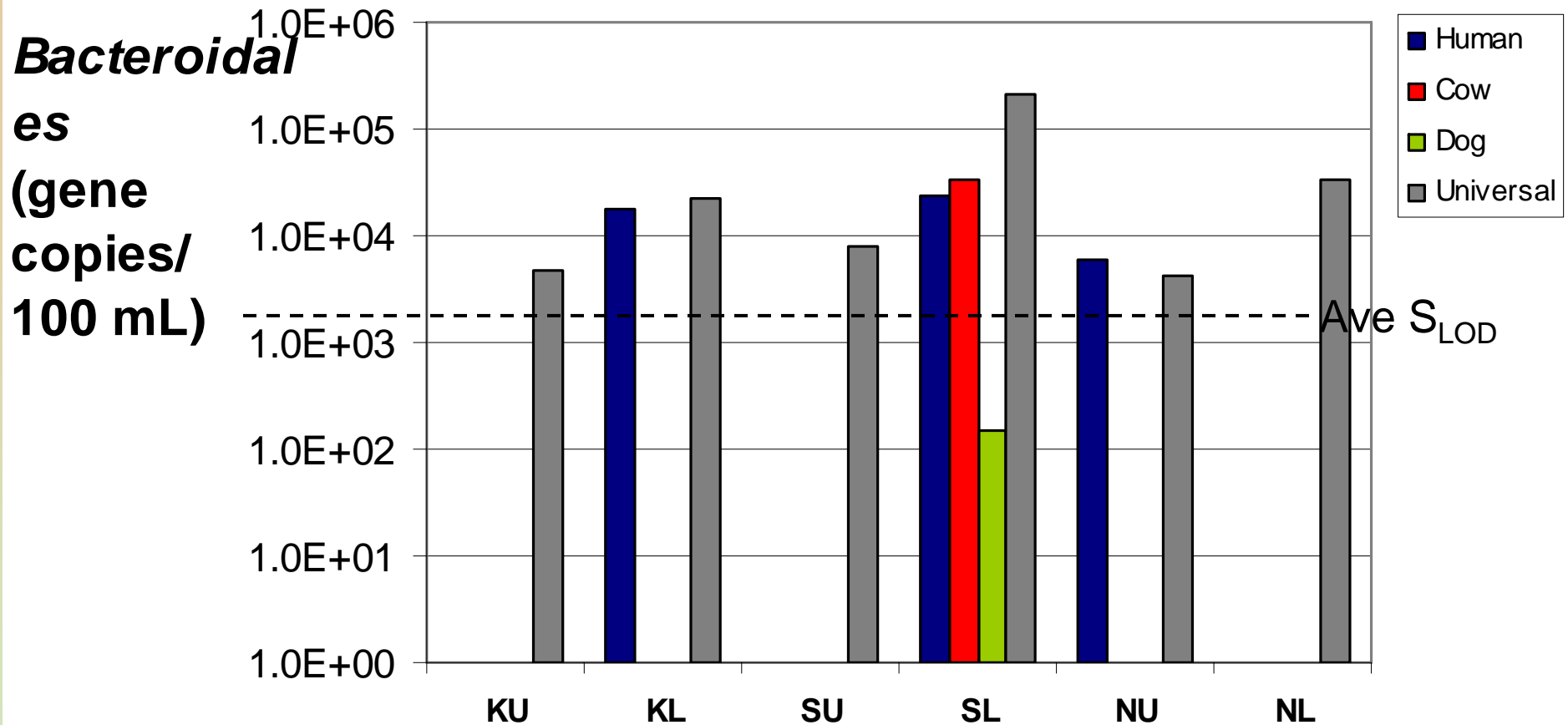


Table 3: Ratio of human to universal *Bacteroidales* during wet and dry weather conditions

| | Human:Universal <i>Bacteroidales</i> (%) | | | | | |
|----------------------|---|-----------|-----------|-----------|-----------|-----------|
| Sampling Site | KU | KL | SU | SL | NU | NL |
| Dry Weather | 0.47 | 1.14 | 0.37 | 1.20 | 2.80 | 0.08 |
| Wet Weather | ND | 80.60 | ND | 11.69 | 146.17 | ND |

Table 4: Presence of pathogens in Sonoma Creek Watershed using Taqman analysis

| Sample | Cryptosporidium | Giardia | Toxoplasma | Francisella | S_{LOD} (gc/100 mL) |
|------------------|-----------------|------------|------------|-------------|--------------------------|
| Dry Event | | | | | |
| KU-01 | Pos | ND | ND | ND | 4.5×10^1 |
| KL-01 | Pos | ND | ND | ND | 2.1×10^2 |
| SU-01 | Pos | ND | ND | ND | 1.6×10^3 |
| SL-01 | Pos | ND | ND | ND | 1.3×10^2 |
| NU-01 | Pos | ND | ND | ND | 1.7×10^2 |
| NL-01 | Pos | Pos | Pos | ND | 8.6×10^3 |
| Wet Event | | | | | |
| KU-02 | ND | ND | ND | ND | 2.1×10^3 |
| KL-02 | ND | ND | ND | ND | 3.1×10^3 |
| SU-02 | ND | ND | ND | ND | 5.2×10^3 |
| SL-02 | ND | ND | ND | ND | 5.3×10^3 |
| NU-02 | ND | ND | ND | ND | 2.5×10^3 |
| NL-02 | ND | ND | ND | ND | 2.2×10^3 |



Relevance to TMDL implementation

- Study demonstrated that septic tanks or leaking sewers are not the only source of indicators in upper watershed (UW)
- Highway runoff in upper watershed (KU) did not contribute human waste during storm but other animal feces must have been washed into the creek
- No viruses were found but Cryptosporidium was ubiquitous during dry event. One site also had Giardia, Toxoplasma, and Francisella.
- No pathogens found during storm event
- Human fecal input solely accounted for the high indicator counts at site KL during wet weather ⇒ **leaking septic tanks likely!**
- Highway runoff in lower watershed maintained signals for all 3 species (human, cow, dog) suggesting a more permanent source of feces from these sources
- Site NU is a residential park that contributed **only human signals** during the wet event! This information can lead to useful implementation strategies.

Conclusions and Applications

- Quantitative molecular determination of viral/bacterial pathogens in place
- Quantitative molecular microbial source tracking in place
- This new technology can help both BMP assessment and TMDL development/implementation



Crystal Ball

- Extend target species for source tracking beyond cow/horse, dog, human
- Establish relationships with other indicators and pathogens
- Extend statistical analysis to determine probabilities of specific loads from non-point sources (animal feces)
- Incorporate into TMDL models

3 years down the road

Low density real time PCR microarrays
for routine source tracking and pathogen
monitoring of highway runoff



Pathogen team

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