

New Ecology Publication and Focus Sheet: Review and Critique of current Microbial Source Tracking Techniques

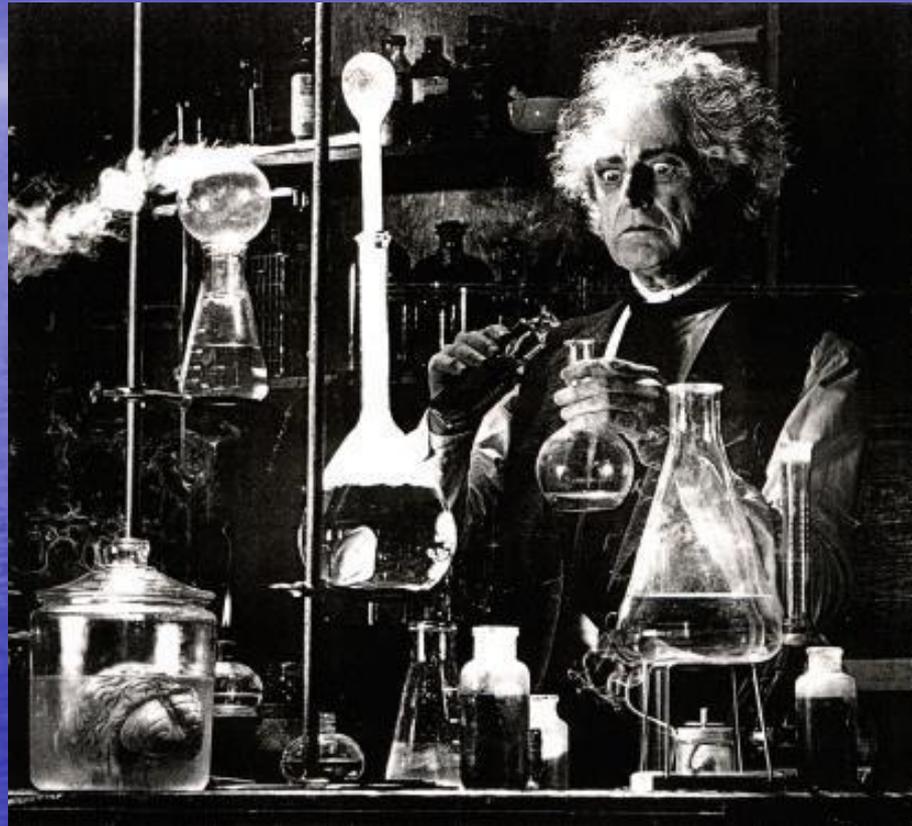


Debby Sargeant, Bill Kammin, Scott Collyard, and
Julie Lowe: Environmental Assessment Program

Background



Major Findings:

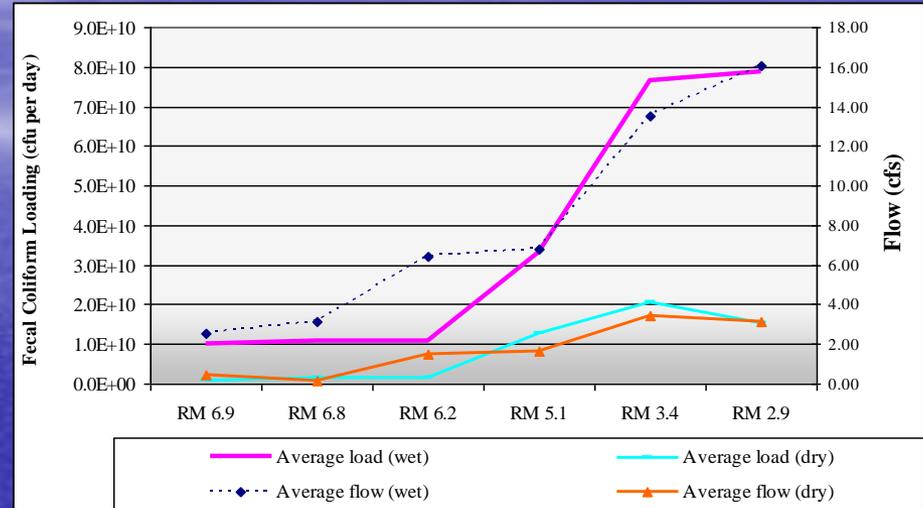


Molecular MST Techniques are experimental science

Conventional fecal source tracking methods should be used first:



Septic system dye testing

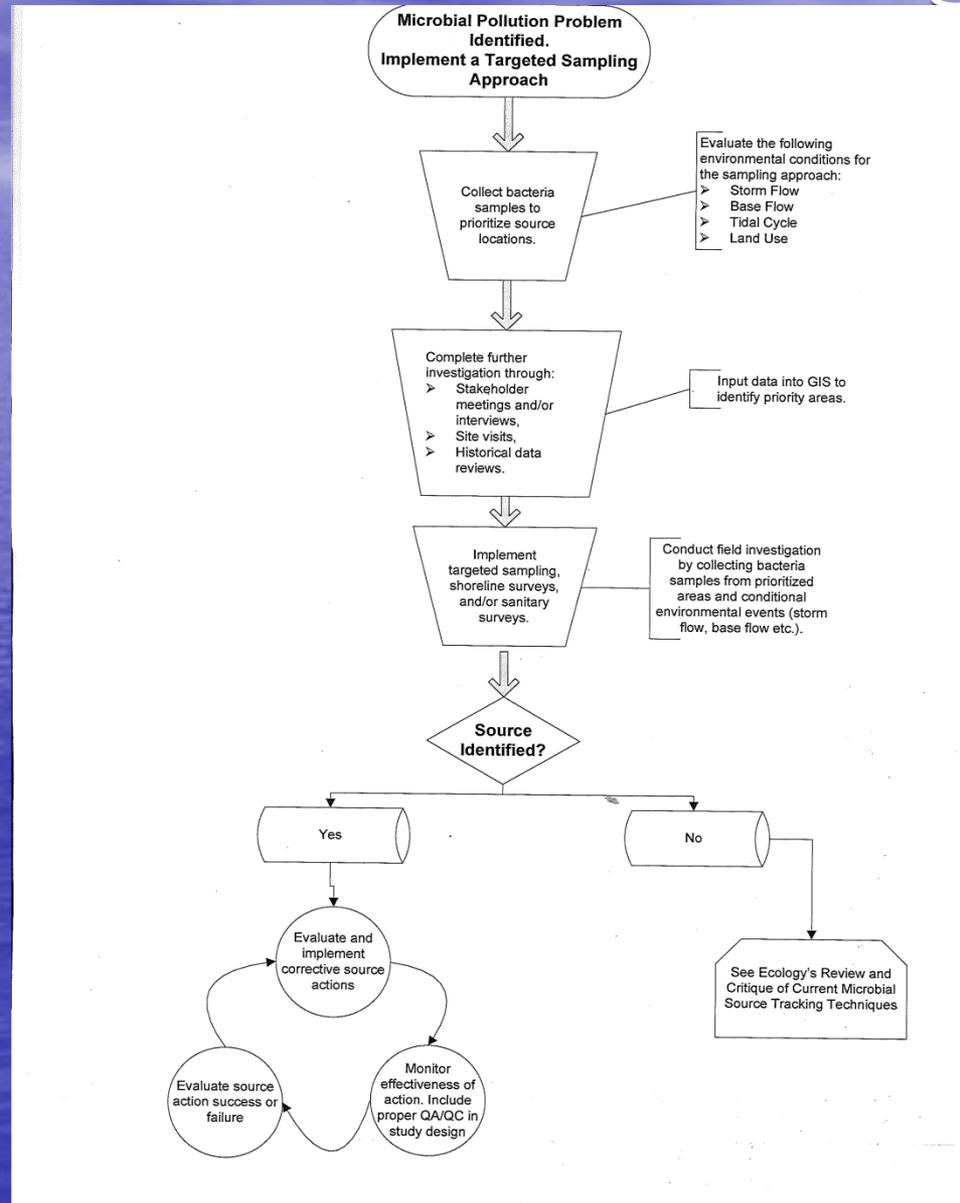


Upstream Downstream sampling

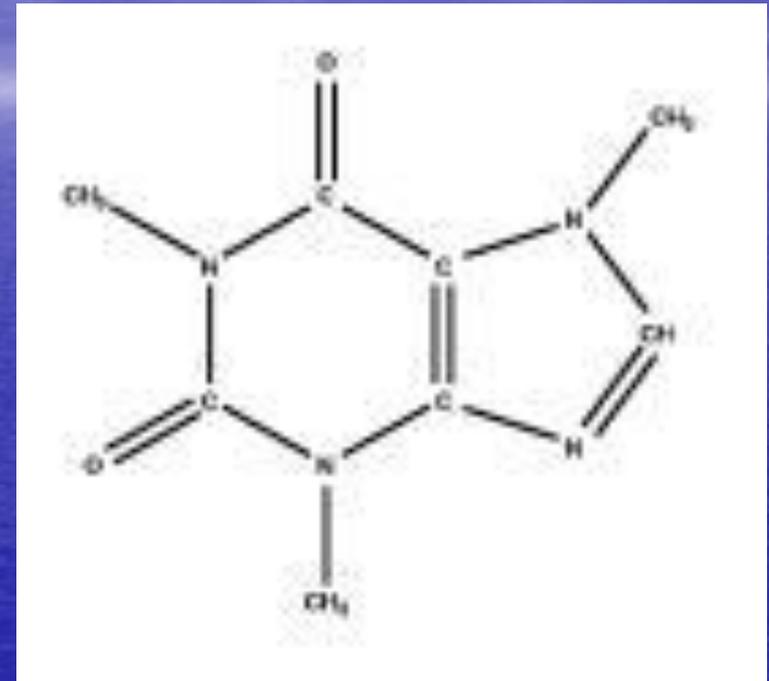
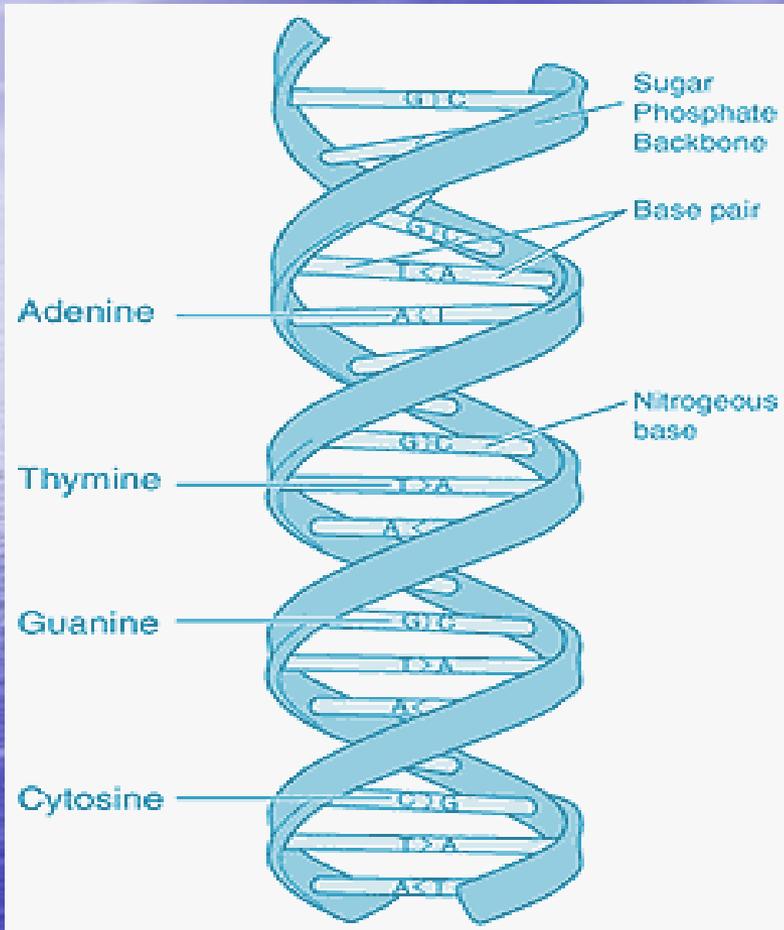


Shoreline Survey

Decision Process for Using MST



Types of Microbial Source Tracking



Chemical methods

Molecular and biochemical based techniques (DNA-based)

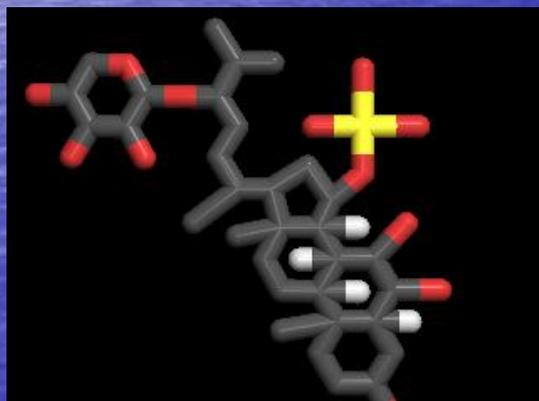
Chemical Methods



Caffeine Detection



Pharmaceuticals



Cholestane-based sterol



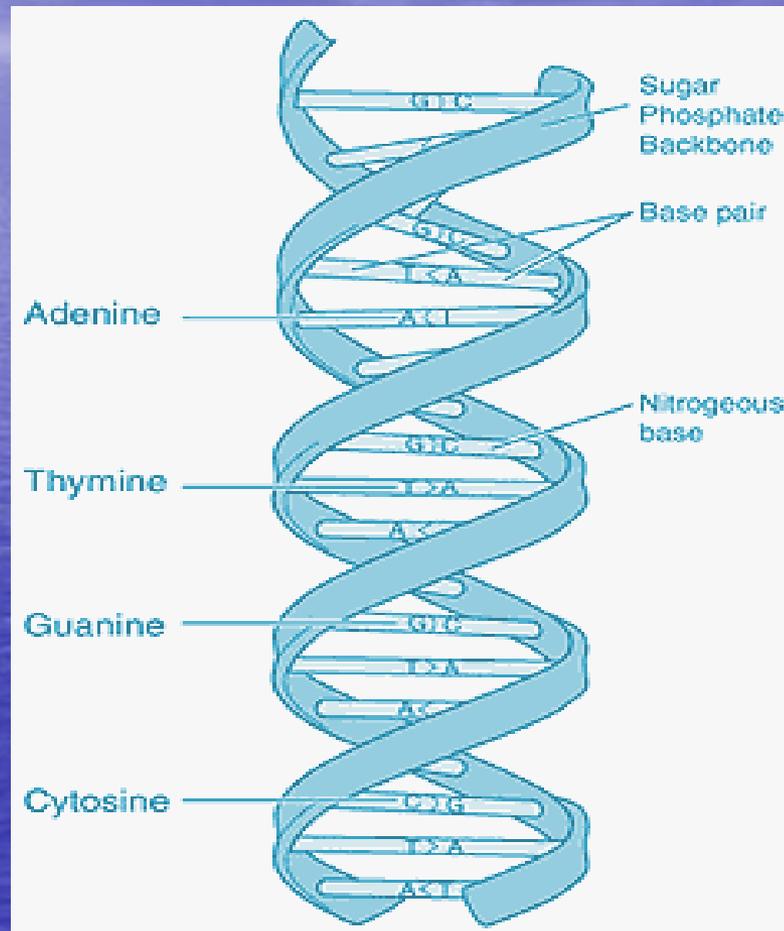
Optical Brighteners

Recommendation:

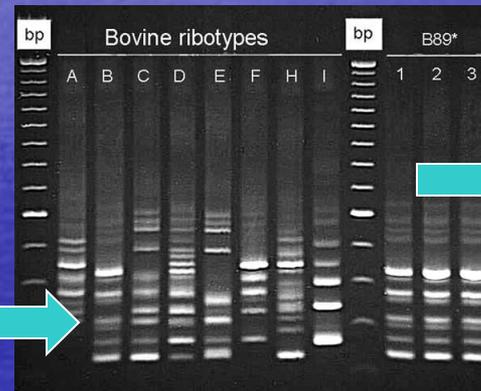


Less expensive techniques such as in-situ monitoring for optical brighteners should be considered for supporting evidence for the presence of human sewage.

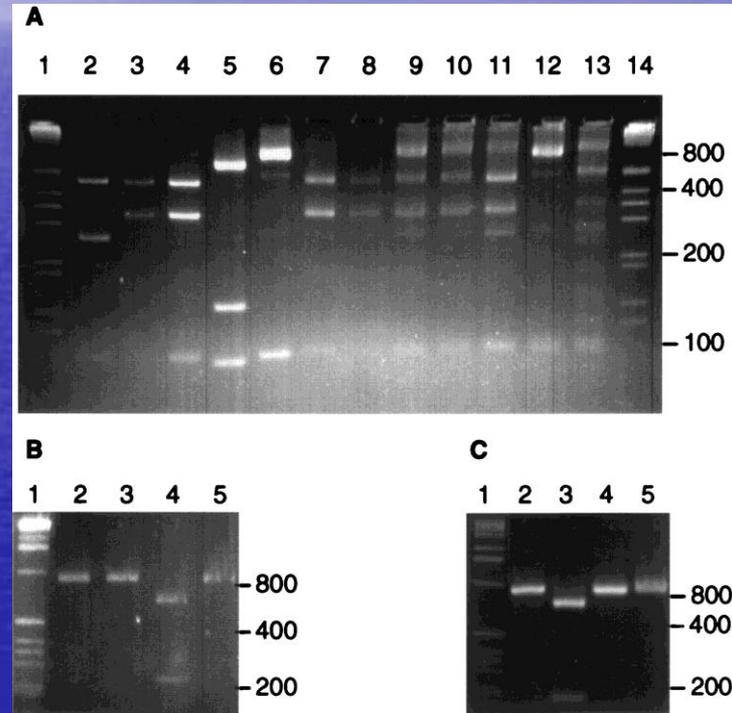
Molecular – biochemical methods



Library-dependent MST methods require development of a source library.



Library Independent Methods target a portion of the genome (DNA or RNA) that is specific to a species



Restriction enzyme cleavage of PCR amplification products from 16S rDNA.

Wood J et al. Appl. Environ. Microbiol. 1998; doi:

Recommendations:

- Library-independent methods recommended over library-dependent.
- MST techniques that have been more frequently tested are preferred over newer methods.
- Fecal samples from the study area should be tested for presence of the proposed MST marker before the study begins.
- Multiple MST markers gives ability to look at more sources and confirm sources.
- Fecal Indicator bacteria (FC) sampling should occur concurrently with MST sampling.
- For detection of human sources: *Bacteroidales*, human polyomaviruses, or *Methanobrevibacter smithii* are recommended.
- MST studies should include adequate field and laboratory QA.

Quality Assurance?

- Is the data reliable?
- Can we use this information?

MST Data Quality

Field Replicates for MST Bacteroides Study

Duplicate	Result 1	Result 2
10314317-8	Human/Ruminant	General
10114055	Ruminant	Human
10114052	General	Human
10194055	General	Human/Ruminant
11015088	Human	General
10194155	General	Human/Ruminant
11015088	Human	General

MST Data Quality

Blind Positive Controls MST Bacteroides Study

Control Number	Control Type	Identified As
1	Cow	Ruminant
2	Cow	Human/Ruminant
3	Dog	Human/Ruminant
4	Cow	Ruminant
5	Dog	Ruminant
6	Dog	General Bacteroides
7	Dog	Human

Field QA Recommendations for MST

- Field duplicates (50%)
- Blind positive controls (fecal material from potential sources) Minimum of one per each potential source per study.
- Blind field negative controls (field blanks) 20% of samples.

Laboratory QA



When is MST useful?



Focus on MST

Ecology Focus sheet on MST

- Ecology's position on MST
- Emphasis on using conventional bacterial tracking methods.
- Decision makers diagram

Review and Critique of Current Microbial Source Tracking methods (2011) at <http://www.ecy.wa.gov/biblio/1103038.html>

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