

Multi-tiered Approach

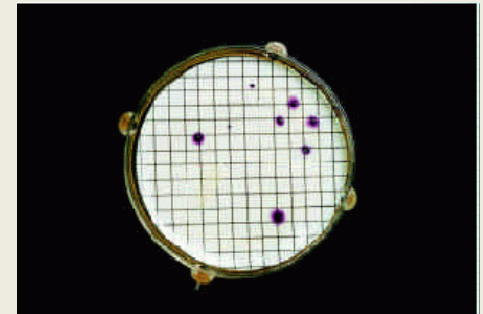
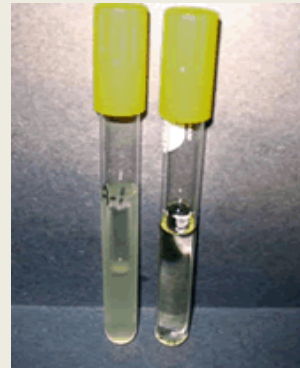
- **Reviews of MST Approaches**
 - Stoeckel, D.M. and Harwood, V.J. (2007). AEM 73(8):2405-2415.
 - Scott, T.M. et al. (2002). AEM 68(12):5796-5803.
- **No one method or microbe alone will identify the source of contamination**
- **Noble et al. (2006). AEM 72(2):1604-1612**
- **Multi-tiered Approach**
 - (1) **Fecal indicators**
 - (2) **Bacterial human markers**
 - (3) **Human Pathogens**

MST: “Lines of evidence”

**(1) Fecal Indicator
Bacteria**

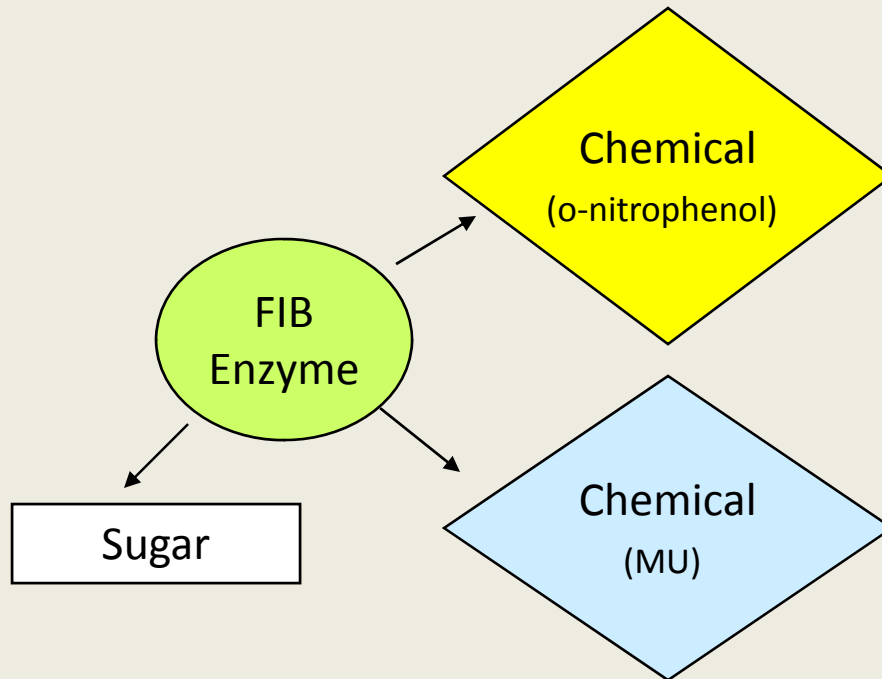
(1) Fecal Indicators

- Reasoning: indication of some type of fecal waste is present, non-human or human
- Fecal Indicators
 - Fecal coliforms, *E. coli*
 - *Enterococcus*
 - Bacteriophages
- Methods
 - Multiple tube fermentation
 - Membrane filtration and plating
 - IDEXX



IDEXX Method

- Add sample to media, shake
- Pour into Quanti-tray[®], incubate overnight
- Count squares and calculate using MPN calculator



(1) Fecal Indicators: References

U.S. EPA Microbiology Home Page

<<http://www.epa.gov/nerlcwww/>>

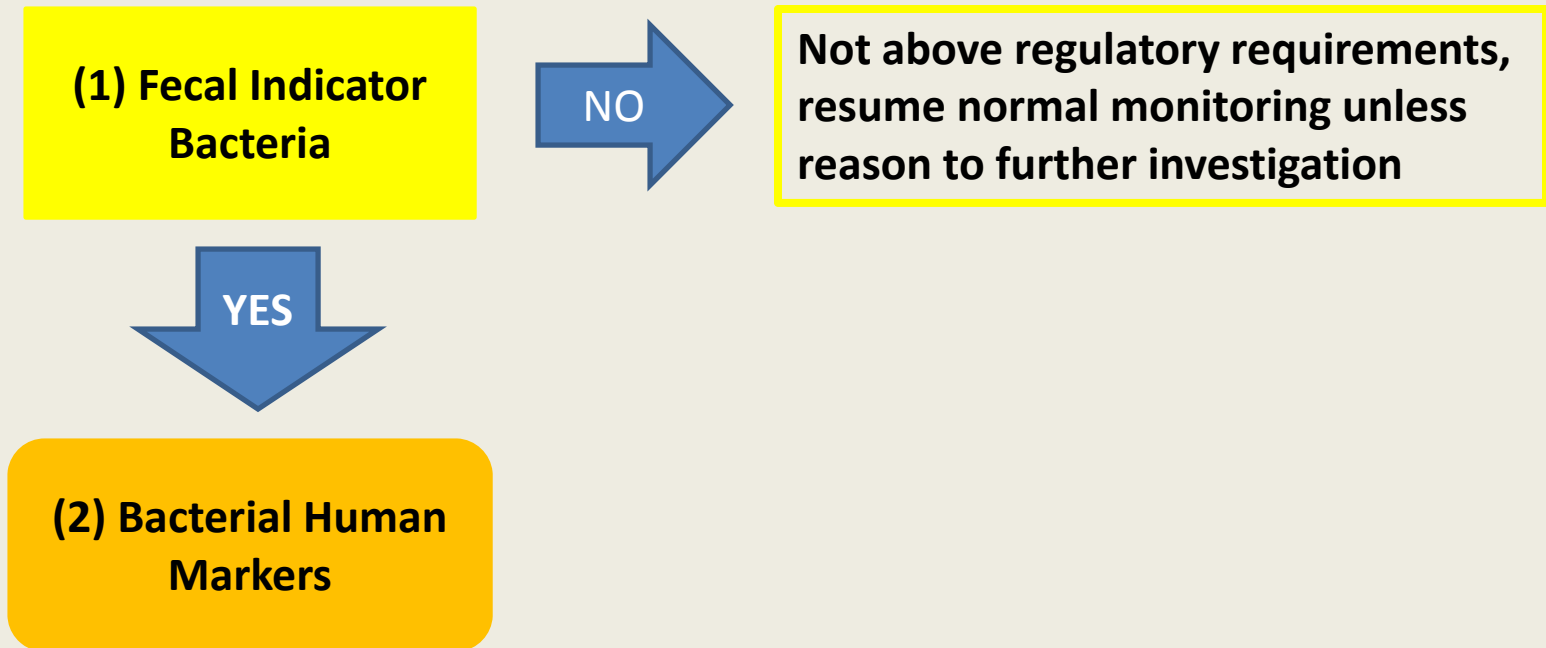
***E. coli* *: Methods 1103.1, 1603, 1604**

***Enterococcus* *: Methods 1106.1, 1600**

Bacteriophage: Methods 1601, 1602

***Methods modified for recreational waters**

MST: “Lines of evidence”



(2) Bacterial Human Markers

- Reasoning: specific genes or sequences identified to be of human origin (i.e. human sewage)
- Bacterial Human Markers
 - *Bacteroides* spp.
 - *Enterococcus esp* gene
- Methods: PCR and QPCR

(2) Bacterial Human Markers

Bacteroides spp.

- Anaerobe
- 100X higher concentration in human gut than FIB
- Total, animal, human markers

Steps

- (1) Filtration
- (2) Extraction
- (3) PCR or QPCR

Few hours with QPCR

Enterococcus faecium esp gene

- Well understood and currently used for water quality management
- Only found in fecal samples from human origin

Steps

- (1) Membrane filtration
- (2) Incubation
- (3) Extraction
- (4) PCR

Molecular Methods



+

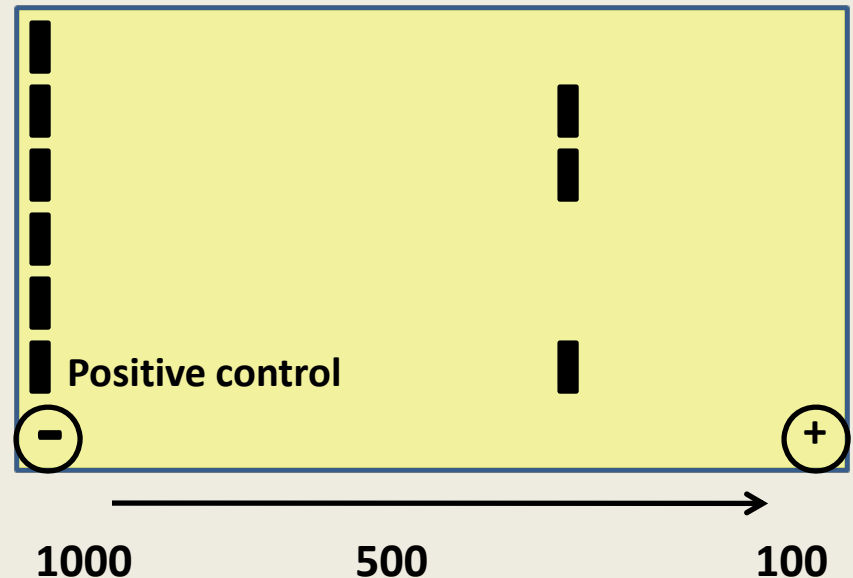
Reagents (buffer, dNTPS)

Primers* (and probes for QPCR) * Specific to assay

Taq polymerase

PCR

- (1) Thermocycler
- (2) Prepare gel
- (3) Mix sample and loading dye
- (4) Add samples to gel
- (5) Run gel

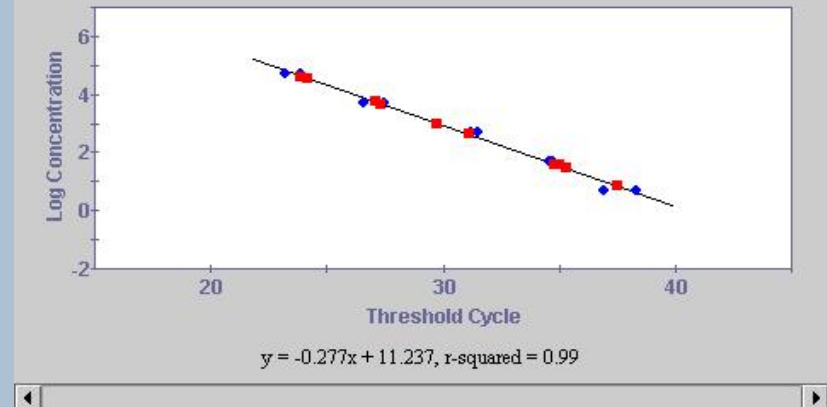


Molecular Methods



- + Reagents (buffer, dNTPS)
- + Primers* (and probes for QPCR) * Specific to assay
- + Taq polymerase

QPCR



(2) Bacterial Human Markers: References

Bacteroides spp.

Bernhard, A.E. and Field, K.G. (2000).

AEM 66(14):1587-1594 and AEM 66(10):4571-4574.

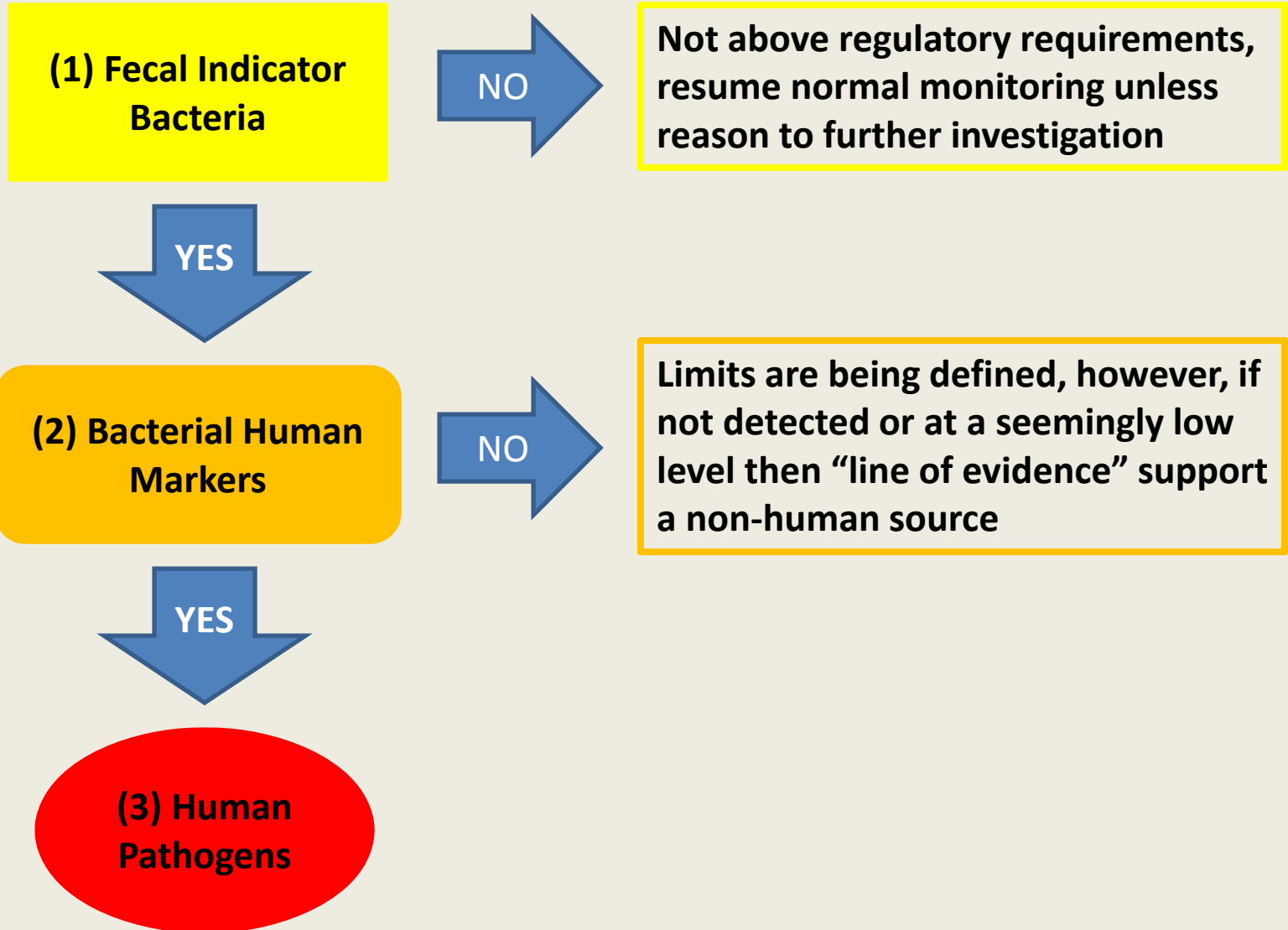
Okabe, S. et al. (2007). AMB 74:890-901

Okabe, S. and Shimazu, Y. (2007). AMB 76:935-944

Enterococcus esp gene

Scott, T.M. et al. (2005). EST 39:283-287.

MST: “Lines of evidence”



(3) Human Pathogens

- **Viruses**

- rotavirus
- coxsackievirus
- echovirus
- calicivirus
- norovirus
- Hepatitis A & E

- **Bacteria**

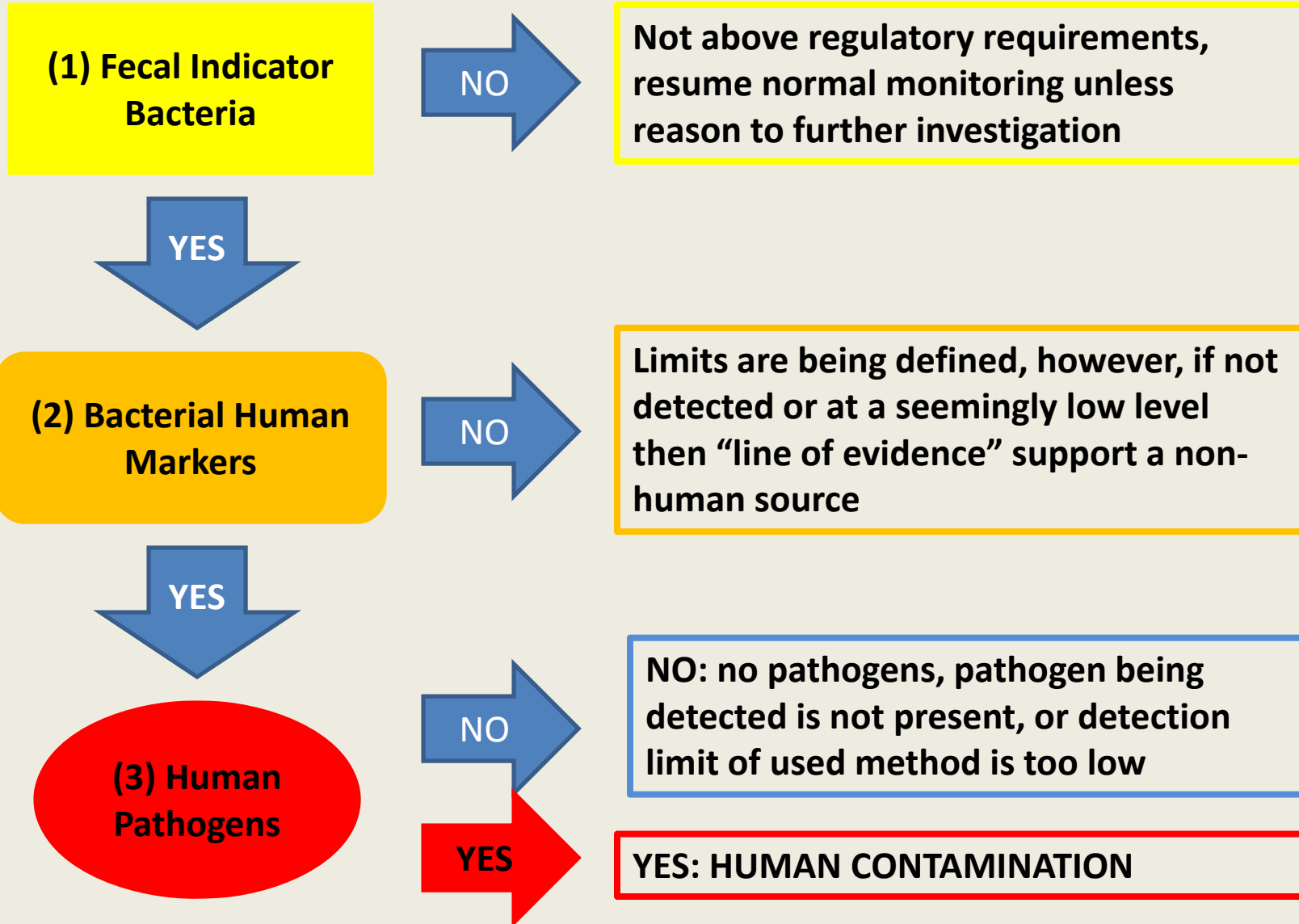
- *E. coli* H7:O157
- *Salmonella* spp.
- *Shigella* spp.
- *Aeromonas hydrophila*
- *Campylobacter jejuni*

- **Protozoa**

- *Cryptosporidium parvum*
- *Giardia lamblia*

- Reasoning: outbreak, concerns, identify source
- Methods: PCR, QPCR, cell culture, microscopy

MST: “Lines of evidence”



Microbial Source Tracking

- **Multi-tiered approach has proven efficient**
- **Conduct steps (1) and (2) simultaneously, confirm with pathogen testing later**

- **Case study**