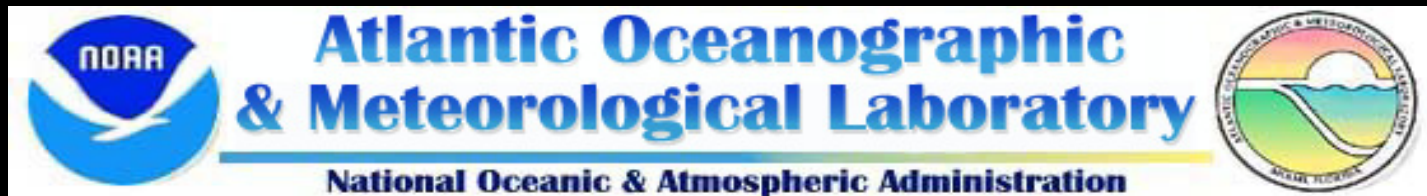


# Development of Molecular Methods to Monitor Waters for Threats to Human Health

**Kelly D. Goodwin (NOAA/AOML)**

**Chris Sinigalliano, David Wanless (UM/RSMAS)**

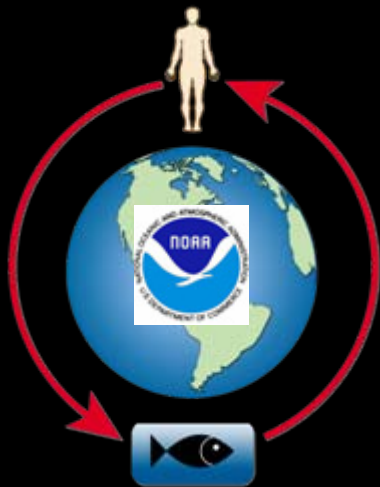


# Oceans & Human Health Centers of Excellence

## NOAA Center at Hollings Marine Laboratory:



- To develop novel techniques capable of rapidly detecting and tracking marine microbes that threaten human health



NOAA  
AOML



## NSF/NIEH Center at UM Rosenstiel:

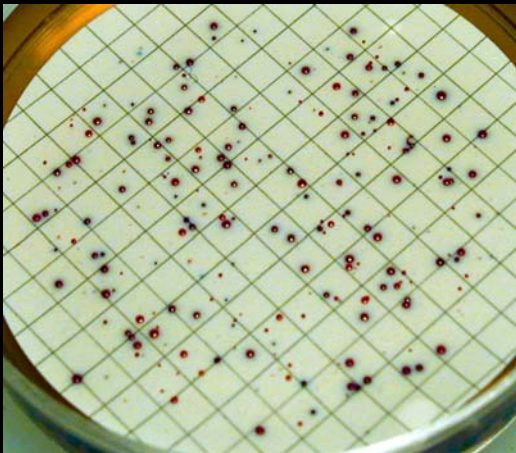
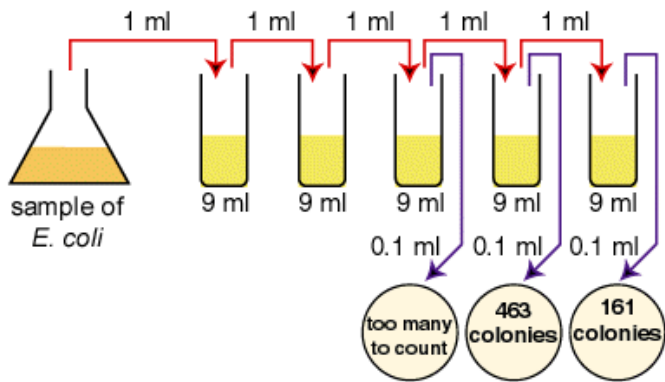
- Identify sources of pollution and develop new monitoring tools, ultimately making beaches safer





# Monitoring & Research Programs Seek Improvements Over Current Methods

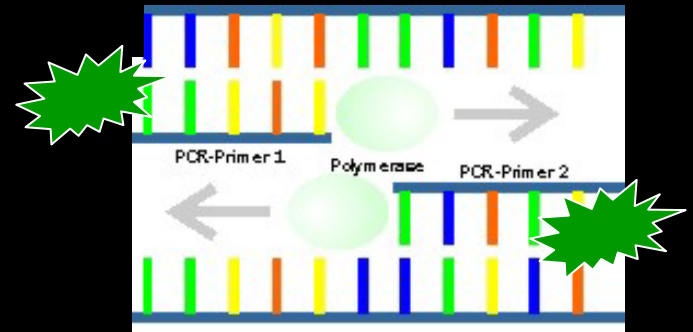
## Traditional Fecal Indicator Assays:



- Too Slow
- Difficult to meet hold time requirement (6 hrs)
- No ability to source track (in time or space)
- No pathogen detection
- Axioms of indicator concept are sometimes violated (regrowth); consequences to human health are unclear

# Use Molecular Techniques to Improve Monitoring of Microbial Contaminants

- ✓ Sensitive & Specific
- ✓ Culture & Microscope Independent



Apply advances in  
clinical biotechnology  
to make environmental monitoring  
faster, easier, cheaper,  
and more automated

# Targets

*Enterococcus*

*Escherichia coli/Shigella* spp.

Bacteroides Fragilis Group

*Bacteroides distasonis*

*Enterococci* human marker

*Bacteroides* human marker

*Bacteroides* dog marker

*E. coli* O157:H7

*Campylobacter jejuni*

*Salmonella* spp.

*Staphylococcus aureus*

adenovirus

standard fecal indicators

alternative fecal indicators

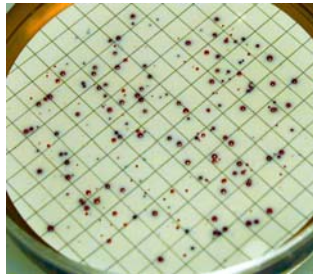
source tracking  
markers

pathogens

intestinal & dermal  
bacteria and virus

# Environmental Samples:

Must Concentrate, Extract, & Amplify Nucleic Acids



focus of work  
to improve sensitivity  
with environmental samples

A) **concentrate targets**

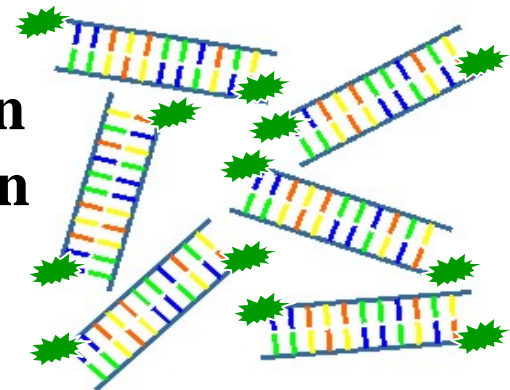
- filtration
- growth

B) **extract DNA**

(or RNA)



C&D) **PCR amplification  
& detection**



# DNA Recovery Protocols

% extraction efficiency, qPCR versus AODC

#ENT cells filtered	Bead-Beat Lysate	Spin kit plant 1:5	Spin kit plant 1:1	Spin kit pellet
1.5E+09	31	2	0.3	27
1.5E+06	16	--	--	NA
4.3E+05	4 - 25	0	0	NA
1.4E+05	23 - 61	0	0	NA
4.5E+04	45	0	0	NA
1.5E+03	79	--	--	NA

**Low & variable efficiencies  
have been observed**

# To Illustrate the Problem

Gene Target	Detection limit (lab) per PCR rx	eluant vol ( $\mu$ l)	dilution factor	% recovery	~ # cells filtered to deliver detect limit
<i>Bacteroides</i> HF8 cluster	1 plasmid	600	5	40	1,500
enterococci <i>esp</i> gene	10 plasmids	600	5	40	15,000
<i>Bifidobacteria adolescentis</i> 16S	5 genome equivalents	50	1	2 30	2,500 167

**THIS IS A CRITICAL AREA FOR WORK**

# Molecular Detection Approaches:



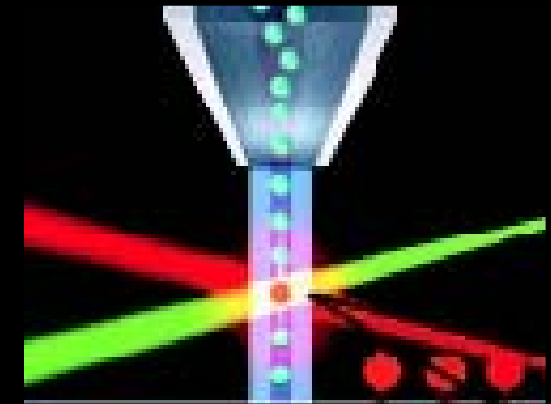
**qPCR**

**(quantitative)**



**electrochemistry**

**(hand-held)**



**Luminex**

**(high throughput)**



**NOAA**

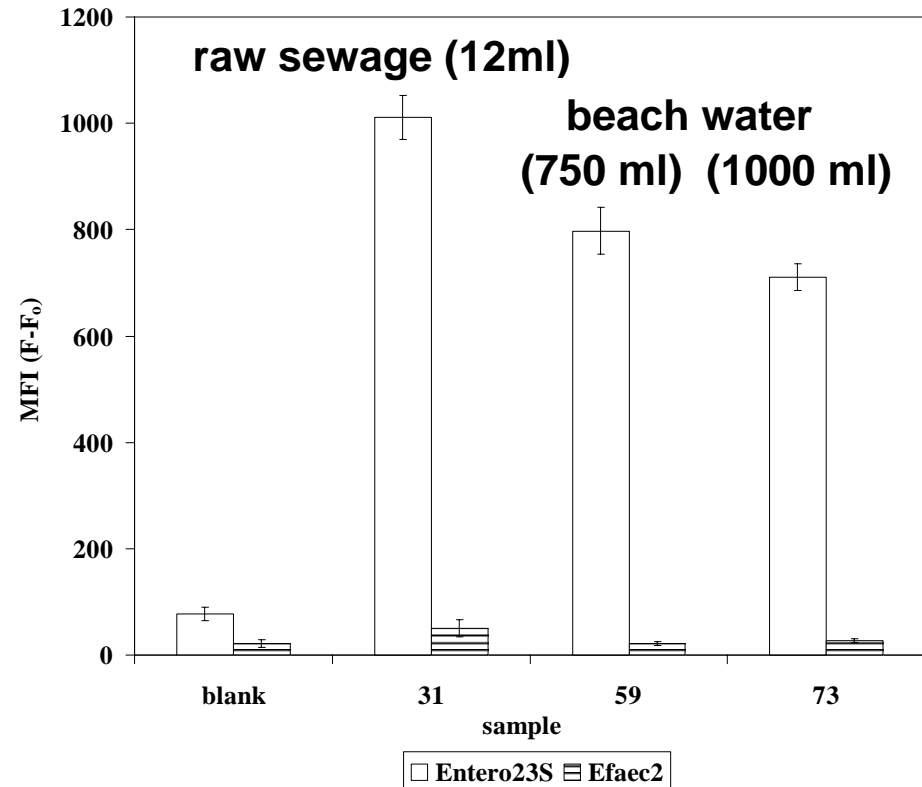
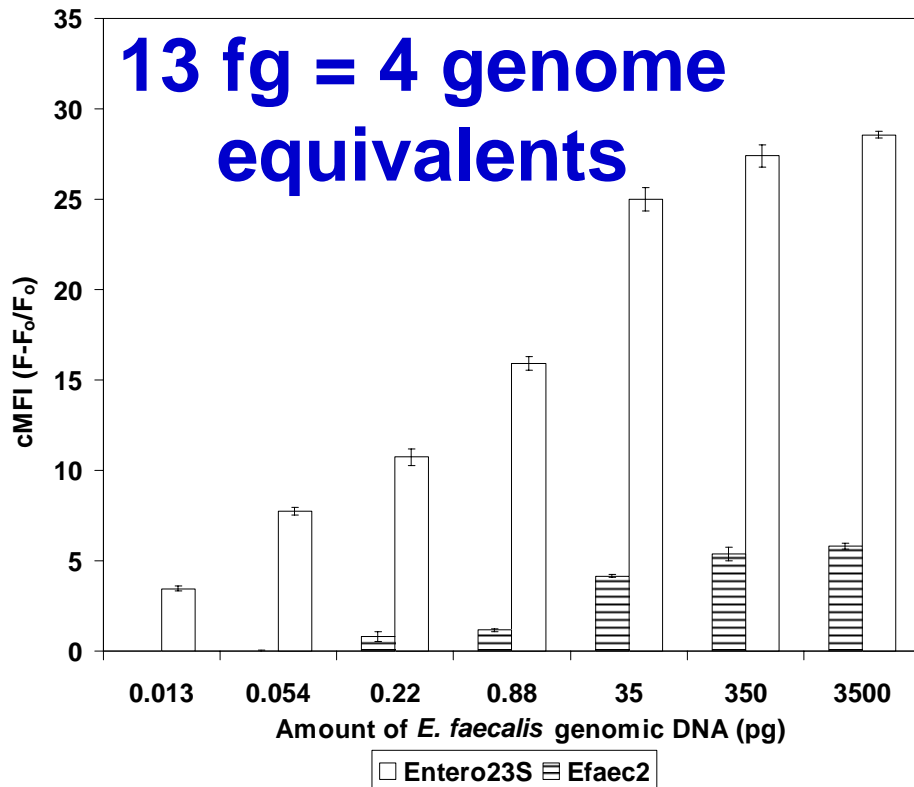


**Alderon**



**U. Miami**

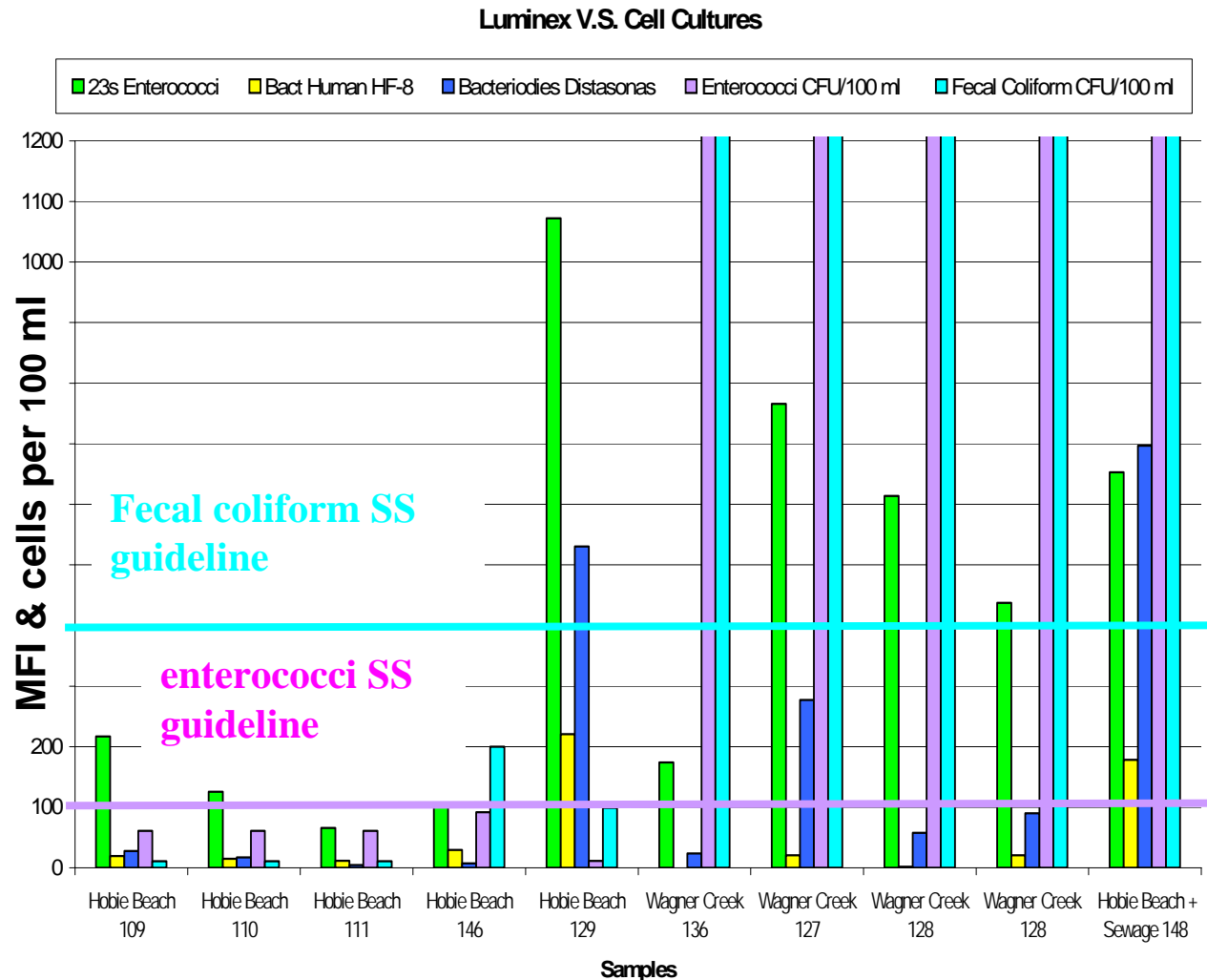
# Sensitivity Improved for Enterococci



switch to 23S target increased sensitivity w/environmental samples

# Correlations between Luminex and culture techniques for environmental samples

- 80% match for samples below the EPA SS
- 75% match for samples above the EPA SS
- Human fecal marker HF8 detected in a beach sample and in a beach sample spiked with 1% sewage



# Source tracking markers

- human *Bacteroides* HF8 gene cluster
- human enterococci *esp* gene
- bird primers
  - cross-react with human feces
  - Search continues (collaboration w/J. Santo Domingo, EPA)
- dog primers (Dick et al. 2005)
  - Chosen focus for Ellender, Wang, Lepo, & Harwood collaboration

# Contact



Kelly Goodwin

305 361 4384

[kelly.goodwin@noaa.gov](mailto:kelly.goodwin@noaa.gov)

Chris Sinigalliano

305 361 4538

[christopher.sinigalliano@noaa.gov](mailto:christopher.sinigalliano@noaa.gov)

David Wanless

305 3614408

[david.wanless@noaa.gov](mailto:david.wanless@noaa.gov)

