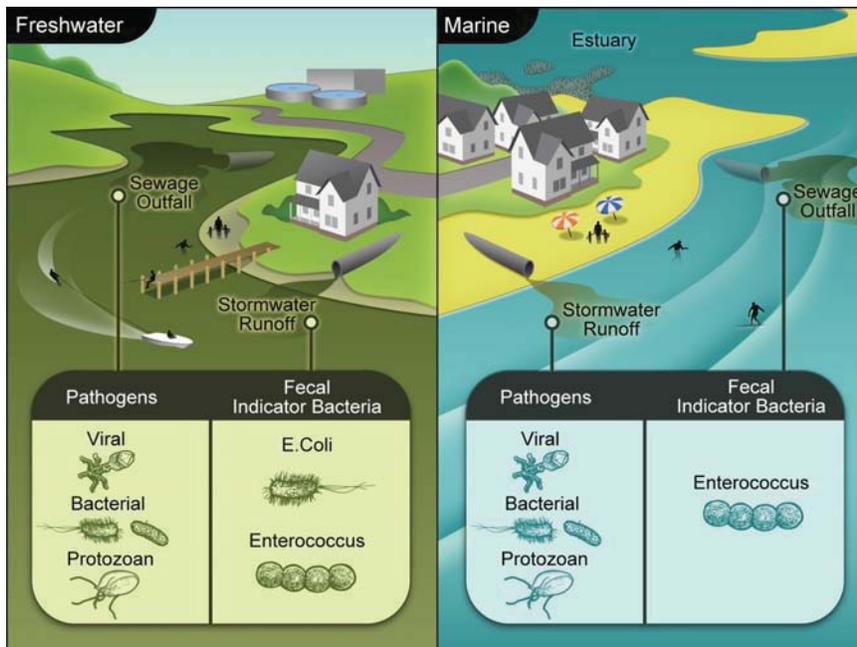




# Pathogen Program: Molecular Tools for Environmental Management

12.2.2015

## Microbial Source Tracking



## Microbial Source Tracking

A **set of techniques** used to determine the sources of **fecal indicator bacteria** in the environment

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## State of the Science

| RATIOS | CHEMICAL/<br>BIOCHEMICAL | LIBRARY DEP.<br>METHODS                                | LIBRARY INDEP.<br>METHODS   | NEXT GENERATION<br>SEQUENCING        |
|--------|--------------------------|--|---|--------------------------------------|
| FC:FS  | Optical Brighteners      | PFGE<br>Ribotyping<br>Rep-PCR<br>Antibiotic Resistance | Bacteriophage<br>Viral PCR<br>Viral qPCR<br>Bacterial PCR<br>Bacterial qPCR | Illumina<br>Roche 454<br>Ion Torrent |



## host-specific qPCR

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## Origins

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HRSD formed to protect public health

- Contaminated oysters were a serious problem

Vision: Future generations will inherit clean waterways and be able to keep them clean

Commission approved Pathogen Program in June 2015

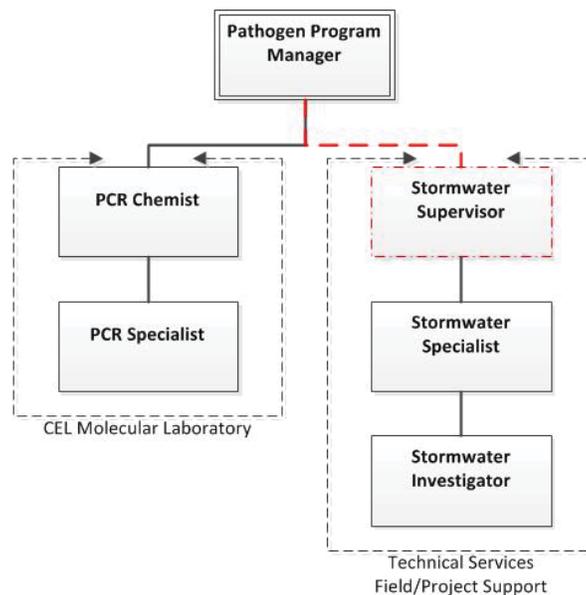
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## Pathogen Program Basics

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## Pathogen Program Basics Cont.

### Focus:

1. **Microbial Source Tracking Studies**
2. Pathogen/Virus Quantification Support

Program Mission: To partner with localities to focus source identification efforts

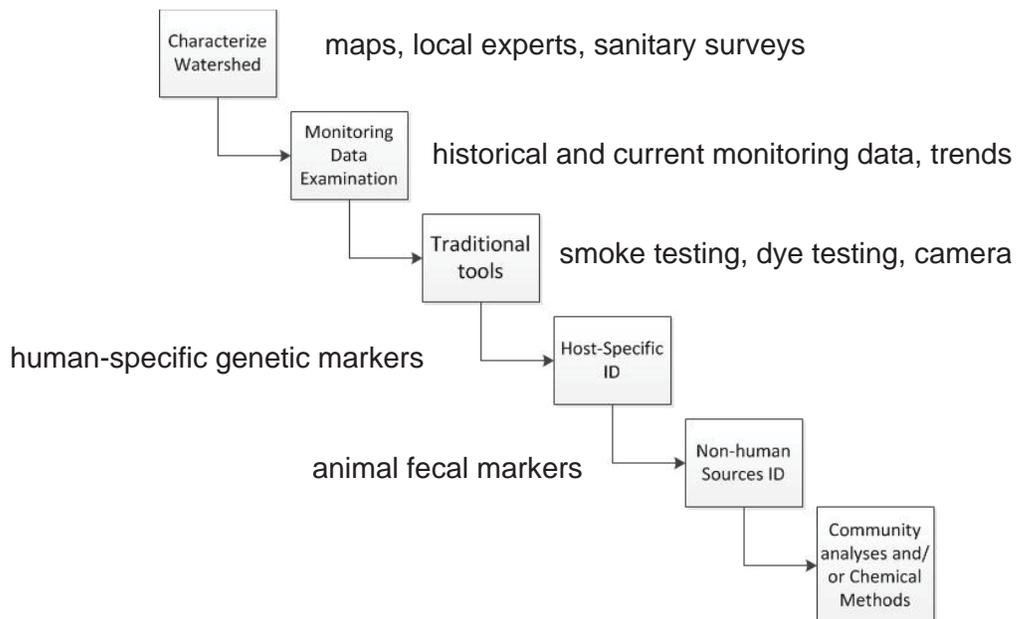
Project Types: infrastructure identification, spill delineation, sewage presence confirmation

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## Chronic Contamination

## MST—The Tiered Approach



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## HRSD's MST Capabilities

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### Currently

- Fecal *Bacteroides* spp.
- HF183
- Human Polyomavirus (HPyV)
- EPA's HumM2 technology

### In the Works

- Other sources
  - Gull
  - Dog
  - Geese
- Pathogens
  - Norovirus (G1 & 2)
  - Adenovirus
  - Hepatitis A Virus

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## Program Logistics

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- **Partnership:** locality must commit resources to put “boots on the ground”
- **Cost:** HRSD pays for sampling and analyses
- **Contract:** Model agreement will be drafted to guide future work
- **Prioritization:** Highest priorities → chronic bacterial exceedances, public health risk
  - Project prioritization: ?

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## Wayne Creek: A Microbial Source Tracking Case Study

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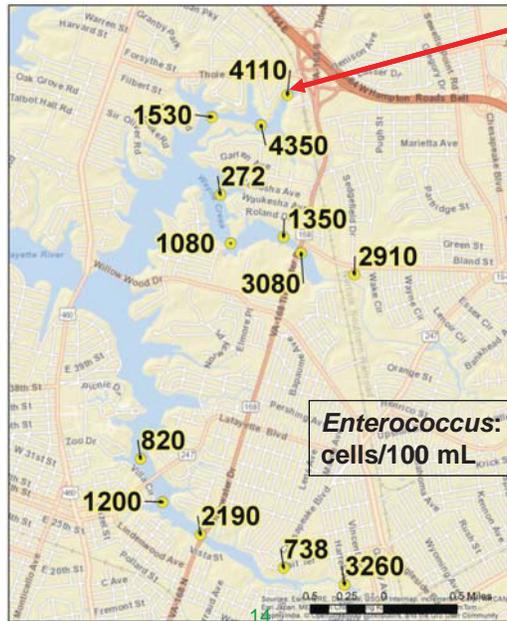
## Chronic Contamination

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## Elizabeth River Project Monitoring

Lafayette Wet Results  
Apr 08, 2014

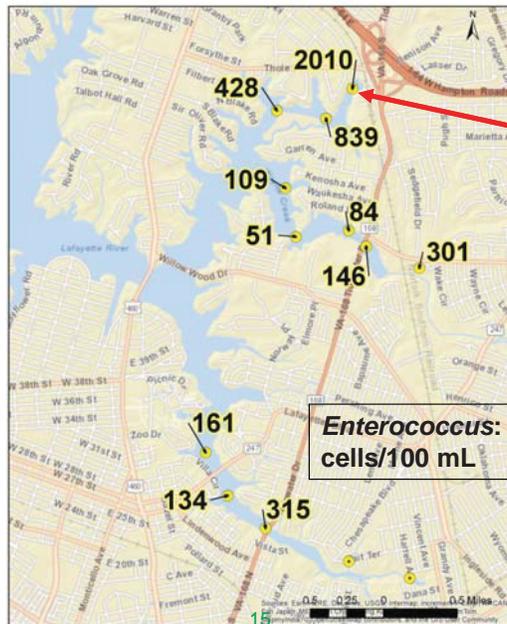


Wet Weather:  
**4110** *Enterococcus*  
cells/100 mL



## Elizabeth River Project Monitoring

Lafayette Dry Results  
Feb 11, 2014



Dry Weather:  
**2010** *Enterococcus*  
cells/100 mL



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## Novel Approach to Understand Causes

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- 1. Alternative indicators
- 2. Targeted in-pipe sampling

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## Sampling Design

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Lafayette Dry Results  
Feb 11, 2014



## Sampling Design

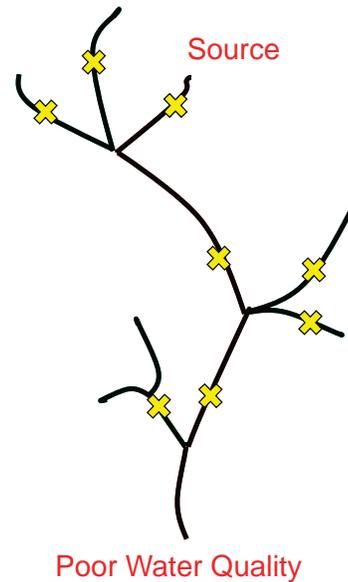


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## Sampling Design

- Downstream to upstream SW pipe network investigation
- Every SW tributary intersection and outfall
- Narrow contamination origin to manageable area



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## Sampling Design

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Sampling design based on infrastructure knowledge

City of Norfolk identified homes still using septic system



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## Results

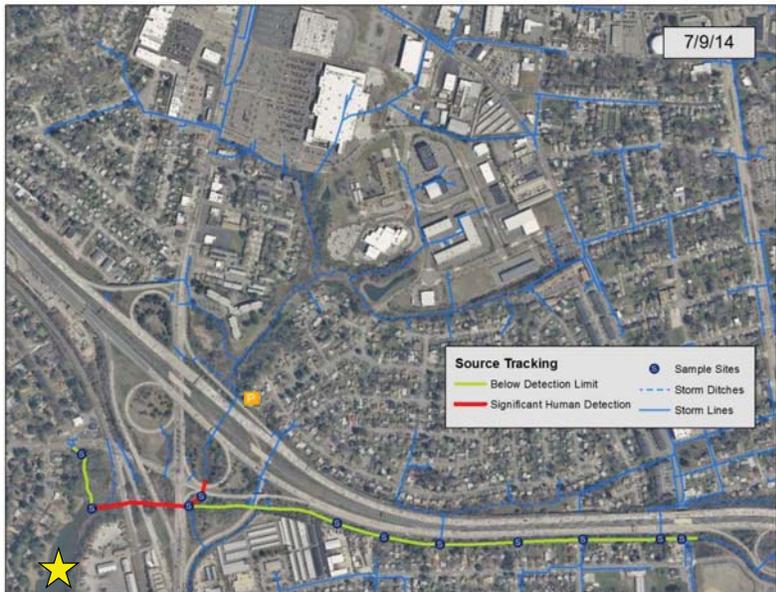
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### 7/9/14—southern segment

Active septic systems—no human signal

Evidence of northern delivery route



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### 9/4/14—northern segment

Human fecal signal found along one stormwater pipe segment

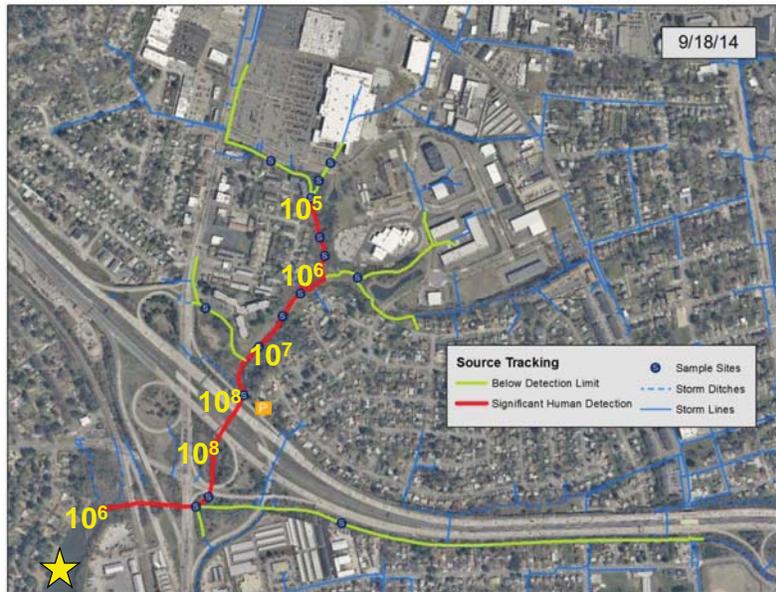


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### 9/18/14—confirmation & delineation

Higher spatial resolution  
Human contamination signal delineated—  
'hot spot' identified



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### 9/18/14—confirmation & delineation

Higher spatial resolution  
Human contamination signal delineated—  
'hot spot' identified  
Human marker levels near pump station levels  
City of Norfolk found a leaking sewer main



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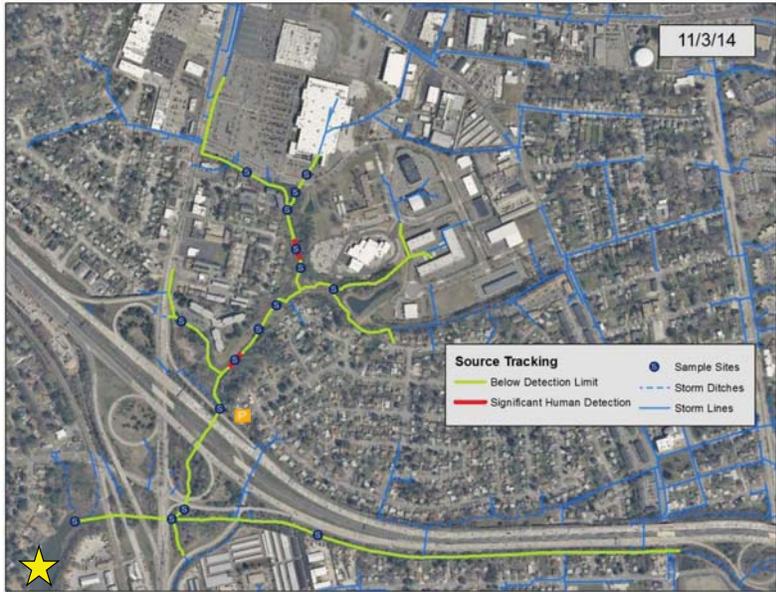


11/3/14—post repair

Water quality improved post repair

2 areas of residual signal

Areas of minimal flushing



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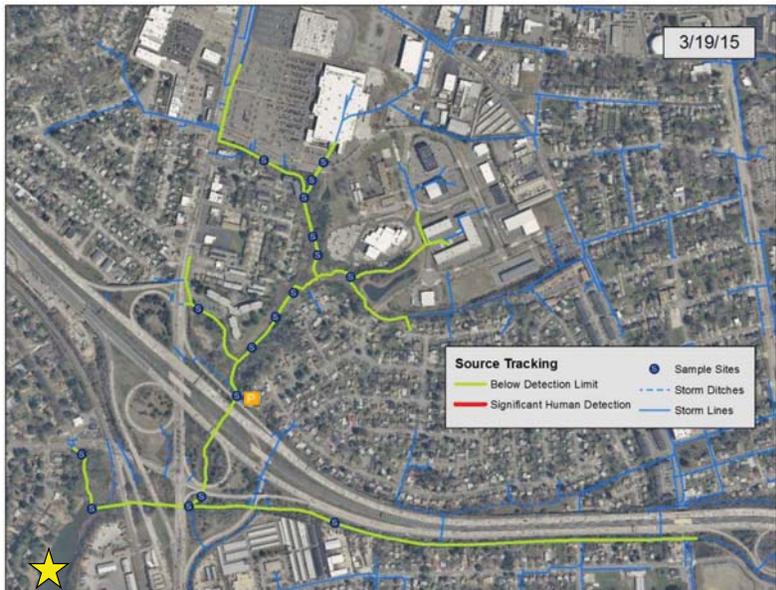


3/19/15—post repair

Excellent water quality:

Low Enterococcus concentrations

No human fecal markers detected



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Questions?

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[www.hrsd.com](http://www.hrsd.com)  
Office: 757 460-7078  
Mobile: 757 613-4956  
Rgonzalez@hrsdc.com

