

U.S. EPA Critical Path Science Plan Projects 19, 20 and 21: Human and Bovine Source Detection

Orin Shanks

Gulf of Mexico Alliance
Microbial Source Tracking & Pathogen Detection Workshop



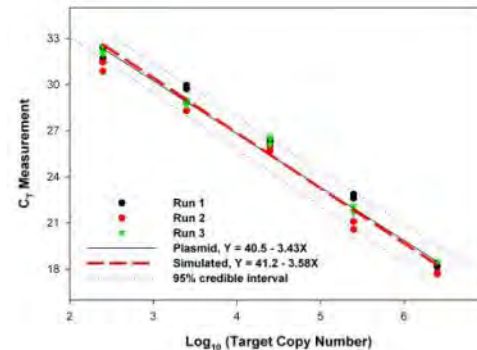
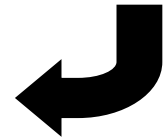


Critical Path Science Plan: Key Science Questions

1. What is the risk to human health from swimming in water contaminated with human fecal matter compared to non-human fecal matter?
2. How well do culture and molecular methods for various indicators correlate with swimming-related illness?
3. Are these indicators...suitable for use in different types of waters?



A Microbial Source Tracking Option

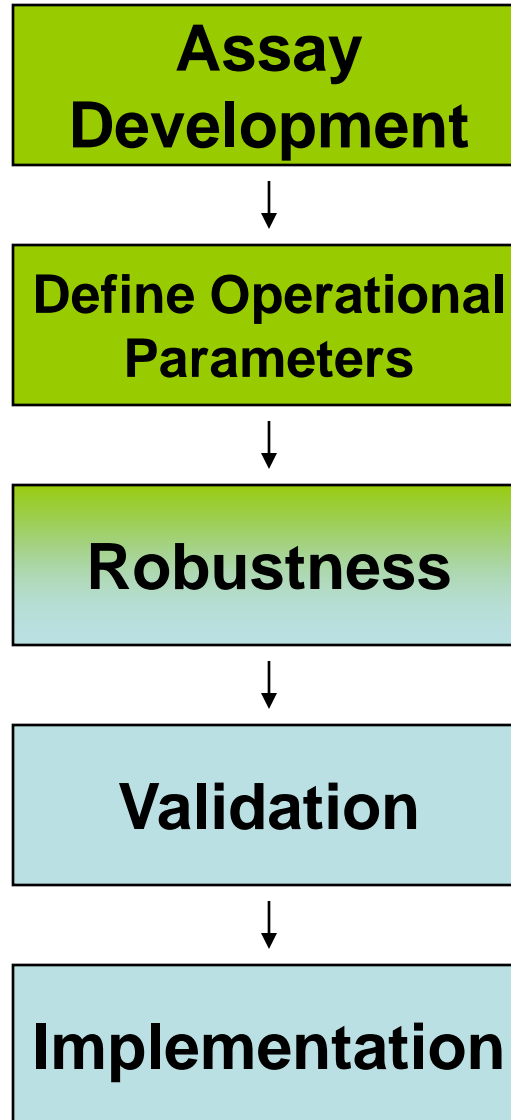
CONCEPT... Match microbe from a polluted site and an animal source to suggest the origin of fecal pollution.



A Map Towards Implementation

- Calibration curve
- Range of quantification
- Precision
- Limit of detection
- Multiplex application

-  Single Laboratory
-  Multiple Laboratories



- Identify host-specific DNA targets
- Design primer/probes
- Controls
- Data analysis

- Specificity
- Host distribution
- DNA target abundance
- Sensitivity
- Sample limit of detection
- Fate and transport
- Link to fecal indicator bacteria
- Link to pathogens
- Link to public health outcomes
- Method comparisons

- AWQC
- TMDLS
- NPDES



Critical Path Science Plan: Human and Bovine Source Characterization Projects

- **Project 19:** Develop novel bovine and human host-specific PCR assays and complete performance evaluation with other published methods.
- **Project 20:** Evaluate human-specific assays with water samples impacted with different levels of fecal pollution from a wide geographic range to supplement site characterization and quantitative sanitary investigation.
- **Project 21:** Evaluate bovine-specific assays with water samples impacted with different levels of fecal pollution to supplement site characterization and quantitative sanitary investigation.

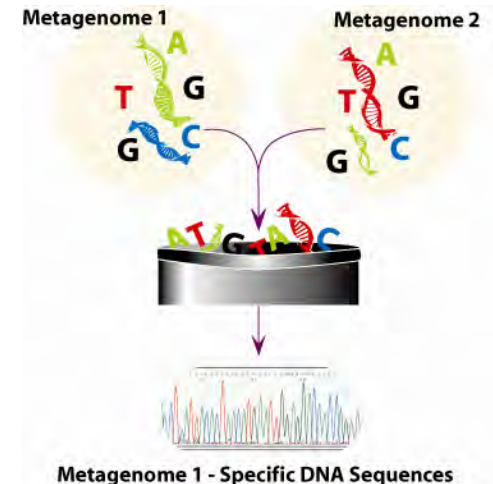
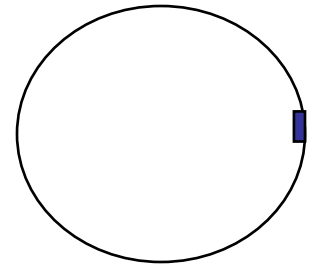
Project 19: Research Goals

- Develop novel host-specific PCR assays
 - rapid technology
 - target bacterial genes
 - appropriate for archived filter testing
 - define operational parameters
- Evaluate performance with other available assays
 - single laboratory
 - primary source
 - end-point and kinetic PCR approaches
 - ribosomal and other gene targets
- Make recommendations for other CPSP projects



Project 19 Assay Development: Identification of Host-Specific Genetic Markers

1. 16S rRNA genes from *Bacteroidales* subpopulations
 - single gene – defined species or related species group
 - targeted sequencing
 - phylogenetic analysis
 - identify 16S rRNA gene sequences specific for target species or related species group
2. Total DNA from fecal microbial community
 - multiple genes -- multiple microorganisms
 - competitive hybridization
 - survey all possible genetic targets
 - identify genes involved in host-specific interactions



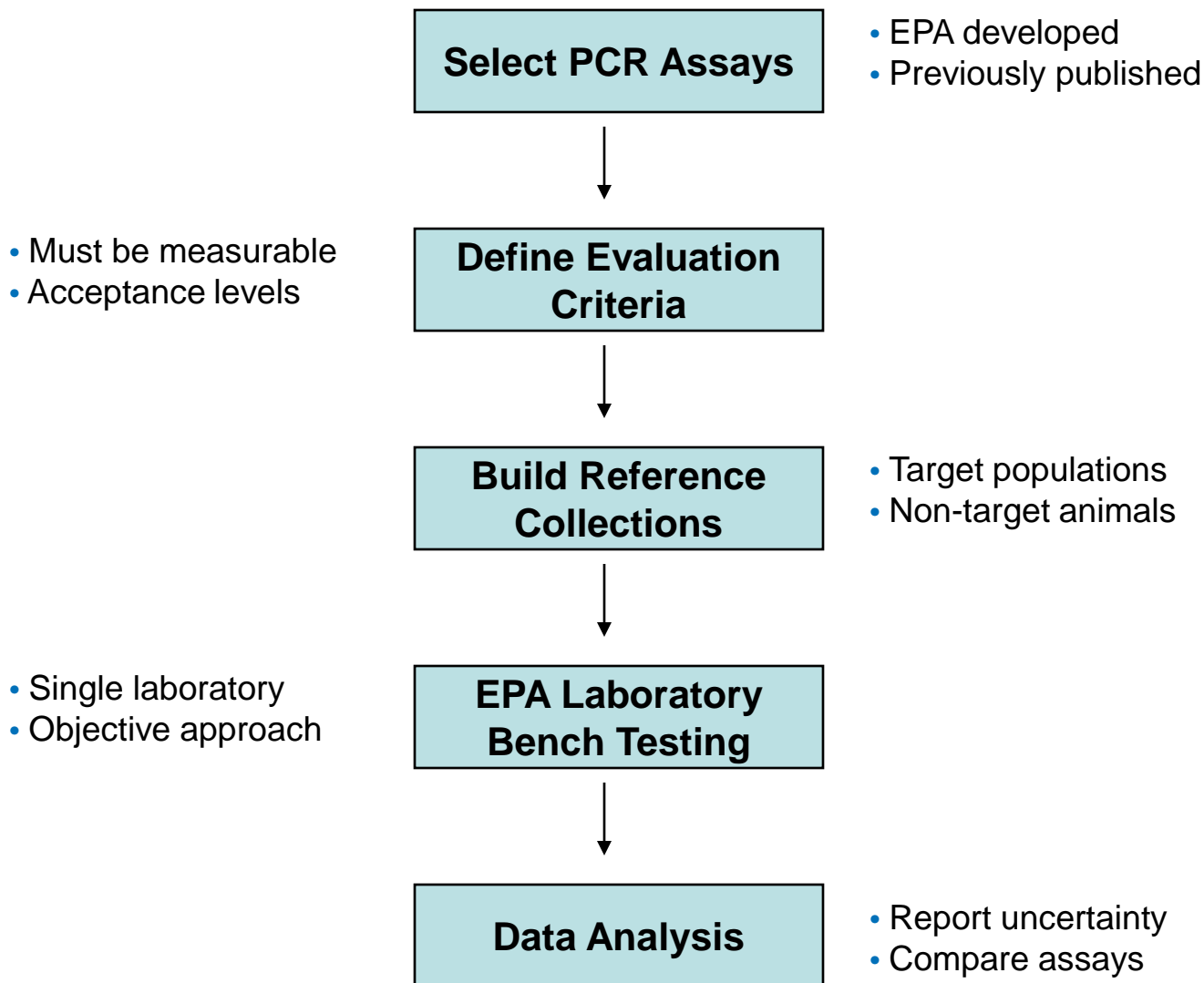


Project 19 Assay Development: Oligonucleotides and inhibition controls

- Primer and Probe Design
 - TaqMan[®] chemistry
 - Primer Express
 - optimization of primer/probe concentrations

- Internal Amplification Controls
 - detection of inhibitory substances
 - competitive approach
 - same primers & reaction conditions
 - possible multiplex application

Project 19 Assay Evaluation: Experiment Design Overview



Project 19 Assay Evaluation: PCR Assay Selection

Focus on bacterial host-associated PCR assays that target DNA sequences from *Bacteroidales* or *Bacteroidales*-like microorganisms

Cattle-Specific		Human-Specific	
<i>PCR</i>	<i>qPCR</i>	<i>PCR</i>	<i>qPCR</i>
4	3	5	12

- 9 End-Point PCR Methods (presence/absence)
- 15 Kinetic qPCR Methods (quantitative)



Project 19 Assay Evaluation: End-Point Criteria

Criterion	Definition	Acceptance Criteria
Sensitivity	Probability that “known” target fecal sample <u>composites</u> yield observed positive result.	High probability score is favorable.
Prevalence	Probability that select “known” <u>individual</u> target fecal samples yield an observed positive result.	High probability score is favorable.
Specificity	Probability that a “known” true negative fecal sample yields an observed negative result.	High probability score is favorable.



Project 19 Assay Evaluation: Kinetic Criteria

Criterion	Definition	Acceptance Criteria
Linearity	The goodness of fit for a regression line plotted from DNA standards and resulting C_T measurements.	Must have $R^2 \geq 0.95$.*
Analytical Precision	The amount of agreement among individually prepared C_T test results; determined by testing multiple times from the same sample material.	Must have mean %CV $\leq 5\%$ across ROQ.*
Range of Quantification	The range of DNA target concentrations that are detectable with an acceptable level of analytical precision.	Must be able to quantify ≤ 25 copies of target DNA.*
Abundance in Target Host	Estimated \log_{10} mean copy number of target DNA from <u>individual</u> samples.	High estimate is favorable.
Abundance in Non-Target Host	Sum of all estimated \log_{10} mean copy number false positives from “known” true negative samples.	Low estimate is favorable.

* Pass or fail acceptance criterion

Project 19 Assay Evaluation: Non-target Fecal Reference Collection



- 33 different species
- 197 individual samples



Project 19 Assay Evaluation: Cattle Fecal Collection



Population	No.	Date Collected
NE2	30	2008
CO1	25	2008
CO2	25	2008
DK Farm	20	2007
NE1	29	2008
5 Star	10	2006
WY	49	2006
USDA	31	2008
OVD	8	2006
GDI	10	2006
Goeres	10	2006
Total	247	

Towards Implementation: Some Research Gaps

- Fate and transport
 - land and water
 - matrix types
 - storm events
 - land use

- Seasonal variability

- Relationship to:
 - fecal indicator bacteria
 - pathogen loads
 - human health
 - general water quality indicators





Project 20 Research Goals

- Conduct series of case studies to help address implementation research gaps
- Supplement ongoing studies:
 - human PCR/qPCR assays
 - qPCR fecal indicator assays
- Provide molecular analysis support for other CPSP projects



Project 20 Case Studies

1. Reanalysis of Great Lakes epidemiology filters (P22)
(NEEAR and US EPA)
2. Avalon Beach 2008 epidemiology study
(SCCWRP and US EPA)
3. Lower East Fork Watershed project
(Clermont County and US EPA)
4. Genetic marker persistence in marine and freshwater mesocosms
(Oregon State University and US EPA)
5. Laboratory support for Urban Runoff (P11) site characterization study (US EPA)



Project 21 Research Goals

- Conduct series of case studies to help address implementation research gaps
- Supplement ongoing studies:
 - bovine PCR/qPCR assays
 - qPCR fecal indicator assays
- Provide molecular analysis support for other CPSP projects



Project 21 Case Studies

1. Iowa cattle Animal Feeding Operation
(Clarkson University and US EPA)
2. North Appalachian Experimental Watershed
(USDA, Clarkson University, and US EPA)
3. Purdue University Animal Science Research and Education Center (USDA, Clarkson University, and US EPA)
4. Genetic marker persistence in sediment microcosms
(USDA, Clarkson University, and US EPA)
5. Laboratory support for Quantitative Microbial Risk Assessment supplemental data (P4) (US EPA)