Determining the Relationship of Microbial Pollution and Associated Health Risks at Freshwater and Saltwater Beaches of Florida

Proposal Prepared for the

Florida Department of Environmental Protection

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Florida Stormwater Association Educational Foundation
Research Advisory Council
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SCOPE OF SERVICES

I. Project Description

In May 2002, the U.S. Environmental Protection Agency (EPA) issued its revised "Implementation Guidance for Ambient Water Quality Criteria for Bacteria," which encourages states to use *E. coli* and/or enterococci as the basis of their water quality criteria for bacteria to protect waters designated for recreation. EPA contends that the use of *E. coli* and/or enterococci is "best suited to prevent acute gastrointestinal illness caused by the incidental ingestion of fecally contaminated recreational water bodies."

Although some states have followed EPA's guidance and have adopted *E. coli* and enterococci criteria, the State of Florida has continued to use fecal coliforms as the basis for its bacteriological water quality criteria, questioning the applicability or appropriateness in Florida of some of the research supporting the use of alternative indicators. Major concerns include the correlation between the indicator organism concentrations and the presence of pathogens and the correlation between these pathogens and the incidence of disease in recreational water users (EPA, 2001). Another key issue is the identification of sources and fate of indicator organisms and pathogens.

The EPA's recent Experts Scientific Workshop has concluded that new or revised criteria are needed based on indicators of fecal contamination, and that *E. coli* and enterococci used without support from any other indicator(s) are probably not appropriate indicators of human health risk from recreational water use in tropical and subtropical environments. Furthermore, it is crucial that revised criteria have a demonstrable

relationship with public health, such as determining the increase in risk of gastroenteritis from recreational water use.

It is likely that at some point in the future, Florida will reconsider joining other states in altering its criteria from fecal coliforms to other indicators. Not only are the indicators themselves a possible target for revision, but more rapid methods of quantification such as quantitative PCR (qPCR) have been proposed to reduce the 24-hour lag time of current culture-based methods

(http://www.epa.gov/waterscience/criteria/recreation/expertsWorkshop.pdf).

Developing and implementing new standards using alternative criteria is an expensive undertaking for the State of Florida and its constituent local governments, and should therefore be initiated only after sufficient research is conducted to guide the state through the process of adopting new bacteriological standards. Such research is necessary in order to resolve some of the many issues that surround the use of fecal indicators in determining the quality of water bodies relative to pathogens and human health, and the appropriate regulatory structure and procedures used to implement any new policies.

It is anticipated that the research results will assist in the development of new or revised criteria for recreational waters. These results will also be applicable for revising and introducing new microbial and chemical indicators for water quality standards and implementation of the total maximum daily load (TMDL) program.

Florida recreational water use is not constrained to designated bathing beaches, but occurs in a widespread pattern at coastal beaches and inland water bodies, therefore studies should be performed at a statewide level at a broad variety of sites that are representative of the diverse recreational waters in the state, which include blackwater rivers, lakes, estuarine bays and marine beaches. However, before embarking on a statewide research program, it is desirable to perform a smaller-scale research study that will lay the foundation for the large-scale study.

The study would be instrumental in answering some of the key questions relative to the statewide effort. Such questions would be:

- What **methodologies** should be used to quantify indicators and pathogens, and are **rapid methods** for detection and quantification useful at this time?
- Are specific indicators of **human fecal contamination** (MST markers) better indicators of public health risk than the generic fecal indicator bacteria currently used?
- What **pathogens** should be included in future studies?
- What is the **correlation** between measured indicators and the presence of pathogens?
- What kinds of **risk assessment** studies are more appropriate to establish a correlation between pathogens and incidence of disease?
- Could **other indicators** be used in lieu of or in combination with *E. coli*/enterococci?

- What are the probable dominant **sources** of fecal indicator bacteria and/or pathogens in a water body where non-point sources are the major contributors of pollution?
- What is the **health risk** to people who use beaches that receive nonpoint source pollution? Can we assess the risk among the most vulnerable fractions of the population, e.g. children?
- Can the relationship between indicators, pathogens, and human health risk be **modeled** using some combination of biological, physical, and chemical measurements?
- Comparison of these results with other Florida and national epidemiology studies (e.g. Miami, southern California) will allow synthesis of the relationship between indicator, pathogen and human health relationships, and determination of the next steps in establishing appropriate recreational water quality standards in Florida.
- It is anticipated that there will be high demand for the laboratory services developed in this proposal, therefore an inter-laboratory calibration study with one or more labs (in addition to USF) will be conducted after the most suitable tests are identified.

The proposed study is timed to take advantage of recent epidemiology studies conducted by the Southern California Coastal Water Research Project (SCCWRP) and the U.S. EPA around the 2006-2008 time frame. Many MST methods were performed in conjunction with the SCCWRP study, and correlation of the MST results with the epidemiology results will facilitate selection of the markers to be used in this study. Q-

PCR of indicator bacteria, particularly enterococci, was used in the U.S. EPA studies (unpublished; Wade et al 2006; Wade et al 2008), which will similarly provide data to optimize the study design in this project.

II. Study Approach

Solutions to the questions above may be best obtained by a two-phased research approach.

1. The first, the **MST-TMDL Phase,** will be planned for a 2.5 year period and will focus on:

- Optimizing and validating recently developed quantitative PCR (Q-PCR) methods for rapidly quantifying enterococci, the fecal indicator bacteria group recommended for use in salt and fresh water by the U.S. EPA. This effort would include correlating the Q-PCR measurements with standard methods of measurement, i.e. membrane filtration and culture. The Q-PCR methods used will be those employed in recent studies by the U.S. EPA (e.g. Haugland et al 2005; Wade et al 2006, 2008).
- Optimizing and validating recently developed Q-PCR protocols for MST markers that quantify microorganisms associated specifically with human fecal pollution, e.g. human *Bacteroides* and human polyomaviruses. This effort would include questions on persistence of the markers, sensitivity and detection limits, and the ability to quantify the target.

- Using field samples to correlate the performance of the Q-PCR methods with standardized, culture-based measurements of indicator bacteria and their ability to quantify sewage pollution in waters that are known to be impacted by various sources of pollution.
- Determining the usefulness of these Q-PCR methods for TMDL assessment and implementation in a watershed study. This component may be able to be "piggy-backed" on an ongoing TMDL study.
- Assessing the robustness of the methods with respect to inter-laboratory usage. Up to three laboratories in Florida will participate with USF in validating the QPCR methods using shared protocols, shared samples and finally, blinded challenge samples (fecal source is known to the distributor but not the recipient).

2. The second, **Epidemiology Phase** will be conducted at bathing beaches at one freshwater and one Tampa Bay site, both of which are known to be impacted by stormwater and which do not (to our knowledge) receive direct sewage impact. It will be planned for a **one-year period,** and will also include measurements of Q-PCR assays for indicator bacteria and human-associated MST markers that were validated in Phase I.

The research will be lead by Dr. Valerie J. Harwood, a member of the FSA Educational Foundation's Research Advisory Council and Professor at the USF Department of Biology, in collaboration with other members of the Research Advisory Council (see last page), and with Dr. Helena Solo-Gabriel and Dr. Lora E. Fleming of the University of Miami.

III. Study Design

This study will be designed in consultation with U.S. EPA scientists and Florida Department of Environmental Protection personnel to ensure that the data generated will be acceptable to the regulatory community. Ideally, this study will be complementary to a recently completed microbiology and epidemiology study in the Miami (Hobie Beach) area in order to allow direct comparison of the results. Major differences between this proposed study and the Miami study include:

(a) use of a freshwater beach site

(b) use of a Gulf of Mexico site

(c) assessment of quantitative (real-time) PCR for quantifying *E. coli,* enterococci and MST markers

(d) assessment of alternative (Bayesian) modeling approaches to predicting pathogen presence

(e) use of physical/chemical indicators of stormwater contamination in modeling indicator concentration, pathogen presence and human health outcomes, and

(f) an inter-laboratory comparison to determine the reproducibility of the QPCR methods will be undertaken.

Task 1. Evaluation of Bacterial Indicator Methods – Conventional vs. QPCR.

Indicators measured by conventional (culture) methods will include fecal coliforms, *E. coli* and enterococci. *E. coli* and enterococci will also be enumerated by quantitative (real-time) PCR, which yields results within two hours (Khan et al 2007; Haugland et al 2005; Siefring et al 2008). Total *Bacteroides* (anaerobic bacterial genus that is a dominant member of the microbial community in the feces of many animals) will also be measured by QPCR (Siegfring et al 2008). This activity will occur in Year 1 (see Timeline below).

QPCR methods will first be validated in the laboratory for sensitivity (ability to detect target when present), specificity (ability to rule out target when absent) and limit of detection (ability to detect target a low concentrations). QPCR assays for *E. coli* and enterococci will be compared to conventional (culture-based) methods. The performance of the QPCR methods will also be evaluated in natural freshwater and saltwater. Inhibition of QPCR assays in environmental waters will be assessed by utilizing internal controls. The laboratory validation of QPCR methods for indicator organisms is expected to take 6 months), and field sampling will be conducted over an additional twelve months. Field sampling will occur at minimum on a monthly basis, and opportunistic sample events will be conducted to take advantage of targets of opportunity (e.g. sewage spills, major rain events). Field study design will be further developed in collaboration with FDEP scientists after field sites are chosen. The rationale for choosing field sites will include the testing of different water matrices (fresh vs. salt water) and waters that are expected to be impacted by anthropogenic activities vs. relatively unimpacted waters (such as protected headwaters or remote beach sites).

Deliverables – Task 1.

a. Laboratory validation methods report: Performance of QPCR for indicator organisms

b. Conventional indicators versus QPCR report

c. Field study design

Task 2. Detection and/or Quantification of genetic markers.

Human sewage is a well-known human health threat and is thus considered to be a high-risk contributor to bacterial loading in surface waters. Human sewage impacts can be readily controlled when identified, unlike less discrete sources such as wild animals. Genetic markers can be used to determine whether human sewage is contributing to microbial concentrations in stormwater and recreational waters. In this study QPCR methods will be used to detect and/or quantify human-associated genetic markers of fecal pollution: e.g. human polyomavirus (HPyV), human *Bacteroides* and *Methanobrevibacter smithii.* These markers will be validated as above. There is also the potential for using markers for fecal pollution from birds and dogs. This activity will begin in the second half of Year 1 and continue through Year 2.

Deliverables – Task 2.

a. Microbial source tracking (MST) optimization and validation report

b. Report on MST field testing results

Task 3 – Field Development of Predictive Models (Bayesian Modeling).

The microbiological and chemical data will be analyzed to determine the best predictive relationship between indicator organisms and MST markers, both on the basis of conventional and QPCR methods for indicator organisms. Approaches included will be Bayesian (probabilistic) modeling of the relationships.

Deliverable – Task 3.

a. Bayesian Model

b. Bayesian Model Results

Task 4 – Inter-laboratory Calibration

This component of the study will validate the performance of the QPCR assays for indicators (*E. coli*, enterococci and total *Bacteroides*) and MST markers (HPyVs, human *Bacteroides* and *esp*. To the best of my knowledge, only three laboratories in Florida have the demonstrated expertise in QPCR and environmental microbiology to participate in this study: University of Miami (Dr. H. Solo-Gabriele), University of West Florida (Dr. J. Lepo) and Biological Consulting Services of North Florida (Dr. T Scott and Dr. J. Lukasik). Each of the three collaborating laboratories will begin the inter-lab calibration study at the beginning of year 2, and the study will continue over the next 18 months.

QPCR protocols optimized and validated by USF and positive control material (organism or plasmid containing the gene of interest) will be distributed to participating laboratories to allow adoption of the assays in the laboratories. Once laboratories are proficient at generating a quantitative standard curve, sensitivity and specificity tests in a simple matrix (phosphate buffer, pH 7.2) will be undertaken by "seeding" samples with fecal material from known sources. The assays will then be conducted on fecal material seeded into fresh, estuarine and marine water to assess their performance in environmental water samples. Finally, water samples seeded with fecal material from known sources that are blinded to the analyzing laboratory will be provided for proficiency testing. This activity will begin in Year 2 and continue through the first half of Year 3 for a total of 18 months.

Deliverables – Task 4

a. Protocols developed by USF

b. Report on Inter-laboratory Calibration

Task 5 – Final Report for Phase I.

A final report for Phase I will be prepared to summarize and present a comprehensive compilation of analysis and results. Individual reports produced within each Task will be included in the Final Report. Prior to publication of the final report, a draft Report will be circulated to the TAC, FDEP, WERF and other interested parties for their review and comment.

Deliverables – Task 5

a. Draft Reports

b. Final Report

PHASE II

Epidemiology. The epidemiology study design is dependent upon the results of the recently-completed University of Miami epidemiology study, and acceptance of the methodology by the U.S. EPA. It is important to develop a study design that is sensitive to low levels of illness in the study population so that relative risk from exposure to recreational water can be assessed. Assuming that the data analysis from the Miami (Hobie Beach) study supports this design, a prospective randomized trial will be conducted in which participants are enrolled in the study for a nominal fee (e.g. $25) and randomly assigned to groups which receive varying exposure to the water. The epidemiology study will mirror the Miami study so that the results can be compared. The freshwater and saltwater study will be conducted in consecutive years. Q-PCR for MST markers and indicator bacteria, as well as specific pathogen assays will be conducted in conjunction with the epidemiology.

Phase II is not included as a part of this specific Scope of Services. Phase II will be performed under separate contract initiated before the end of Phase I.

SUMMARY TIMELINE

	Year 1 (2009) Phase I		Year 2 (2010) Phase I		Year 3 (2011) Phase I & II		Year 4 (2012) Phase II		Year 5 (6 months) Wrap-up
Task 1 – Evaluation of Indicator Models	→	→							
Task 2 – Genetic Markers		→	→	→					
Task 3 – Modeling		→	→	→	→				
Task 4 - Inter-Lab Calibration			→	→	→				
Task 5 – Phase I Report					→				
Epidemiology: Freshwater					→	→			
Epidemiology: Saltwater							→	→	
Final Data Analysis & Report									→

TECHNICAL ADVISORY COMMITTEE

Both phases of the Project will incorporate the use of a Technical Advisory Committee or TAC. The TAC will be composed of persons with expertise in the subject matter from those governments or agencies that have an interest in the successful completion of the research project. The TAC would meet on a regular basis throughout the duration of both phases of the project. Entities that would be asked to appoint experts to the TAC include but are not limited to the following:

- Florida Department of Environmental Protection
- US Environmental Protection Agency – Region IV
- US Environmental Protection Agency – Headquarters
- Florida Department of Health
- Water Environment Research Foundation
- Various Florida local governments or representatives thereof

References

Haugland RA, Siefring SC, Wymer LJ, Brenner KP, Dufour AP. 2005. Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Res 39(4):559–568.

Khan IUH, Gannon V, Kent R et al. 2007. Development of a rapid quantitative PCR assay for direct detection and quantification of culturable and non-culturable *Escherichia coli* from agriculture watershed. J. Microbiol. Methods 69:480-488.

Siefring S, Varma M, Atikovic E, Wymer L, Haugland RA. 2008. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. J Water Health. 2008 Jun;6(2):225-37.

Wade TJ, Calderon RL, Sams E, et al. 2006. Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. *Environ Health Perspect.* 114:24 –28.

Wade TJ, Calderon RL, Brenner, KP et al. 2008. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. Epidemiology 19:375-383.

PHASE I - ESTIMATED BUDGET[a]

(30 months - Not including Epidemiology Study)

Indicators and MST (USF only)

Personnel	$183,660
Tuition (grad students)	$ 10,332
Lab Supplies	$ 45,000
Travel	$ 5,000
Equipment (Q-PCR)	$ 15,000[a]
Indirect costs	$ 109,820
SUBTOTAL	**$368,813**

Inter-Laboratory Calibration[b]

3 labs @ $25,000 each	$75,000
USF coordination	$ 5,000
SUBTOTAL	**$80,000**

[a]Partial cost (approximately ½) is requested to purchase a QPCR thermocycler; partial funding is available from a U.S. EPA grant.

[b]Cost is estimated for 4 laboratories (USF and 3 others) for the inter-laboratory calibration.

FSAEF Administrative and Miscellaneous Costs

Administrative	$ 15,000
Miscellaneous	$ 5,000
TAC Travel (2 mtgs of 6)	$ 7,200
SUBTOTAL	**$27,200**

GRAND TOTAL……..……$476,013

This proposal seeks funding for the MST-TMDL phase only. A separate cost proposal for the epidemiological study will be submitted during the course of the MST-TMDL phase in consultation with Dr. Solo-Gabriele and Dr. Fleming.

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