

**San Juan Creek Watershed Bacterial Study  
Orange County Public Health Laboratory**

**Description of Study  
Part 1**

**Introduction**

The beach water at the mouth of San Juan Creek and the portion of the creek immediately upstream of the beach fails State bacteriological standards regularly. While there are suspected sources of bacterial contamination, no comprehensive study has been carried out to address this problem. In addition, no bacteriologic survey has been carried out for the remainder of the watershed so there is no information available on potential sources of pollution. This study has three purposes:

- 1) Provide a bacterial survey of the water quality of the San Juan Creek watershed in dry weather conditions and locate any areas with bacteriologic water quality problems. Survey problem areas in detail.
- 2) Determine the source of the bacteria found in the problem areas using bacterial source tracking.
- 3) Compare two different techniques of bacterial source tracking, Ribotyping and Antimicrobial Resistance Testing (ARA) to determine the accuracy of these techniques.

This is the majority of the work required by Agreement 9-182-190-0 between the State Water Resources Control Board and Orange County PFRD.

**Study Design**

**Phase 1: Bacteriological Survey of Watershed and Adjacent Beach Recreational Water**

Phase 1 is designed to quickly survey the watershed to locate areas with bacterial pollution problems. Sites will be selected to sample tributaries, storm drains and to sample known or suspected problem areas and recreational water at the mouth of the creek. Approximately 30 sites will be sampled weekly for 10 weeks (300 samples).

Bacteriological testing will include total coliform and fecal coliform by MTF or membrane filtration and enterococcus by Enterolert or membrane filtration.

PFRD, environmental health and public health laboratory staff will determine the sampling sites with input from the technical advisory committee. PFRD will provide necessary access information for sampling sites. The Public Health Laboratory will do the sampling, testing and analysis.

**Phase 2: Detailed Bacteriological Survey of Identified Problem Areas**

Phase 2 is designed to further characterize locations with water quality problems identified in Phase 1. These sites will be tested in detail to determine maximum and minimum bacterial counts as well as temporal and geographic boundaries of problem. Existing data indicates that one known problem area is the very lower end of the creek, which is often prevented from flowing into the ocean by a sand berm. Samples will be taken from at least one known problem area and a second area geographically removed. E. coli and enterococcus isolates from a sub-set of samples will be saved to use in Phase 3.

It is expected that approximately 200 samples will be collected over 10 weeks. From a sub-set of these, 5 isolates of E. coli and enterococcus per sample for each bacterium will be saved for phase 3 analyses.

The public health laboratory will perform sampling and testing. Sampling strategy will be determined by public health laboratory staff and with input from PFRD, environmental health and the technical advisory committee.

### **Phase 3: Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination**

Phase 3 is designed to build the necessary databases and determine the accuracy of two methods of bacterial source tracking, ARA (Antimicrobial Resistance Analysis) and Ribotyping. Overall, this will be done by constructing a library of bacterial isolates from known species, performing the source analysis testing to build or add to an existing database and determining the accuracy of the methods utilizing bacterial strains from known sources not included in the database.

#### **ARA Technique:**

ARA differentiates bacteria from different species of animals by comparison of their resistance to antibiotics. Fecal samples from known animal species and human sources are taken, *E. coli* and enterococcus bacteria are isolated and a collection of isolates is constructed. Each bacterial isolate is tested against 4 concentrations of 8 different antibiotics. Resistance patterns by species analyzed by discriminate analysis are used to classify individual strains into the most likely group (species).

Several recent published studies done by several researchers on different watersheds have shown the utility of the ARA technique and have documented the accuracy as being between 75% and 85% (Wiggins, Hagedorn, Parveen, Harwood). In this study, dual analysis of both *E. coli* and enterococcus isolates is expected to improve the accuracy somewhat. In addition, isolates will be frozen so they can be re-analyzed by other techniques in the future. The technology is fairly simple resulting in lower cost per isolate and an increased probability that the technique can be rapidly imported into the Public Health Laboratory for future studies if it proves to be an accurate technique. This technique produces matches based on the local database of known isolates. This requires a large database be constructed. However, the database can be utilized for future bacterial pollution problems.

The initial part of this technique is to construct a library of strains from known species. Constructing the library will require 500 strains of each bacterium from human sources and from each animal species that may be a major contributor. This will be done by collecting 100 samples per species and picking 5 isolates from each sample for each bacterium. This will result in 2500 *E. coli* strains and 2500 Enterococcus strains included in the database if humans and 4 species of animals are considered potential sources.

Quality assurance is carried out first by determining the initial accuracy of the technique by holdout analysis, a statistical study of the database. Additional quality assurance is covered under the Quality Assurance section below.

Samples will be collected by public health laboratory staff and any other organizations that can assist (animal control, sewage treatment plant personnel, volunteers, humane societies). Bacteria will be isolated and identified and frozen by the public health laboratory staff. Valerie Harwood, Ph.D., Assistant professor, University of South Florida, and Tampa, FL will subcontract with the public health laboratory to perform the ARA analysis.

#### **Ribotyping Technique:**

Ribotyping differentiates bacteria by detecting changes in restriction enzyme cutting sites in the bacterial genome. Restriction fragments are separated by size on an agarose gel and the fragments containing r-RNA (ribosomal RNA) gene sequences are detected using a complimentary probe. The patterns of different sized fragments are compared between isolates. Isolates with the same patterns are considered related.

Similar to ARA, a library of *E. coli* strains from known animal species and human sources is utilized to identify the source of strains isolated from problem areas. Ribotype patterns, analyzed by discriminant analysis are used to classify strains into the most likely group (species). Unlike ARA, large isolate libraries, maintained by the testing laboratory have been traditionally utilized for matching along with the local library. However, a local isolate library is necessary to identify up to one third of strains. The accuracy of a geographically separated database is not established at this time. In the one published study utilizing ribotyping, an accuracy of 82% was achieved (Parveen 1999).

To construct the local library will require 100 strains of E. coli from human sources and from each animal species that may be a major contributor. This will be done by collecting 100 samples per species and picking 1 isolate from each sample. This will be a subset of the 5 samples/specimen taken for ARA analysis. This will result in 500 strains for analysis if 4 animal species and humans are considered potential sources. This library will be utilized with the existing library by the testing laboratory to determine sources.

The samples that will be utilized are the same as ARA analysis. Bacteria will be isolated, identified and frozen by the public health laboratory staff. The ribotyping technique published by S. Parveen of the University of Florida (Parveen, 1999) will be utilized for analysis. The testing laboratory will be George Lukasik, Ph.D., Biological Consulting Services of North Florida, Gainesville, Florida or another acceptable laboratory.

#### **Quality assurance:**

Two types of quality assurance tests will be carried out:

1) Reproducibility. 20 strains of each bacterium will be submitted to the testing laboratory 3 different times blinded. The percentage of reproducibility will be determined for each strain and summed.

2) Accuracy. Final accuracy of each technique is determined by an analysis of 100 isolates from known species (which are not residing in the database) that are submitted to the reference laboratory blinded. These bacterial strains will be isolated from collected along side the data base samples and held back. The analysis of rate of correct classification will be presented in a table similar to tables 1 and 2 of Harwood et. al. 2000. After this analysis adjustments can be made in the analysis technique to maximize accuracy if necessary. The same strains will be utilized for both typing techniques so a direct comparison can be made.

#### **Phase 4: Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates.**

Phase 4 is the analysis of bacterial isolates from problem areas that have been collected in phases 2 and 3 utilizing the two source tracking techniques and a comparison of the results of the techniques. Approximately 200 bacterial isolates (per species) from the problem areas will be provided to the two reference laboratories and will be compared to the library of isolates from known species to determine potential source. For ARA, a comparison is made between the results for the two different bacterial databases (E. coli and enterococcus) to determine a final result. For ribotyping the analysis will be done comparing the unknown E. coli strains to the local database and to the testing laboratory's database. The ARA results will be compared to that of the ribotyping analysis and written report issued. The report is also prepared for publication in a peer-reviewed journal.

Public health laboratory staff will do the sampling and bacterial isolation and identification. Dr. Harwood, Dr. Lukasik and Dr. Moore will write the final report.

#### **Schedule**

Phase 1: April 16 - June 22

Phase 2: June 22 - August 10

Phase 3: June 22 - November 16

Phase 4: November 16 - May 15, 2002

#### **Budget**

See spreadsheet

#### **References**

**Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Baneau.** 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. *Applied and Environmental Microbiology*. 65(12):5522 - 5531.

**Harwood, V.J., J. Whitlock and V. Washington.** 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Appl Environ Microbiol*. 66:3698-3704.

**Parveen, S., R. L. Murphree, L. Edmiston, C. W. Kaspar, K. M. Portier, and M. L. Tamplin.** 1997. Association of multiple-antibiotic-resistance profiles with point and non-point sources of *Escherichia coli* in Apalachicola Bay. *Appl. Environ. Microbiol*. 63(7):2607-12.

**Parveen, S., K. M. Portier, K. Robinson, L. Edmiston and M. L. Tamplin.** 1999. Discriminant Analysis of Ribotype Profiles of *E. coli* for Differentiating Human and Non-Human Sources of Fecal Pollution", *Appl Environ Microbiol*. 65:3142-3147.

**Wiggins, B. A., R. W. Andrews, R. A. Conway, C. L. Corr, E. J. Dobratz, D. P. Dougherty, J. R. Eppard, S. R. Knupp, M. C. Limjoco, J. M. Mettenburg, J. M. Rinehardt, J. Sonsino, R. L. Torrijos, and M. E. Zimmerman.** 1999. Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. *Appl Environ Microbiol*. 65:3483-6.

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**Contract Scope of Work  
Part 2**

**1. Project Officials:**

The State Water Board's Contract Manager shall be Chris Means of the San Diego Regional Water Quality Control Board. The Contract Manager shall be the day-to-day representative for administration of this agreement, and, except as otherwise specifically provided, shall have full authority to act on behalf of the State Water Board with respect to this agreement. The State Water Board's Executive Director, or designee, may also perform any and all acts which could be performed by the Contract Manager under this agreement. Except as otherwise expressly provided, all communications relative to this agreement shall be given to the Contract Manager.

The Contractor's Project Director shall be Vicki L. Wilson, Director, County of Orange, Public Facilities and Resources Department. The Project Director shall be the Contractor's representative for the administration of the agreement and shall have full authority to act on behalf of the Contractor. All communications given to the Project Director shall be as binding as if given to the Contractor.

The parties may change their Contract Manager or Project Director upon providing ten (10) days written notice to the other party.

**2. Work To Be Performed**

**A. Scope and Objectives**

The beach water at the mouth of San Juan Creek and the portion of the creek immediately upstream of the beach fails State bacteriological standards regularly. While there are suspected sources of bacterial contamination, no comprehensive study has been carried out to address this problem. In addition, no bacteriologic survey has been carried out for the remainder of the watershed so there is no information available on potential sources of pollution. This study has three purposes:

1. Provide a bacterial survey of the water quality of the San Juan Creek watershed in dry weather conditions and locate any areas with bacteriologic water quality problems. Survey problem areas in detail.
2. Determine the source of the bacteria found in the problem areas using bacterial source tracking.
3. Compare two different techniques of bacterial source tracking, Ribotyping and Antimicrobial Resistance Testing (ARA) to determine the accuracy of these techniques.

The San Diego Regional Water Quality Control Board (RWQCB) has identified San Juan Creek as an impaired water body for pathogens in the lower reach. The primary bacteriological contaminant that has led to the impaired designation is fecal coliform. Fecal coliform is a group of bacteria that is commonly associated with the digestive tracts of warm-blooded animals; including humans, domestic and wild mammals, and birds. The presence of fecal coliform within surface waters indicates the likely presence of fecal wastes from such warm-blooded animals. If the coliform is associated with human waste, it may indicate the presence of a wide variety of human pathogens (e.g., viruses, bacteria, etc.) that cause human disease. Although fecal contamination from non-human sources presents a reduced human health threat, some threat may still exist. Salmonella, Listeria, Campylobacter and Cryptosporidium (spp.) are some of the bacteria that can be found in animal wastes, can be carried via contaminated water, and can be contagious to humans.

The Orange County Health Care Agency (OCHCA) monitors beaches throughout Orange County to determine concentrations of various indicators of bacteriological and pathogenic water pollution. The OCHCA uses this monitoring data to determine if the water quality at the beaches is suitable for body contact recreation. The OCHCA will close beaches to body contact recreation when the concentrations of indicators exceed water quality objectives. Beach water quality is monitored for total and fecal coliform bacteria, and enterococcus. Sanitary surveys are conducted to determine the sources of pollution when the indicators exceed water quality objectives.

Based on the water quality monitoring conducted by OCHCA, and the history of closures of body contact recreation, the RWQCB has listed the lower reach of San Juan Creek (Creek) as water quality impaired in accordance with Section 303(d) of the Clean Water Act. The RWQCB determined that water quality in the Creek does not currently meet water quality objectives for fecal and total coliform established by the RWQCB for the protection of contact water recreation (REC-1).

The federal Clean Water Act requires the State of California (through the RWQCB) to identify impaired water bodies and to implement corrective measures to restore water quality areas that have been identified as impaired. These corrective measures include establishing Total Maximum Daily Loads (TMDLs) for contaminants and when necessary, implementation of enforcement actions such as a Cleanup and Abatement Order (CAO). Unless prior actions are taken to identify and reduce the bacteriological impairment of lower San Juan Creek, the RWQCB can be expected to ultimately utilize its enforcement authority.

When the State of California adopted Assembly Bill (AB) 411 (Wayne, 1997), it required county health officers to test recreational beach waters for three indicators of possible human pathogens. These include total coliform, fecal coliform and enterococcus. The AB 411 requires county health officials to post beaches if testing shows the presence of these indicators above certain standards. This sampling study will provide the County of Orange with valuable information on the relative magnitudes of the bacteria loadings and the sources of the bacteria. From such information, an effective correction program can be developed and implemented.

In late fall 1997, the City of Coronado attempted to determine the causes of high fecal coliform counts at the outfall of a dewatering system associated with their groundwater collection network. With high readings coming from the outfall area, the RWQCB adopted a cease and desist order that required the City to correct the high counts or stop the dewatering. The City contracted with Woodward Clyde to both engineer a source identification program and to study alternatives that would resolve the problem. After conducting DNA tests of input sources, the City was able to show that the fecal coliform came from a variety of human, animal and bird sources. Later Woodward Clyde helped design an ultraviolet radiation structure that treated the groundwater before release and that brought coliform counts down to acceptable levels for water recreation. Although the San Juan Creek storm drain system is much larger and more complex than the single dewatering outfall at Coronado, the same types of source identification can be expected to assist the County in meeting the standards of AB 411.

Although stream flows within the lower San Juan Creek watershed frequently contain concentrations of indicator bacteria that exceed bathing standards, health experts do not currently know the degree to which that bacteria may be associated with the presence of pathogens that are harmful to humans. All existing sampling has utilized methods that are not able to determine if the bacteriological contamination is from human sources. Identifying and stopping the bacteriological contamination will be much easier once the specific sources are known.

This project is designed to conduct a state-of-the-art study of existing bacteriological contamination within the San Juan Creek watershed. An extensive sampling program will be implemented to identify the relative contribution of bacteria loading from each of the major tributaries within the watershed. The sampling program will also utilize genetic fingerprinting to identify the most likely animal sources of the bacteria loading. The relative proportions of the bacteria loadings that are from humans, pets, shore birds, horses, or wildlife are needed in order to assess the level of human health threat that is posed by the contamination as well as to develop an effective strategy to reduce the loadings. In addition, this project will compare two different techniques of bacterial source tracking, Ribotyping and Antimicrobial Resistance Testing (ARA) to determine the accuracy of these techniques.

## **B. Work**

The work to be performed under this contract includes the collection and analysis of water samples for total and fecal coliform, enterococcus, and *Escherichia coli* (*E. coli*). In addition, sample cultures of *E. coli* and Enterococcus will be analyzed using bacterial source tracking to qualitatively identify sources of fecal coliform found in the discharges. These analyses will determine whether there are human sources of bacteriological contamination, as well as identify the types of animals that contribute to the concentrations of bacteria in the discharges to the creek and beach. The specific number of samples and analyses will be determined within a sampling plan to be developed by the Technical Advisory Committee. The County will subcontract with the OCHCA, and/or other certified laboratories for the analysis of the water samples. For the ARA bacterial source tracking technique, samples will be collected by public health laboratory staff and any other organizations that can assist (animal control, sewage treatment plant personnel, volunteers, humane societies). Bacteria will be isolated and identified and frozen by the public health laboratory staff. Valerie Harwood, Ph.D., Assistant professor, University of South Florida, and Tampa, FL will subcontract with the public health laboratory to perform the ARA analysis. For the Ribotyping bacterial source tracking technique, samples that will be utilized are the same as ARA analysis. Bacteria will be isolated, identified and frozen by the public health laboratory staff. The ribotyping technique published by S. Parveen of the University of Florida (Parveen, 1999) will be utilized for analysis. The testing laboratory will be George

Lukasik, Ph.D., Biological Consulting Services of North Florida, Gainesville, Florida or another acceptable laboratory. The contractor shall be responsible for the performance of work as set forth herein below and for the preparation of products and a final report as specified below.

## **Study Design**

### **Phase 1: Bacteriological Survey of Watershed and Adjacent Beach Recreational Water**

Phase 1 is designed to quickly survey the watershed to locate areas with bacterial pollution problems. Sites will be selected to sample tributaries, storm drains and to sample known or suspected problem areas and recreational water at the mouth of the creek. Approximately 30 sites will be sampled weekly for 10 weeks (300 samples).

### **Phase 2: Detailed Bacteriological Survey of Identified Problem Areas**

Phase 2 is designed to further characterize locations with water quality problems identified in Phase 1. These sites will be tested in detail to determine maximum and minimum bacterial counts as well as temporal and geographic boundaries of problem. Existing data indicates that one known problem area is the very lower end of the creek, which is often prevented from flowing into the ocean by a sand berm. Samples will be taken from at least one known problem area and a second area geographically removed. E. coli and enterococcus isolates from a sub-set of samples will be saved to use in Phase 3.

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#### **ARA Technique:**

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traditionally utilized for matching along with the local library. However, a local isolate library is necessary to identify up to one third of strains. The accuracy of a geographically separated database is not established at this time. In the one published study utilizing ribotyping, an accuracy of 82% was achieved (Parveen 1999).

Quality assurance: Two types of quality assurance tests will be carried out:

1) Reproducibility. Twenty (20) strains of each bacterium will be submitted to the testing laboratory 3 different times blinded. The percentage of reproducibility will be determined for each strain and summed.

2) Accuracy. Final accuracy of each technique is determined by an analysis of 100 isolates from known species (which are not residing in the database) that are submitted to the reference laboratory blinded. These bacterial strains will be isolated from samples collected alongside the data base samples and held back. The analysis of rate of correct classification will be presented in a table similar to tables 1 and 2 of Harwood et. al. 2000. After this analysis adjustments can be made in the analysis technique to maximize accuracy if necessary. The same strains will be utilized for both typing techniques so a direct comparison can be made.

#### **Phase 4: Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates.**

Phase 4 is the analysis of bacterial isolates from problem areas that have been collected in phases 2 and 3 utilizing the two source tracking techniques and a comparison of the results of the techniques. Approximately 200 bacterial isolates (per species) from the problem areas will be provided to the two reference laboratories and will be compared to the library of isolates from known species to determine potential source. For ARA, a comparison is made between the results for the two different bacterial databases (E. coli and enterococcus) to determine a final result. For ribotyping the analysis will be done comparing the unknown E. coli strains to the local database and to the testing laboratory's database. The ARA results will be compared to that of the ribotyping analysis and written report issued. The report is also prepared for publication in a peer-reviewed journal.

#### **References:**

**Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Beneau.** 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. *Applied and Environmental Microbiology*. **65**(12):5522 - 5531.

**Harwood, V.J., J. Whitlock and V. Washington.** 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Appl Environ Microbiol*. **66**:3698-3704.

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**Contract Scope of Work - Tasks  
Part 3**

**Task 1. Project Management and Administration**

1.1 Provide all technical and administrative services as needed for contract completion; monitor, supervise and review all work performed; and coordinate budgeting and scheduling to assure that the contract is completed within budget, on schedule and in accordance with approved procedures, applicable laws and regulations.

1.2 Ensure that contract requirements are met through completion of Quarterly Progress Reports submitted to the Contract Manager by the 10th of the month following the end of the calendar quarter (March, June, September, and December) and through regular communication with the Contract Manager. The Quarterly Progress Reports shall describe activities undertaken and accomplishments of each task during the quarter, milestones achieved, and any problems encountered in the performance of the work under this contract. The description of activities and accomplishments of each task during the quarter shall be sufficient in detail to provide a basis for the payment of invoices and shall be translated into percent of task completed for the purpose of calculating invoice amounts.

1.3 Document steps taken in soliciting and awarding the subcontract and submit them to the Contract Manager for review.

1.4 Project Survey Form - At the completion of the project and prior to final payment, the Project Director shall complete and provide a project survey form to be included with the final report.

**Task Products:**

Quarterly progress reports, subcontractor selection, project survey form.

**Task 2. Assemble a Technical Advisory Committee**

Form a Technical Advisory Committee (TAC) comprised of technical experts representing various agencies (Orange County Health Care Agency, Regional Water Quality Control Board and California Department of Health Services), and the scientific community. The Contractor will facilitate the meetings. The TAC will be request to provide recommendations for development of the storm drain and tributary sampling plan (Sampling Plan).

2.1 Meeting Agendas - The Contractor will develop and distribute written meeting agendas. Discussion items will include the Sampling Plan.

2.2 Meeting Participants - At a minimum, the following representatives shall be invited to participate in the meetings:

Agency Technical Representatives of  
Orange County Health Care Agency;  
Regional Water Quality Control Board, San Diego Region;  
California Department of Health Services;

2.3 Meetings - The TAC group will meet on an as-needed basis throughout this project. Contact may also be accomplished by email and telephone. At the meetings, the details of the Sampling Plan will be finalized (e.g., sampling sites, sampling periods, water quality constituents and so on).

**Task Products:**

2.1 Meeting Agenda(s) and minutes  
2.3 TAC participant sign-up sheet(s)

### **Task 3. Bacteriological Survey of Watershed and Adjacent Beach Recreational Water (Phase 1)**

3.1 Sampling Plan for Storm Drains and Tributaries - Prepare a Sampling Plan for dry weather site locations. The Sampling Plan shall identify who will collect the samples and the time and frequency of sample collection at each location specified. The Sampling Plan shall provide for the analysis of the samples for total and fecal coliform and enterococcus bacteria. Bacteriological testing will include total coliform and fecal coliform by MTF (Multiple Tube Fermentation) or membrane filtration and enterococcus by Enterolert or membrane filtration. A Quality Assurance/Quality Control (QA/QC) program shall be developed and implemented as part of the Sampling Plan.

3.2 Conduct Phase 1 Storm Drain and Tributary Sampling - The Sampling Plan developed in Task 3.1 will be conducted. This task includes sample collection and analyses of bacteriological samples, plus any other data relating to water quality and conditions at the time of sampling. Sampling data may include data on flow rate, electrical conductivity (EC), total suspended solids (TSS), or other parameters as prescribed within the Sampling Plan. The County of Orange Public Health Laboratory shall conduct the sampling, testing, and analysis.

3.3 Watershed Bacteriological Survey Report - Prepare a report that describes the results from Task 3.2. The report shall include analytical results, as well as other data on water quantity and quality. The report shall also identify problem areas to be further studied in a detailed bacteriological survey. The report shall be submitted within a timely manner of receiving the analytical results.

#### **Task Products:**

- 3.1 Watershed Sampling Plan
- 3.2 Conduct Watershed Storm Drain and Tributary Sampling Plan
- 3.3 Watershed Bacteriological Survey report

### **Task 4. Detailed Bacteriological Survey of Identified Problem Areas (Phase 2)**

4.1 Sampling Plan for Problem Areas - Prepare a Sampling Plan for problem areas. The Sampling Plan shall identify who will collect the samples and the time and frequency of sample collection at each location specified. The sampling Plan shall identify collection sites from at least one known problem area and a second area geographically removed. The Sampling Plan shall be determined by Public Health Laboratory staff and with input from PFRD, Environmental Health and the Technical Advisory Committee. The Sampling Plan shall provide for the analysis of the samples for total and fecal coliform, E. coli, and enterococcus bacteria. The Sampling Plan shall provide for the collection of approximately 200 samples over approximately 10 weeks and for the saving isolates of E. coli and enterococcus for analysis in Phase 4 of this study.

4.2. Conduct Phase 2 Bacteriological Survey - The Sampling Plan developed in Task 4.1 will be conducted. This task includes sample collection and generation of E. coli and enterococcus isolates from bacteriological samples, plus any other data relating to water quality and conditions at the time of sampling. Sampling data may include data on flow rate, electrical conductivity (EC), total suspended solids (TSS), or other parameters as prescribed within the Sampling Plan. The County of Orange Public Health Laboratory shall conduct the sampling, testing, and analysis.

4.3. Problem Area Bacteriological Survey Report - Prepare a report that describes the results from Task 4.2. The report shall include analytical results, as well as other data on water quantity and quality. The report shall also identify the temporal and geographic boundaries of the problem at the problem areas. The report shall be submitted within a timely manner of receiving the analytical results.

#### **Task Products:**

- 4.1 Problem Area Sampling Plan
- 4.2 Conduct Problem Area Sampling Plan
- 4.3 Problem Area Bacteriological survey report

**Task 5. Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination. (Phase 3)**

5.1 Construct Bacterial Isolates Library from Known Species. - Construct a local bacteria isolates library. The library will contain strains of E. coli and enterococcus bacteria isolated from approximately 100 samples of known species, including humans and animal species that may be a major contributor to bacteria pollution in the watershed. For the ARA (Antimicrobial Resistance Analysis) technique approximately 500 strains of each bacterium will be developed by picking 5 isolates from each sample for each bacterium. For the Ribotyping technique approximately 100 strains of E. coli per species will be developed from 1 isolate per sample. Isolates shall be frozen to permit analysis by other techniques in the future. Bacteria will be isolated, identified, and frozen by the public health laboratory staff. A Quality Assurance (QA) program shall be developed and implemented as part of the library preparation that includes reproducibility and accuracy tests.

5.2 Accuracy Determination Report of Bacterial Source Identification Methods - Prepare a report on the accuracy of the source identification methods based on the results obtained from the accuracy tests. Final accuracy of each source identification technique shall be determined for each species in the local bacteria isolate library. The same strains will be used for both typing techniques.

**Task Products:**

- 5.1 Bacteria Isolates Library
- 5.2 Report of Source Identification Accuracy Determination

**Task 6. Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates. (Phase 4)**

6.1 Source Identification Report - Prepare a source identification report that describes, identifies and quantifies the major sources of bacteriological contamination in the problem areas of the watershed based on the results obtained from the bacterial source tracking identified above. The report shall include an explanation of the work performed and results of all phases of the study. The report shall include the analytical results for the source identification methods as well as other data on water quantity and quality. The report shall include the Quality Assurance/Quality Control (QA/QC) tests performed. The report shall include a discussion of the data that has been obtained and the implementation measures which may be used to correct the identified loadings. The report shall identify and compare results of both the ARA and Ribotyping methods and include a discussion of the comparison of the two bacterial source tracking techniques. A final draft report shall be submitted upon completion of all phases of the study and shall not be delayed pending final preparation of the manuscript for the peer-reviewed journal.

6.1.b Interim Source Identification Report - Prepare an interim source identification report by March 1, 2002 that describes the status of the source identification of watershed isolates. The interim report shall include results of source identification performed to date and the status and expected conclusion date of Phase 4 of the study.

**Task Products:**

- 6.1 Source Identification Report
- 6.1.b Interim Source Identification Report

**C. Schedule of Completion Dates.**

Task #	Task Description	Completion Date
Task 1	Project Management & Administration	On-going
Task 2	Assemble Technical Advisory Committee	4/01
Task 3	Phase 1 - Bacteriological Survey of Watershed and Adjacent Beach Recreational Water	9/01

Task 4 Phase 2 - Detailed Bacteriological Survey of Identified Problem Areas	11/01
Task 5 Phase 3 - Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination	2/02
Task 6 Phase 4 - Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates	5/02

**D. Reports**

1. Not later than June 1, 2001 and quarterly thereafter, during the life of this agreement, the Project Director shall provide a written progress report to the Contract Manager describing activities undertaken, accomplishment of milestones, and any problems encountered in the performance of the work under this agreement, and delivery of intermediate products, if any.
2. The Project Director shall submit to the Contract Manager reports upon the completion of each Phase of the Study and an interim report for Phase 6 as described in this Scope of Work. In the event completion dates for the Phases are delayed, then a progress report containing the results of the work performed in the 3 months following the last report shall be submitted.
3. Not later than February 28, 2002, the Project Director shall submit to the Contract Manager two (2) copies of a draft final report describing the work performed pursuant to Section B of this Exhibit for review and comment.
4. Within four (4) weeks of receipt of the draft report, the Contract Manager will submit his final comments to the Project Director.
5. On or before the termination date of this agreement, the Project Director shall submit to the Contract Manager for approval one (1) reproducible master and two (2) copies of the final report containing the results of the work performed and addressing the comments submitted to the Project Director by the Contract Manager.
6. The report will not be considered final until approved and accepted by the Contract Manager.

**A. BUDGET:**

Task	Item	Cost
1	Project Management and Administration	\$0.00
2	Assemble Technical Advisory Committee	\$0.00
3	Phase 1 - Bacteriological Survey of Watershed and Adjacent Beach Recreational Water	\$27,000.00
4	Phase 2 - Detailed Bacteriological Survey of Identified Problem Areas	\$42,300.00
5	Phase 3 - Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination	\$57,600.00
6	Phase 4 - Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates	\$61,600.00
<b>Total Cost</b>		<b>\$188,500.00</b>

**B. INVOICING PROCEDURES:**

The Contractor shall be paid quarterly in arrears, upon submission of an original and two copies of the invoice which properly details all charges, expenses, direct and indirect costs. Invoices shall be submitted to:

Chris Means  
California Regional Water Quality  
Control Board, San Diego Region

9771 Clairemont Mesa Blvd., Suite A  
San Diego, CA 92124-1324

1. The original and one (1) approved copy of the invoice or payment request will be forwarded to the State Water Board's Accounting Operations Section by the Contract Manager. Contractors who are certified as small businesses or recognized as non-profit organizations by the Office of Small Business Certification and Resources will be paid in accordance with California Government Code, Title 1, Section 926.15. Invoices for all other contractors shall be paid within 45 calendar days. In either situation, payment of any invoice will be made only after receipt of a complete, adequately supported, properly documented and accurately addressed invoice or payment request. Failure to use the address exactly as provided above may result in return of the invoice or payment request to the Contractor. Payment shall be deemed complete upon deposit of the payment, properly addressed, postage prepaid, in the United States mail. All invoices must be approved by the Contract Manager.

2. Payments prior to satisfactory completion of all work required by the agreement shall not exceed, in the aggregate, ninety percent (90%) of the total earned with the balance to be paid upon satisfactory completion of the agreement, and provided, further, that the State Water Board shall retain from the Contractor's earnings for each period for which payment is made an amount equal to ten percent (10%) of such earnings, pending satisfactory completion of the agreement.

3. The invoice shall contain the following information:

(a) The word "INVOICE" should appear in a prominent location at the top of the page(s);

(b) Printed name of the Contractor;

(c) Business address of the Contractor, including P.O. Box, City, State, and Zip Code;

(d) Name of State Water Board/Regional Water Board being billed;

(e) The date of the invoice;

(f) The number of the agreement upon which the claim is based; and

(g) An itemized account of the services for which the State Water Board is being billed:

(1) The time period covered by the invoice, i.e., the term "from" and "to";

(2) A brief description of the services performed;

(3) The method of computing the amount due. The invoice shall detail the percentage of each task completed during the invoice period multiplied by the agreed upon cost to perform each task, as specified in the Budget less the ten percent (10%) withhold, if any;

(4) The total amount due; this should be in a prominent location in the lower right-hand portion of the last page and clearly distinguished from other figures or computations appearing on the invoice; the total amount due shall include all costs incurred by the Contractor under the terms of this agreement; and

(5) Original signature of Contractor (not required of established firms or entities using preprinted letterhead invoices).

## **Quarterly Report #1**

### **Agreement No. 9-182-190-9, San Juan Creek Watershed Study August 10, 2001**

Orange County PFRD in conjunction with the HCA Public Health Laboratory

#### **Study Tasks Completed This Quarter:**

- 1) Task 1, Project Management and Administration (Ongoing)
- 2) Task 2, Assemble a Technical Advisory Committee (Ongoing)
- 3) Task 3.1, Bacteriological Survey of Watershed and Adjacent Beach Recreational Water - Prepare sampling plan. Study protocol attached.
- 4) Task 3.2, Bacteriological Survey of Watershed and Adjacent Beach Recreational Water - Conduct sampling and testing. Sampling and testing completed.

#### **Study Tasks in Progress:**

- 1) Task 3.3. Bacteriological Survey of Watershed and Adjacent Beach Recreational Water - Watershed Bacteriological Survey Report.

Raw data has been transferred to the contracting statistician, Medical Data Coordinating Center, for analysis and table and graph preparation. Report is in process of being prepared.

- 2) Task 4.1, Detailed Bacteriological Survey of Identified Problem Areas -

Site visit by Dr. Harwood: Dr. Harwood visited the San Juan watershed May 18 reviewed the testing protocol with Dr. Moore and presented a seminar on source tracking.

#### Sampling Plan for Identified Problem Areas

Proposed sampling plan is to sample at 4 locations: 1) Trabuco Creek below Ramos St, 2) San Juan Creek below Trabuco Creek, 3) San Juan Creek at the beach (behind berm), 4) Ocean at San Juan Creek. This will allow the subsequent source-tracking portion of the study to compare the source species of bacterial pollution upstream vs. the creek mouth at the beach vs. the ocean water. This plan was discussed with the regional board representative Jeremy Haas on Aug 10.

#### **Contract Activities:**

- 1) Scope of work in contract modified to contain costing by task. State board is in final approval process. Scope of work is attached.
- 2) MOU between Orange County PFRD and HCA Public Health Laboratory is in the process of being finalized.
- 3) The Orange County Health Care Agency obtained contracts with the following scientists. Each of the scientists is a subcontractor to Orange County Public Health Laboratory for this study

Dr. Harwood, University of South Florida, Tampa, Florida,  
Dr. Lukasik, Biological Consulting Services of North Florida, Gainesville, FL.  
Dr. Lewis, Medical Data Coordinating Center, Encinitas, CA

**TAC Meetings:**

Meeting # 1 Held on October 12, 2000. Study proposal reviewed and input received for selecting study test sites.