Quality Assurance Project Plan

for

Survey of selected microbial pathogens in the Salton Sea

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Institution: USGS Biological Resources Division, National Wildlife Health Center

Section A: PROJECT MANAGEMENT

A1 Project Title and Approval Sheet

Survey of selected microbial pathogens in the Salton Sea

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Dr. Tonie E. Rocke

Dr. Mark J. Wolcott
Technical Project Managers Date

Dr. Milton Friend
Salton Sea Project Manager Date

Dr. Barry Gump
Salton Sea QA Coordinator Date

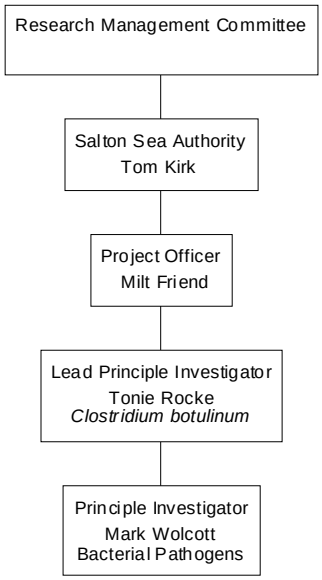
A2 Table of Contents

A3 Distribution List

USGS Biological Resources Division, National Wildlife Health Center
Salton Sea Authority

A4 Project/Task Organization and Responsibilities (organizational chart)

The project is part of the An Environmental Reconnaissance of the Salton Sea study. The project was initiated by the Salton Sea Research Management Committee and is under the direction of the Salton Sea Authority. The project reports through the study Project Officer to the Executive Director of the subcommittee.



A5 Project Definition and Background

See: Summary *In* Proposal to Conduct: An Environmental Reconnaissance of the Salton Sea submitted to the Salton Sea Research Management Committee by the USGS/BRD National Wildlife Health Center, Title: Survey of selected microbial pathogens in the Salton Sea (attached).

A6 Project/Task Description

See: Objectives *In* Proposal to Conduct: An Environmental Reconnaissance of the Salton Sea submitted to the Salton Sea Research Management Committee by the USGS/BRD National Wildlife Health Center, Title: Survey of selected microbial pathogens in the Salton Sea (attached).

PROJECT TASK LIST AND TIMELINE

Task No.	Task	Responsibility	Start Date	Completion Date
1	The distribution and abundance of *Clostridium botulinum* type C spores and toxin-producing cells at various times of year in the sediments of Salton Sea ecosystem.	Rocke	1 January 99	30 December 99
2	The prevalence of the selected bacterial pathogens in the sediments and water of the Salton Sea ecosystem at various times of year.	Wolcott	1 January 99	30 December 99

A7 Quality Objectives and Criteria for Measurement Data

Data to be collected:

Task 1 Data will be the determination of the distribution and abundance of *Clostridium botulinum* type C spores and toxin-producing cells.

Task 2 Data will be presence or absence of selected bacterial pathogens in the sediments and/or water.

Conditions under which data are to be collected:

Data will be generated by laboratory analysis of samples procured from the Salton Sea. Data will be generated under laboratory conditions that are process controlled within the extent of experimental research and biological analysis. The objective of the data collection for this study is to produce data that represents, as closely as possible, *in situ* conditions at the Salton Sea.

MEASUREMENT PERFORMANCE CRITERIA

The measurements taken during this study are qualitative measurements only. The methods used in the performance of determination of the distribution and abundance of *Clostridium botulinum* type C spores and toxin-producing cells has been previously shown to detect approximately 400 cells per gram of sediment. The methods used in the performance of determination of presence or absence of selected bacterial pathogens in the sediments and/or water have been previously shown to detect 100 viable cells per milliliter of water. Sufficient sample size and detection capabilities are in place to insure the satisfactory accuracy and precision for the application of this method to the qualitative determination of the presence or absence of the organisms of interest in this study. The overall presence and/or absence of the various pathogens being determined in this study will be in part a reflection of their presence and/or absence during the repeated sampling events during the course of this study.

Data Representativeness:

The data collected during this study will encompass sampling points and sampling times that provide for data that is representative of the pathogenic population of the Salton Sea.

Data Comparability:

Bacterial pathogens are biological in nature and hence are subject to variations consistent with other physical and biological impacts on the Sea. The data collected during this study will be representative only of the population of the selected pathogens in the Sea during this study. Comparison to previous data is not intended.

A8 Special Training Requirements/Certification

Although there are no specific prerequisite training and certification requirements required to perform the tasks outlined in the proposal, sufficient skills, knowledge, and abilities sufficient to perform advanced level microbiological analysis in support of wildlife health/environmental diagnostics and research are necessary.

See: Personnel Qualifications *submitted with* Proposal to Conduct: An Environmental Reconnaissance of the Salton Sea submitted to the Salton Sea

Research Management Committee by the USGS/BRD National Wildlife Health Center, Title: Survey of selected microbial pathogens in the Salton Sea.

A9 Documentation and Records

Data reported for this project will consist of the results of the laboratory analysis for the selected pathogens (presence) by location (sample site) and date. The predominate data type will be qualitative data. Data that will not be reported but will be retained and made available if necessary will be the sample collection information (field records), intermediate laboratory analysis results, and internal quality control data.

Section B: MEASUREMENT/DATA ACQUISITION

B1 Sampling Process Design (Experimental Design)

Sampling Design, Assumptions and Rationale:

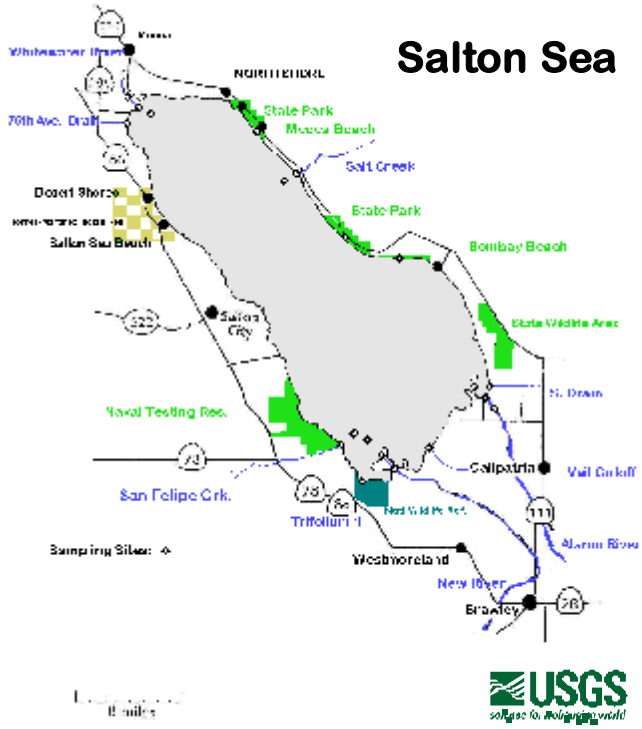
See: Sampling methodology and frequency *In* Proposal to Conduct: An Environmental Reconnaissance of the Salton Sea submitted to the Salton Sea Research Management Committee by the USGS/BRD National Wildlife Health Center, Title: Survey of selected microbial pathogens in the Salton Sea (attached).

There will be 20 sampling sites for this project at the Sea. Three of these sampling sites will be monitored monthly. These three sites are the primary in-flows to the Sea and best represent areas where changes in the bacterial pathogens would first occur. Six other sites will be sampled on a quarterly basis for the detection of selected pathogens. The quarterly sampling should afford an economical sampling that provides for seasonal variations, should they occur. These six sites represent parameter locations around the Sea that afford access and should be representative of the conditions at the Sea. All 20 sites will be sampled for the detection of botulism at the Sea on a quarterly basis and include the additional months of August, September and October. Botulism has been associated primarily with the summer months but the additional sampling on the other months will provide information on the residual latency of botulism at the Sea.

Location and Frequency of Samples:

Site Location	Description	Sample Matrix	No. of Samples per Station	Sampling Method	Frequency of Sampling
1	New River	water/sediment	3	grab	monthly
2	New River delta	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
3	Sea near New River	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
4	Alamo River	water/sediment	3	grab	monthly
5	Alamo River Delta	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
6	Sea near Alamo River	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests

7	Whitewater River	water/sediment	3	grab	monthly
8	Whitewater River Delta	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
9	Sea near Whitewater River	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
10	San Felipe Creek	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests
11	Sea near San Felipe Creek	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
12	Salt Creek	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests
13	Sea near Salt Creek	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
14	76th Ave Drain	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests
15	S. Drain (Davis Road)	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
16	Vail Cutoff	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
17	Trifolium 1	sediment	1	grab	quarterly plus Aug Sept & Oct for botulism tests
18	Mecca Beach	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests
19	Salton Sea State Park	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests
20	Bombay Beach	water/sediment	2	grab	quarterly plus Aug Sept & Oct for botulism tests



B2 Sampling Method Requirements

Sampling Methods:

Types of Samples to be Collected:

Samples will consist of grab samples of both water and sediment (mud) as identified previously. Water samples will be near surface samples obtained according to the general procedures of Part 9000 of Standard Methods for the Examination of Water and Wastewater (20th Edition, 1998, APHA). Sediment samples will consist of the top 10 cm of sediment.

Sampling Method's Requirements:

Water samples. Water samples will be collected in presterilized, nontoxic polypropylene (or similar) bottles. Water samples will be cooled on wet ice until frozen at -20 to -180EC and transported to the Center for analysis. Samples will not exceed 6 hours on wet ice before being frozen and once frozen, will not be allowed to thaw until analyzed.

Sediment samples. Sediment samples will be collected in presterilized, nontoxic specimen containers. One sediment sample will be collected for pathogen screening and five samples will be collected for botulism testing. Each sample will be thoroughly mixed. For pathogen screening, an approximate 0.25 g sample will be transferred to a 5 ml cryovial containing DMSO (final concentration 10%), placed on wet ice, and frozen at -20 to -180EC within 6 hours of collection. For botulism testing, sediment will be

aliquoted into a 15 ml cryovial tube and three 12 ml LDPP sample tubes that will be used for DNA extraction. These and the remainder of the samples will then be placed on wet ice and frozen at -20 to -180EC within 6 hours of collection. Samples will be shipped to the Center for analysis and will not be allowed to thaw until analyzed.

Sampling System Failure Response and Corrective Action Process:

Due to constraints at the Sea for adequate refrigeration and freezing, continual assessment of the sample collection method will be made and adjustments consistent with good practices for microbial analysis may be made. Internal controls for the collection and shipment of the samples will be included and alterations to the sample collection methods will be made consistent with the internal control verifications.

B3 Sample Handling and Custody Requirements

PARAMETER TABLE

Parameter	Number of Samples	Matrix	Sample Preservation	Holding Time[1]
Botulism	600	Sediment	Frozen	#6 hrs
Pathogens	72	Water/sediment	Frozen	#6 hrs

[1] Holding time on ice after initial collection

Sample Handling:

All samples will be packed in appropriate shipping containers so that they will be maintained frozen at -20 to -180EC during all times (consistent with federal regulations regarding shipment of hazardous materials). All caps and lids will be checked for tightness prior to shipping. All shipments will be packaged to insure containment incase of thawing and leakage. All samples will be handled, prepared, transported and stored in a manner so as to minimize loss, contamination, or degradation. Sample containers will be clearly labeled with an indelible marker.

All samples remaining after successful completion of analysis will be either disposed of properly or retained for archival purposes where deemed appropriate (sediment only). All archival samples will be maintained for a minimum of one year after the conclusion of this study.

Custody Procedures:

Custody logs will be maintained to track each sample collected and to analyze or preserve each sample within the specified holding times.

B4 Analytical Methods Requirements

Sample Parameter	Matrix	Analytical Method Reference
Botulism	sediment	Williamson, J. L. 1998. Genetic characterization of *Clostridium botulinum* type C C_1 toxin gene in strains 468C and 96-SAC and detection of the C_1 toxin gene in wetland sediments. M.S. Thesis. University of Wisconsin.
Pathogens	water/sediment	Part 9000 of Standard Methods for the Examination of Water and Wastewater (20^{th} Edition, 1998, APHA)

Data's ability to meet the QC acceptance criteria:

Each principle investigator is responsible for the appropriateness and quality of the data generated, changes in the protocols, and all corrective actions during the course of this study. The Salton Sea Project Officer will be notified of any changes and corrective actions.

Sample preparation procedures:

Sample preparation procedures are included with the analytical methods referenced previously.

Deviations or selection of method options:

Part 9000 of Standard Methods for the Examination of Water and Wastewater (20^{th} Edition, 1998, APHA) outlines standard methods for the analysis of only two of the pathogens being studied. As such, the general provisions of the Standard Methods will be used for the analysis of the other pathogens of interest in this study. Comparable methods will be used to insure that the data generated meets the analytical needs of the study.

B5 Quality Control Requirements

QC Procedures:

Quality assurance and quality control procedures will be used to evaluate the effectiveness of all phases of performance under this study. Field and laboratory QC checks will be incorporated to insure the fidelity of the data generated. All work performed during the course of the contract will adhere to the general tenants of Good Laboratory Procedures (GLP) as described in 40 CFR 160, Part 9000 of Standard Methods for the Examination of Water and Wastewater (20^{th} Edition, 1998, APHA), or as institutional policy provides. Logs will be maintained on all laboratory equipment, reagent, and media checks performed. Manufacturers' Certificates of Analysis (CofA) will be used as much as possible to document reagent and media quality control. Due to the type of data being collected, data will be validated against the internal reference of reasonability and the investigator's experience. Any data that is not consistent with the internal reference will attempt to be rechecked and verified before any data are described/interpreted or shared with others. Any recheck or verification that does not support the initial data will be noted and the data qualified in all reports. The final report will include a detailed analysis of the data, including statistical analysis as appropriate.

Field QC checks

The purpose of the field QC checks is to insure that sampling, handling and transportation of the study samples does not compromise the data being sought. A field quality control blank will be included in the collection of the samples. A preparation of known bacterial suspension will be prepared in the laboratory and used as the control blank. The bacterial suspension will be added to a control sample collected and handled under identical conditions as the study samples. The control blank will be analyzed in the laboratory for recovery of the organism to insure appropriateness of collection, handling, transportation, and analysis of the study samples. This is analogous to the traditional spiked sample recovery studies but only qualitative in nature under these study conditions.

Laboratory QC checks

The purpose of analyzing laboratory control samples is to demonstrate the accuracy of the analytical method. Laboratory controls will consist of analyzing known organisms of the same genera as being sought in the selected pathogens. All media and procedures will be tested to insure adequate performance prior to analysis of the field samples. Due to the biological nature of the analysis, only positive and negative controls will be used. Concurrent use of controls will be used at the principle investigators discretion consistent with the guidance of Good Laboratory Procedures (GLP) as described in 40 CFR 160, Part 9000 of Standard Methods for the Examination of Water and Wastewater (20th Edition, 1998, APHA), or as institutional policy.

Corrective actions:

Due to the type of data being collected, data will be validated against the internal reference of reasonability and the investigator's experience. Any data that is not consistent with the internal reference will attempt to be rechecked and verified. Any recheck or verification that does not support the initial data will be noted and the data qualified in all reports.

Failure of the field QC may indicate sampling, handling, or transportation problems. Due to the nature of the field QC, concurrent analysis of the study samples may yield results. Results obtained from study samples whose corresponding field QC check failed will require assessment of the collection, handling, and transport of the study samples. Samples whose field QC check failed would normally require resampling for the study samples. Due to the analysis times and collection requirements, resampling may be waived at the principle investigators discretion. Corrective actions will include an analysis of the sampling, handling, or transportation methods.

Failure of the laboratory check may indicate analytical method problems. Any laboratory QC check that fails will not normally compromise the study samples and should be corrected and resolved prior to the analysis of study samples.

All corrective actions will be documented and documentation retained for a minimum of one year after the conclusion of this study. Corrective action documentation will include a description of the problem, analysis or investigation of the problem, and the proposed remedy of the problem. All corrective actions will also include a verification that the remedy of the problem did in fact result in the elimination or mitigation of the

problem.

B6 Instrument/Equipment Testing, Inspection, and Maintenance Requirements

All instruments and equipment used in the testing or analysis of the study samples will include testing, inspection, and/or maintenance consistent with the provisions of Good Laboratory Procedures (GLP) as described in 40 CFR 160, Part 9000 of Standard Methods for the Examination of Water and Wastewater (20th Edition, 1998, APHA), or institutional policy. All instruments and equipment checks will be documented as per institutional policy and all records and documentation retained for a minimum of one year after the conclusion of this study. Any instrument and equipment failures noted prior to the analysis of study samples will be corrected prior to the analysis of the study samples. Any instrument and equipment failures noted subsequent to the analysis of study samples will result in laboratory QC check failure and the initiation of a corrective action as noted previously.

B7 Instrument Calibration and Frequency

All instruments and equipment used in the testing or analysis of the study samples will include calibration consistent with the provisions of Good Laboratory Procedures (GLP) as described in 40 CFR 160, Part 9000 of Standard Methods for the Examination of Water and Wastewater (20th Edition, 1998, APHA), or institutional policy. All instruments and equipment calibration will be documented as per institutional policy and all records and documentation retained for a minimum of one year after the conclusion of this study. Any instrument and equipment calibration failures noted prior to the analysis of study samples will be corrected prior to the analysis of the study samples. Any instrument and equipment calibration failures noted subsequent to the analysis of study samples will result in laboratory QC check failure and the initiation of a corrective action as noted previously.

B8 Inspection/Acceptance Requirements for Supplies and Consumables

All supplies and consumables for the study will be procured from reputable suppliers. When practical, all suppliers will provide certificates of analysis stating the acceptance criteria and the results of any testing against the acceptance criteria. Supplies and consumables not provided with a certificate of analysis will be subject to inspection prior to use. Nonperformance supplies and consumables (ie gloves, bottles, etc.) will be visually inspected to insure that the material is the appropriate item and does not contain any notable defects. Defective materials will be quarantined and returned to the vendor/supplier. Performance supplies (ie reagents, media, etc) will typically have quality control checks of performance prior to, or concurrent with, the analysis of the study samples. Any performance supply failure will result in the initiation of a corrective action as noted previously. Inspection protocols and acceptance criteria will be documented and retained for a minimum of one year after the conclusion of this study.

B9 Data Acquisition Requirements (Non-direct Measurements)

The only data from nonmeasurement sources expected to be used during this

study is data and information representing historical information. Typically this data will be used to support courses of actions taken during this study, as supplemental information for this study, or as other references for this study. This data will be scrutinized by the principle investigators for inclusion in this study and will be validated against the internal reference of reasonability and the investigator's experience. Any data or information that is not consistent with the internal reference will attempt to be verified. Any verification that does not support the initial data or information will be noted and the data or information qualified in all reports or uses.

B10 Data Management

Data generated during this study will be maintained consistent with the provisions of Good Laboratory Procedures (GLP) as described in 40 CFR 160, Part 9000 of Standard Methods for the Examination of Water and Wastewater (20th Edition, 1998, APHA), or institutional policy. All original paperwork will be retained by the principle investigators and retained for a minimum of one year after the conclusion of this study. Data and information for this study will be maintained primarily as hard copy information.

Errors in paperwork, including data entry, will be detected through the normal review process. After data generation, data entry, or data transfer, data will be reviewed for transcription errors and corrected as appropriate. Errors that are detected prior to the use of information or data in an external report will be corrected through a non-destructive mechanism and all corrections will bear the date and initials of the corrector in a conspicuous manner as appropriate. Errors that are detected after the data or information has been used in an external report will be corrected through a non-destructive mechanism and all corrections will bear the date and initials of the corrector in a conspicuous manner as appropriate and an addendum or errata will be published and forwarded to all recipients of the report for inclusion with the report.

All data and information collected or generated in support of this study will be safe guarded against loss, destruction, or altercation at all times. Photostatic copies of critical data will be maintained in an alternate location pending the analysis and/or completion of reports or summaries.

In cases where results are less than the reporting limit for a parameter, the results will be reported consistent with the reporting parameter. Concentrations of chemicals and all numerical biological parameters will be reported using metric measurements. Data and information will be maintained consistent with the data reporting requirements of the database manager for this project.

Section C: ASSESSMENT/OVERSIGHT

C1 Assessments and Response Actions

Assessments of compliance with the quality control procedures will be undertaken by the principle investigators on a routine basis during the data collection. Corrective actions shall be carried out by the principle investigators as appropriate to maintain sample and analytic quality and shall then be reported to the project Quality Assurance Manager. Routine procedures to assess the quality of the data generated

has been discussed previously.

C2 Reports to Management

Study progress reports will be prepared monthly and highlight the progress and any problems identified in the study. Routine quality assurance reports will be incorporated into the monthly study progress reports submitted to the project officer. Reports specific to a quality assurance issue will be prepared as necessary.

Section D: DATA VALIDATION AND USABILITY

D1 Data Review, Validation, and Verification Requirements

As discussed previously, all data and information generated as part of this study will have ongoing review and verification. Due to the type of data being collected, data will be validated against the internal reference of reasonability and the investigator's experience. Any data that is not consistent with the internal reference will attempt to be rechecked and verified. Any recheck or verification that does not support the initial data will be noted and the data qualified in all reports.

D2 Validation and Verification Methods

The principle investigators, in conjunction with the project quality assurance officer, will be responsible for validating and qualifying all data based on the evaluation of quality assurance and quality control procedures previously described. The final report will include a detailed analysis of the data, including statistical analysis as appropriate.

D3 Reconciliation with Data Quality Objectives

Data generated from this study will be periodically reviewed to insure the data is consistent with the tasks outlined in the study. Any data that is inconsistent with the study tasks will be flagged and reviewed in conjunction with the project officer and the quality control officer. Inconsistent data will be evaluated for relevancy and a decision to include or exclude the data will be made. All decisions on the relevancy and inclusion or exclusion of the data will be documented by the principle investigator, project officer and the quality control officer and the documentation will be retained with the study's files for at least one year after the conclusion of this study.