Quality Assurance Project Plan
for
Microbial Source Tracking of *E. coli* in the Rio Fernando de Taos

Prepared by:

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Approval Sheet

Amigos Bravos Project Manager: Shannon Romeling

Shannon Romeling
Signature

11/15/18
Date

Amigos Bravos QA Manager: Joseph Zupan

Joseph A. Zupan
Signature

11-15-18
Date

EPA Project Manager:

_____
Signature

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Date

EPA QA Manager:

_____
Signature

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Date

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1.0 PROJECT MANAGEMENT

This Quality Assurance (QA) Project Plan has been prepared for the Microbial Source Tracking (MST) and Water sampling Portion of the EPA Environmental Justice Collaborative Problem Solving Grant. This project will be located in Taos, New Mexico. This section of the QA Project Plan describes how the project will be managed, organized and implemented.

The project will include two distinct aspects: 1) Streamside and E. coli sampling to be conducted in Taos and 2) Microbial Source Tracking to be conducted at Source Molecular Laboratory in Miami Lakes, Florida.

1.1 Distribution List:

The following is a list of organizations and persons who will receive copies of the approved QA Project Plan and any subsequent revisions:

1. Region 6 Project Officer Debra Tellez
2. Source Molecular Laboratory
3. Matthew Bogar, oversight
4. Members of the Rio Fernando de Taos Revitalization Collaborative

1.2 Project organization

The responsible agency for this project is Amigos Bravos. The Rio Fernando de Taos Revitalization Collaborative will be integrally involved in all decisions made, however Amigos Bravos is the lead organizer and takes full responsibility for the project. Formed in March 2017, the RFdT Revitalization Collaborative is a partnership of diverse local and regional stakeholders who are united around the common goal of resolving differences and improving the health and vitality of the RFdT watershed. This group is committed to the collaborative process and recognizes the social, environmental and economic benefits of a healthy watershed to both the urban and rural context.

Matthew Bogar, MS will act as independent oversight for this project. Matthew will oversee data collection and data interpretation.

Source Molecular Corporation is a private commercial laboratory founded in 2002 with a mission to fill the void between source identification research and real-world implementation of the technology. The laboratory is dedicated to offering innovative technology for solving pathogenic water pollution problems through microbial source tracking genetic and molecular techniques. Source Molecular will be processing all Microbial Source Tracking samples that are collected during this project. Daron Stein is the primary contact at Source Molecular for this project.

The US Forest Service will also be a close partner for this work, as the majority of the Rio Fernando flows through Carson Forest land. Local volunteers will be used to do the stream-side portion of the sampling under supervision of the project manager. Only the

project manager Shannon Romeling, Matthew Bogar will collect samples for Microbial Source Tracking purposes.

The participating agency is the EPA. The roles and responsibilities of those involved in the water quality sampling portion of the project are described below. An organizational chart for the project is shown as Figure 1-1.

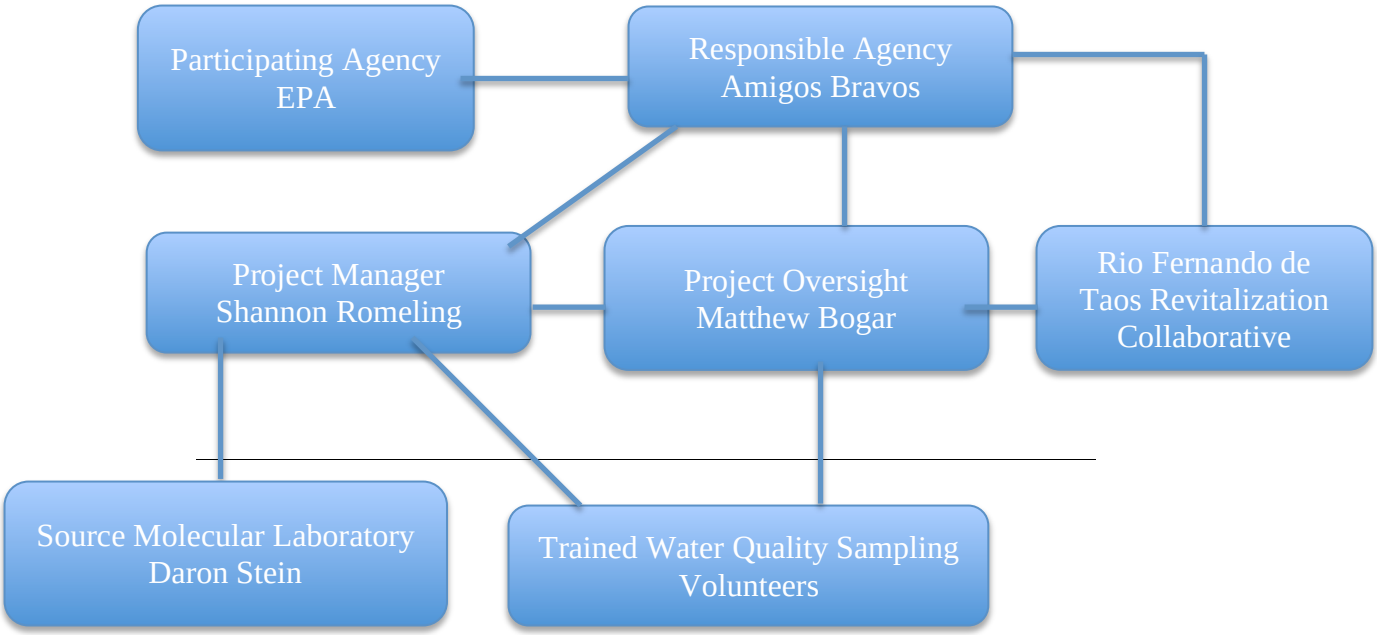


Figure 1-1. Organizational Chart

1.2.1 Key Persons and Coordination Roles and Responsibilities

Project Manager: ***Shannon Romeling*** of Amigos Bravos will use her skills as a Biologist to manage this project. Shannon will conduct preparation, sampling, and data processing to ensure that the project is executed as planned. Shannon is a full time employee of Amigos Bravos and is responsible for researching, writing, submitting, and tracking proposals and reports regarding grants from foundations, government agencies, and other contractual funders. She is also responsible for any project related work delegated to her by the ED or Projects Director and for guiding and assisting the Membership Coordinator when needed. Contact information: Shannon Romeling. P.O. Box 238 Taos NM 87571 sromeling@amigosbravos.org / 575-758-3874

Amigos Bravos: A non-profit water conservation organization dedicated to protecting the ecological and cultural richness of the Río Grande and other wild rivers in New Mexico, will serve as the project's fiscal sponsor.

Source Molecular Corporation: A private commercial laboratory founded in 2002 with a mission to fill the void between source identification research and real-world implementation of the technology. The laboratory is dedicated to offering innovative technology for solving pathogenic water pollution problems through microbial source tracking genetic and molecular techniques. To provide the highest quality of service, Source Molecular secured a license from the U.S. Environmental Protection Agency (EPA) allowing it to use the MST technology the EPA developed and patented. Their lab is located at: 15280 NW 79th Court Suite 107, Miami Lakes, FL 33016 USA. Contact Information: Daron Stein, dstein@sourcemolecular.com / 786 404 1675

Rio Fernando de Taos Revitalization Collaborative: To undertake the work to revitalize the river, a collaborative of individuals, organizations, and government agencies have come together. This Collaborative includes diverse community leaders, groups, businesses, and government agencies, attracting money and resources to help our community, acequias and the river, resources often not available to one entity alone. Collaborative members and lead partners include:

- Amigos Bravos
- LOR Foundation
- Taos County
- Taos Land Trust
- Taos Valley Acequia Association
- The Nature Conservancy
- Town of Taos
- US Forest Service

Contact Information: Laura McCarthy, The Nature Conservancy, (505) 231-9740 / lmccarthy@tnc.org; Kristina Ortez, Taos Land Trust, (575) 751-3138 / kristina@taoslandtrust.org

Matthew Bogar: Matthew received a Master's degree in geology from New Mexico State University in 2005. His study focused on collecting and analyzing metamorphic rock samples both physically and chemically to determine their origin. Matthew will be working independently for this project. He is also currently employed as an Environmental Scientist/Specialist with the New Mexico Environment Department (NMED): Environmental Health Bureau where he permits and inspects onsite liquid waste treatment systems, food retail establishments, and public swimming pools and spas. With NMED he routinely tests well water samples for nitrate, sulfate, iron, and fluoride concentrations and field parameters for pool and spas. He also has experience with soil chemistry, titration chemistry in a Thermal Ionization Mass Spectrometry clean lab, and X-ray Fluorescence Spectrometry. Matthew has demonstrated an expertise in the design of environmental sampling and analysis plans which include sampling techniques and testing methodologies, the fundamentals of quality assurance and quality control, and data and error analysis.

1.2.2 Volunteer Sample Collector Contact Information:

Shannon Romeling will collect and hold all volunteer collector contact information. Contact Shannon as shown above.

Sample collectors will conduct sample collection activities according to the methods identified by this QAPP. Responsibilities include:

- Calibration, maintenance and utilization of field equipment for analysis of dissolved oxygen (DO), temperature, pH, and conductivity.
- Obtaining needed sample containers and preservatives for sampling events.
- Following quality assurance procedures for sample collection identified by this QAPP.
- Filling out chain of custody (COC) forms when necessary.

1.2.3 Sample Transport Contact Information

Shannon Romeling, Matthew Bogar

Shannon and Matthew will ensure that water samples are delivered to the Amigos Bravos office in a secure and timely manner. Trained volunteers will also transport samples to the Amigos Bravos office when necessary.

Responsibilities include:

- Keeping samples secure between sampling site and the office.
- Maintaining COC document according to procedures identified.
- Delivering samples within specified holding times.

1.2.4 Sample Analysis Contact Information:

Amigos Bravos Office
114 Des Georges Place, Taos, NM 87571

Shannon Romeling will ensure that samples are analyzed in a manner that provides the most accurate data possible.

Responsibilities include:

- Analyzing samples according to EPA protocols.
- Analyzing samples within established holding times.
- Analyzing samples with appropriate calibration standards and blanks.
- Reporting Quality Assurance-validated results to the Rio Fernando de Taos Revitalization Collaborative

1.2.5 Data Reporting Contact Information

Shannon Romeling, Amigos Bravos Projects and Foundation Coordinator
P.O. Box 238
Taos, NM 87571
575-758-3874
sromeling@amigosbravos.org

Data reporting will ensure the data collected by the project is stored appropriately and disseminated to interested parties. Data will be backed up on the Amigos Bravos in-house server. Data will be backed up and shared on the Amigos Bravos server. This server is backed up incrementally using a cloud backup every day.

Responsibilities include:

- Organization of final report on data collected by the project.
- Dissemination of report to specified local, state and federal agencies.
- Dissemination of report to newspapers and other local news media and presentation of project information to the public upon request.
- Entering data into Amigos Bravos' water quality database.

1.3 Problem definition/background

In 2006, the Upper Rio Grande Watershed Restoration Action Strategy and Non Point Source Abatement Plan was developed collaboratively under a 319 Grant administered by The Meridian Institute. That WRAS included separate sub-plans for the Rio Fernando and the Rio Hondo. The Rio Don Fernando de Taos NM WRAS Water Restoration Action Strategy & Non Point Source Abatement Plan document can be found at: http://www.nmenv.state.nm.us/swqb/wps/WRAS/UpperRioGrandeWRAS.pdf.

The Rio Fernando WRAS identified grazing, recreational activities, removal of riparian vegetation, stream bank modification/destabilization, runoff from roads and/or parking lots, pollution from municipal point sources, as well as natural leaching as having affected water quality in the Rio Fernando. This combination of sources has resulted in increases in the levels of nutrients, conductivity, pH, temperature, and stream bottom deposits that exceed established water quality standards.

Amigos Bravos and the Water Sentinels-Rios de Taos have been conducting water quality monitoring in the Taos watershed (Rio Hondo, Rio Pueblo de Taos, and the Rio Fernando) for a number of years. The Taos Water Quality Sampling Reports – Rio Hondo, Rio Fernando de Taos, and Rio Pueblo de Taos can be found at: http://amigosbravos.org/on-the-ground-restoration. This monitoring documented the *E. coli* impairment in the Rio Fernando de Taos, which the New Mexico Environment Department accepted and included in its 2012-14 303d list of impaired waters. The list includes the three segments of the Rio Fernando de Taos for the following:

- Tienditas Creek to the Headwaters (in the RFP, AU_ID NM-98.A_001): *E. coli* (with sources from grazing and "unknown")
- USFS boundary to Tienditas Creek (NM-2120.A_513): *E. coli* (with source from "unknown")
- Confluence with the Rio Pueblo de Taos to USFS boundary (NM-2120.A_512): *E. coli*, Nutrient/Eutrophication Biological Indicators, Sedimentation/Siltation, Specific Conductance, and Temperature (with a wide variety of typically "urban" sources: highway/ road/bridge runoff, irrigated crop production, natural sources, other recreational sources, rangeland grazing, source unknown, and stream bank modification/destabilization)

TMDLs were established in 2012 for the *E. coli* impairments identified. The sedimentation/siltation and nutrient impairments on the middle segment (USFS boundary to Tienditas Creek) do not yet have established TMDLs. This creates somewhat of a disconnect in dealing with water quality on the Rio Fernando, since the segments above and below this segment have established TMDLs for all of their recognized impairments.

In 2016, Amigos Bravos began the Watershed Based Planning process for the Rio Fernando using Clean Water Act 319 grant funding. This plan is due to be completed in the Spring of 2019. While the 319 funds could not directly fund the Microbial Source Tracking work, the NMED and EPA expressed their full acceptance of including the work in the Watershed Based Plan if other funding could be acquired. The MST results will be added to the watershed based plan as soon as the results are complete and reported under this grant.

The MST project will focus on determining detailed sources of the on-going *E. coli* contamination on the Rio Fernando de Taos. While there is much speculation about the sources primarily responsible for high *E. coli* levels in the Rio Fernando, studies have yet to positively identify the main contributors. We will use the MST bacteria genotyping technology to identify the animal group source (human, cattle, dog, beaver, etc.) of bacteria in river water samples. By identifying and focusing resources on the most significant bacteria sources, the Collaborative will be better equipped to plan projects that achieve measurable reductions of *E. coli* in the river.

1.4 Project task description/schedule

Total *E. coli* samples taken will be taken at the same time that the MST samples for Source Molecular Corporation are collected. All samples will be collected as grab samples from the banks and all sampling locations will be recorded using global positioning system (GPS) equipment. Samples will be sent to the Amigos Bravos office for analysis using the IDEXX Laboratories, Inc. Colilert method.

Sampling Schedule:

Sampling will be conducted at 4 distinct sites, which have consistently been plagued with E. coli exceedences since our sampling began in 2007.

1. Collect samples and send to Source Molecular
    a. Milestone: 4 sites have been sampled for *E. coli* and those samples have been sent to Source Molecular for analysis. Each location will be sampled once a week for 5 months (October, April, June and August, 14 weeks total), totaling 70 samples collected at each location between September 1 and July 30. A sixth sample will be collected a needed (funds are budgeted for 1 extra test at 1 site for 14 events, most likely bird feces).
    b. Milestone: Send known fecal samples to Source Molecular. Samples

of human, cattle, elk, canine, beaver, feces have been sent to Source Molecular.
    c. Milestone: Independent Expert monitors sampling
    d. Timeline: Water samples and known fecal samples conducted during the water sampling period of October 1, 2018 – August 30, 2019. Independent Expert will help throughout the process.
2. Conduct two public/community meetings during the sampling period.
    a. Milestone: Outreach is performed and two public meetings are executed
    b. Timeline: September 1, 2018 – August 30, 2019
3. Interpret results
    a. Milestone: Source Molecular has sent the results and Amigos Bravos has had time to interpret them along with the independent expert.
    b. Timeline: August 30 - September 30, 2019
4. Include results in the Rio Fernando de Taos Watershed Based Plan.
    a. Milestone: Amigos Bravos is able to add the MST results to the Rio Fernando Watershed Based Plan before it is complete.
    b. Milestone: MST results inform the proposed implementation projects in the Watershed Based Plan and inform work of the Rio Fernando Revitalization Collaborative.
    c. Timeline: Into year 2 funding - Winter, 2019.

1.5 Special training requirements

No special certification is required to implement this QAPP, however, proper training of field personnel is a critical aspect of Quality Control. All Amigos Bravos staff, Wet Water Environmental Services, and volunteers with the responsibility of collecting water quality data will have sufficient training and experience.

Additionally, all newly trained volunteers will undergo a period of apprenticeship where they will be accompanied by experienced staff when collecting samples or field measurements until the Project Mamanger determines that the person is appropriately trained and qualified to collect quality data.

All people who are responsible for collecting and/or managing data/information and producing planning or reporting documents are required to be familiar with this QAPP.

1.6 Documents and records

This QAPP and referenced procedures includes methods related to the collection, processing, analysis, and reporting and tracking of environmental data. This QAPP is updated annually and made available to EPA staff and contractors responsible for collecting, processing, and analyzing data. Data generated from projects covered by this QAPP must be of sufficient quality to withstand challenges to their validity, accuracy, and legibility.

The documentation of all environmental data collection activities must meet the following minimum requirements: Data and associated information must be documented

directly, promptly, and legibly. All original data records include, as appropriate, a description of the data collected, units of measurement, unique sample identification, location identification, name (signature or initials) of the person collecting the data, and date of collection. Any changes to the original (raw data) entry must not obscure the original entry. The reason for the change must be documented, the change must be initialed and dated by the person making the change and approved by the Program Manager.

Field records will be documented on designated forms to provide a secure record of field activities, observations and measurements during sampling. Lab data and observations will be recorded in real time on a lab specific data form. Entries are never erased and mistakes are lined out and initialed by the data recorder. Completion of appropriate field and lab documentation and forms for each sample is the responsibility of the Project Coordinator or designee.

Water quality survey project files are maintained by the Project Manager; however, the file is used by numerous staff within the section for various purposes. To ensure consistency and accessibility to all users, water quality survey project files are maintained in three ring binders as follows:

Label each binder on spine and front cover with the following information:

- Survey Title Survey Year(s)
- Project Coordinator [Binder X of X]
- Hydrologic Unit Code/Watershed

Create tab dividers with labels for the sections listed below and place all associated documents and records in the applicable section of the binder in the same order as listed below:

- Introductory Information
    - Map(s) of survey area
    - Field Sampling Calendar

- Background Information
    - Site access information
    - Supplemental information pertinent to the survey (land-use, land activities, ect.)

- Field Data – Physical and Biological info
    - Water Sampling field Forms (See Appendix B- Field Sampling Form)
    - Flow field forms and flow calculation worksheets (See the Flow Sampling Form in Appendix C)

- IDEXX Lab Data
    - Analytical IDEXX Laboratory submittal forms
    - Chain of custody forms

- Source Molecular Lab Data
    - Results sent back from Source Molecular

2.0 Data Generation and Acquisition

2.1 Sampling design (experimental)

We will continue determining ways to address the *E. coli* impairment by determining which species are dominating E. coli samples. We will sample for levels of *E. coli*, *E. coli species*, temperature, dissolved oxygen, conductivity, and measure stream flow at each site. This intensive sampling will provide a picture of water quality at each site over the year.

If observation leads us to suspect specific septic tanks as contributors of *E. coli* in certain locations, we will use a septic dye test (a tracer) to look for potential leaks, following owner consent.

2.2 Sampling methods

Samples will be collected using the containers, preservatives, volumes and holding times identified in Table 2-1. There are three distinct sampling methods that will be used:

1) Stream side sampling using a water quality meter
2) IDEXX method for determining *E. coli* levels
3) Source Molecular Lab analysis for conducting Microbial Source Tracking (speciation of *E. coli*).

E. coli levels will be determined using the NMED Surface Water Quality Bureau IDEXX method (Standard Methods, Part 9000 -APHA 2005)

Streamflow is determined using one of four methods depending on the site:

1. Float method – See Appendix C for the datasheet.
2. The Notch Weir Method – a standard notch wier is used to block off the waterway and measure flow.
3. The Bucket method – a 5 gallon bucket and a timer is used to estimate flow.
4. The Visual Method – visual estimates are made in gallons per minute.

Parameter	Optimum Volume	Container Type	Preservation Method	Holding Time
E. coli And Fecal Coliform	150 mL	Sterile Bottle	Cold (on ice)	**8 Hours**
Dissolved Oxygen	Determined On-Site			**None**
Temperature	Determined On-Site			**None**
Conductivity	Determined On-Site			**None**
pH	Determined On-Site			**None**
Stream Flow	Determined On-Site			**None**

Table 2-1: Parameter information

2.2.1 Field Sample Collection Procedures

Samples will be collected:

- Midstream just below the water's surface.
- Facing upstream to avoid disturbances caused by the sample collector.
- Upstream of minor temporal or spatial impacts, such as bridges and campsites.
- When sampling downstream of certain impacts (sources) care will be taken, using best professional judgment, to sample well-mixed waters.
- Free of floating debris.
- Using appropriate sample containers and preservatives.

Field Equipment:
Temperature, pH, conductivity – Euteck Instruments PCTestr 35 from Oakton
Dissolved Oxygen – CHEMets Dissolved Oxygen Kit, Model K-7512

At a minimum, equipment should be wiped down with sanitizing wipes after use to minimize *E. coli* exposure.

2.2.2 IDEXX Laboratory Analysis Procedures

Field sampling procedures will follow the New Mexico Surface Water Quality Bureau Standard Operation Procedure 9.1 for Bacteriological Sampling and Analysis. This SOP is available in Appendix A.

Quality assurance of laboratory methods is the sole responsibility of the sample analysis contact previously identified – Shannon Romeling and Matthew Bogar

Samples will be collected:

- Midstream just below the water's surface.
- Facing upstream to avoid disturbances caused by the sample collector.
- Upstream of minor temporal or spatial impacts, such as bridges and campsites.
- When sampling downstream of certain impacts (sources) care will be taken, using best professional judgment, to sample well-mixed waters.
- Free of floating debris.
- Using IDEXX sample containers and preservatives.

Samples will be tagged appropriately with identifying number/information and delivered to appropriate laboratory personnel accompanied by appropriately completed and signed Chain of Custody (COC) forms (Part of the Field Sampling Form - Appendix B).

Amigos Bravos uses the IDEXX Laboratories, Inc. Colilert® procedures for enumeration of total coliform and *E. coli* by the most probable number (MPN) method. The procedure is explained in Standard Methods, Part 9000 (APHA 2005). Background on the MPN method can be found in Oblinger and J. A Koburger (1975).

IDEXX Colilert Lab Equipment:

• IDEXX 110V Incubator
• Fluorescent UV lamp, 6-watt, 365 nm
• IDEXX Quanti-Tray sealer
• IDEXX Quanti-Tray/2000 rubber insert
• Appropriate Personal Protective Equipment, “PPE” (i.e., UV protective eyewear, Nitrile gloves)

Because *E. coli* can be an indicator of pathogens harmful to humans, precautions should be taken when sampling potentially contaminated water. Avoid accidental ingestion, contact with mucous membranes, eyes and skin to the extent possible, especially areas with cuts and abrasions. Wear splash protection and eye protection (i.e., goggles, gloves, and aprons) while working with bacteriological samples. Wash hands with soap and water or disinfecting hand cleaner as soon as possible after collecting samples and working with equipment. Equipment exposed to potentially contaminated water should be cleaned using a dilute (1:10) bleach solution and rinsed in clean water if possible. In the field, at a minimum, equipment should be thoroughly rinsed in clean water (e.g. the stream receiving the effluent above the point of discharge) immediately after use.

2.2.3 Source Molecular Lab Analysis

Water Samples: Each submitted water sample is filtered through 0.45-micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, are run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Please See Appendix D for more details.

2.2.4 Independent Expert

Amigos Bravos will hire an independent contractor to oversee data collection methods and result analysis. Tasks will include:

- Participate in first two sampling events to oversee and recommend method tweaks (approximately 20 hours total)
- Analyze results from Source Molecular Laboratory (approximately 10 hours)
- Consult with Amigos Bravos on their written report of results as they come in (approximately 20 hours)

Work will be conducted in short intervals as samples are taken and results are received. . Work will occur between the dates of Nov 1 – October 1 2019. Up to $2,500 will be paid to the contractor. Additional matching funds may be secured from Taos Soil and Water Conservation District or Amigos Bravos, but are not confirmed.

2.2.5 Site Locations

Four site locations have been chosen. The Rio Fernando Revitalization Collaborative will decide what species of *E. coli* is checked for at each site. Choices include human, elk, cattle, beaver, dog, bird, and others.

Station Number (Headwaters to end)	Station Name	Latitude	Longitude	Station Rationale
1	FLJ	N 36 25.149	W 105 20.590	La Jara Canyon. Headwater at the U-turn before Angelfire. About 100 yards from the parking lot.
2	FRE	N 36 24.2298	W 105 20.7081	The Riparian Exclosure – a cattle pasture that is grazed 10 days a year. Straight to river from gate.
3	F16	N 36 24.010	W 105 35.013	Richard Duncan Property –At the end of Santistevan Lane in Taos
4	F4	N 36 23.971	W 105 35.367	Fred Baca Park, near the footbridge at the bend.

Table 2-2: Sampling Site Locations

2.3 Sample handling and custody

All trained staff and volunteers will sign chain of custody forms before handing off samples to be analyzed for *E. coli*. See Appendix B for the Chain of Custody section of the Field Data Sheet that is used.

2.4 Quality control requirements

Measurements will be made using the following equipment:

- CHEMets Dissolved Oxygen Kit, Model K-7512 – measures dissolved oxygen
- Euteck Instruments PCTestr 35 from Oakton – measures pH, temperature, and electrical conductivity
- IDEXX Colilert System
    - IDEXX 110V Incubator
    - Fluorescent UV lamp, 6-watt, 365 nm
    - IDEXX Quanti-Tray sealer
    - IDEXX Quanti-Tray/2000 rubber insert
    - PPE

PARAMETER	DETECTION LIMIT	ACCURACY
Dissolved Oxygen	1 to 12 mg/L	+/- 1 ppm
Temperature	0^o to 50^o C	+/- 0.5^o C
Conductivity	0 to 1999 µS/cm	+/-10 µS/cm
pH	0.00 to 14.00 ph units	+/-.001 pH units
E. coli	NA	

NA=Not Applicable

Table 2-3: Measured parameters and their detection limits

Field instruments will be calibrated according to manufacturers' instructions <24 hours prior to each sampling event. Chemicals used for dissolved oxygen will be replaced according to expiration dates provided by the manufacturer.

New field volunteers will learn sampling techniques through training and apprenticeship with experienced Amigos Bravos staff. All personnel who collect environmental data must be familiar with this QAPP and collect data in accordance with the procedures.

An additional check on the quality of field activities includes periodic Quality Assurance Audits. Quality Assurance Audits will be performed periodically as resources allow. Field crews to be audited will be randomly selected and the audits will be performed by the QA Officer or designee.

3.0 DATA REVIEW AND USABILITY

Data verification involves examining the data for errors, omissions, and compliance with quality control (QC) acceptance criteria. Once measurement results have been recorded, they are verified to ensure that:

- Data are consistent, correct, and complete, with no errors or omissions.
- Data specified in Sampling Process Design were obtained.
- Methods and protocols specified in the QA Project Plan were followed.
- Results for QC samples accompany the sample results.
- Established criteria for QC results were met.
- Data verification should be performed as close in time as possible to when the sample data was either retrieved from the field. The purpose of rapid data verification/validation is to provide the project coordinator with the most options for re-deployment/additional field collections if problematic data is encountered.

The project coordinator Shannon Romeling is responsible for verifying that field data entries are complete and correct (e.g., decimal point missing from an entry or something doesn't look right, based on experience). The project coordinator will also examine lab results for errors, omissions, and compliance with QC acceptance criteria, as soon as possible, as outlined above

Results that do not meet quality assurance requirements will be labeled with appropriate qualifiers, and an explanation will be provided and attached to the data package.

Data usability determination will follow verification. This determination is parameter-specific and involves a detailed examination of the data package. Professional judgment will be used to determine whether data quality objectives have been met.

APPENDICES

Appendix A: SOP 9.1 for Bacteriological Sampling and Analysis
Appendix B: Field Sampling and Chain of Custody Form
Appendix C: Flow datasheet - Float method
Appendix D: Source Molecular QA/QC Summary

Appendix A

Title: **Bacteriological Sampling and Analysis**	No: SOP 9.1 Revision 1	Page 1 of 6
Effective Date: 3/01/2013	Next Revision Date 3/01/2015	

New Mexico Environment Department
Surface Water Quality Bureau

Standard Operating Procedure

for

Bacteriological Sampling and Analysis

Approval Signatures

Gregory Huey
Subject Matter Expert — Date: 4/3/13

Jodey Kougioulis
Quality Assurance Officer — Date: 4/3/13

Shelly Lemon
Acting Program Manager — Date: 4-3-2013

1.0 Purpose and Scope

This procedure describes the collection and analysis of ambient water and wastewater samples for total coliform and *Escherichia coli* (*E. coli*) bacteria using the IDEXX Laboratories, Inc. Colilert® method for both water quality standards assessment and permit compliance monitoring purposes.

2.0 Personnel Responsibilities

All personnel who collect or process samples for total coliform or *E. coli* analysis are responsible for implementing this procedure.

One individual within SWQB is designated as the "Bacteriological Equipment Manager." The manager is responsible for keeping the equipment used for bacteriological sampling in working order and ready for use. The manager is responsible for verifying that the dates on the lots have not exceeded their expiration dates.

3.0 Background and Precautions

The SWQB and the New Mexico State Laboratory Division (SLD) both use the IDEXX Laboratories, Inc. Colilert® procedures for enumeration of total coliform and *E. coli* by the most probable number (MPN) method. The procedure is explained in the Colilert® reagent snap packs and in *Standard Methods*, Part 9000 (APHA 2005). Background on the MPN method can be found in Oblinger and J. A. Koburger (1975).

Because *E. coli* can be an indicator of pathogens harmful to humans, precautions should be taken when sampling potentially contaminated water. Avoid accidental ingestion, contact with mucous membranes, eyes and skin to the extent possible, especially areas with cuts and abrasions. Wear splash protection and eye protection (i.e., goggles, gloves, and aprons) while working with bacteriological samples. Wash hands with soap and water or disinfecting hand cleaner as soon as possible after collecting samples and working with equipment. Hepatitis vaccinations are available for staff, if desired. Equipment exposed to potentially contaminated water should be cleaned using a dilute (1:10) bleach solution and rinsed in clean

water if possible. In the field, at a minimum, equipment should be thoroughly rinsed in clean water (e.g. the stream receiving the effluent above the point of discharge) immediately after use.

4.0 Definitions

None.

5.0 Equipment and Supplies

Equipment

There are two primary sets of IDEXX equipment available through the SWQB laboratory: a lab kit and a field kit.

Lab Kit:
- IDEXX 110V Incubator
- Fluorescent UV lamp, 6-watt, 365 nm
- IDEXX Quanti-Tray® sealer, “lab”
- IDEXX Quanti-Tray®/2000 rubber insert

Field Kit:
- Portable Incubator with DC power plug for vehicle operation
- IDEXX 6 watt fluorescent UV lamp, 365 nm
- IDEXX Quanti-Tray® sealer, “field”
- IDEXX Quanti-Tray®/2000 rubber insert
- 12 V Deep Cycle or Regular Automotive Starting Battery
- AC/DC inverter to power the portable incubator via a typical 110V outlet
- DC/AC 800 watt inverter and: +/- alligator clips to allow connection of sealer to vehicle alternator
- DC/DC adapter allowing vehicle power port conversion to +/- alligator clips for deep cycle battery power

Supplies

Materials for Samples Processed by SWQB

- IDEXX Colilert® Reagent “Snap Packs” for 24-28 hour incubation
- IDEXX Colilert®-18 Reagent “Snap Packs” for 18-22 hour incubation
- IDEXX Quanti-Tray®/2000 bacterial enumeration trays
- IDEXX Shrink Banded Sample Bottles

Materials for SWQB QC
- Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator

Materials for Samples processed by SLD

- 125 ml, sterile polypropylene sample bottles (available from SLD)
  Note: SLD does not accept samples in IDEXX bottles
- “Do not tamper” seals for lids (available from SLD)
- Submittal form (download from http://www.sld.state.nm.us/Documents/waterform.pdf)

The Environmental Microbiology section at SLD should be consulted (505-383-9129) at least 2-3 weeks in advance of the proposed sampling date to assure availability of media and incubator space.

6.0 Step-by-step Process Description

E. coli sample collection

Before going in the field, fill out a "Bacteria Record Sheet" (attachment to this SOP) and use this form to record all requested information for each sample.

Collect both routine and quality control (blank) samples. In most cases, collect one set of quality control samples with each group of samples collected on consecutive days within one week. Generally, no less than 5% of the samples should be quality control samples. Prepare the field blank using distilled water and process quality control samples at the same as a routine sample.

Collect samples in sterile bottles. Do not rinse sample bottles before use and do not remove the caps or shrink wrap until immediately before sampling. For compliance sampling, the samples must be collected directly into the sample bottles. Otherwise, collect samples directly into the sample bottles whenever possible. If it is necessary to transfer to the sample bottle from another vessel, note this in the comment field when the sampling event is entered into NMEDAS.

Verify that the bottle is properly labeled and remove and dispose of the shrink band or sealing tape. In a stream, it is preferable to sample where the stream is flowing, well mixed and more than 6 inches deep. Stand downstream of the sampling bottle to avoid getting streambed sediment in the sample. In a lake or reservoir, collect the samples from the side of a boat at the sampling station or from the water's edge.

Wear disposable gloves if you are sampling effluent water or are working downstream of a wastewater treatment plant. Uncap the sample bottle. Holding the cap in one hand and the bottle in the other, submerge the bottle in as upright a position as possible to retain the sodium thiosulfate chlorine neutralizer. Collect a sample from the water column, minimizing the amount of surface material entering the bottle. Attempt to fill the bottle just to the 100-mL line. If the bottle is filled above the 100-mL line, immediately decant excess water. Carefully replace the cap.

After collecting the sample, ice or refrigerate it at a temperature less than 10°C. Use insulated containers to assure proper maintenance of storage temperature. Ensure that sample vessels are not totally immersed in water during transit.

Samples for Submittal to SLD

Use 125 ml, sterile polypropylene sample bottles containing sodium thiosulfate (available from SLD).
Note: SLD will not accept samples in IDEXX bottles. Contact SLD in advance of delivery of the samples to verify that the samples can be processed within the required time.

Note the temperature of the samples when they are submitted to SLD receiving. Fill out one submittal form for each sample and apply "Do Not Tamper" seals to bottles before submitting. Maximum transport time is 6 hrs from time of collection until time of delivery at SLD, and 8 hours from collection to the start of incubation.

Figure 1. IDEXX Bottle

Samples for IDEXX processing

Use IDEXX sample bottles (120-mL shrink-banded containers with sodium thiosulfate, IDEXX Part Number WV120SBST).

Processing IDEXX Samples in the SWQB Laboratory

1. Remove the samples from the cooler approximately 30 minutes prior to arriving at the processing site and allow to warm to ambient temperature. Add the IDEXX Colilert® reagent packet. Although the reagent will dissolve in chilled samples, it dissolves better if the samples are at room temperature.

2. Switch on the Quanti-Tray® Sealer and allow it to warm up until the green light on the cover comes on. Warm up times greater than 14 minutes improve sealing.

3. Fill out a "Bacteria Record Sheet" (attachment to this SOP) and use this form to record all requested information for each sample.

4. As appropriate based on read-time, select either Colilert®-18 (18-22 hr incubation) or Colilert®-24 (24 - 28 hr incubation) reagent.

5. Add one reagent packet directly to each sample bottle. Invert gently at 2-3 min intervals until completely dissolved.

6. Pour the solution into a Quanti-Tray®2000 incubation tray while holding it vertically. Gently tap the incubation tray to dislodge any bubbles that may be trapped in the cells.

7. Place the tray into the red rubber frame and insert the frame and tray into the bay in the front of the sealer.

8. Carefully ease the frame and tray forward until the sealer activates and feeds the tray automatically. The sealer may occasionally jam while processing the tray. If this occurs, press the reverse button on the cover and the track will reverse, depositing the frame on the bay. Wait until the green light activates again and repeat the loading process. The tray will be deposited on the counter behind the sealer.

9. Record initial incubator temperature on the Bacteria Record Sheet.

10. Start the incubation within 8 hours of sample collection. Place the sealed tray into the 35˚C incubator and incubate 18-22 hrs for Colilert®-18 reagent or 24-28 hrs for Colilert ® reagent.

11. After the appropriate incubation period, record the final incubator temperature on the Bacteria Record Sheet. Remove the sealed tray and record the incubation time. Do not read trays that have been incubated beyond the appropriate incubation time, as they may produce false positives.

12. Enumerate total coliform bacteria by counting the number of large and small cells that turn yellow after incubation. Enumerate *E. coli* by counting the number of large and small cells that fluoresce under UV illumination with the black light. For both total coliform and *E. coli*, the Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator should be used to verify positive or negative cells. The large window at the top of the tray should be counted as the 49th large cell.

13. Enter the sample date, method, sample ID (RID#), and the number of positive large and small cells counted into the IDEXX MPN generator (P:\SWQB PUBLIC\MAS Core Documents\IDEXX MPN Generator\IDEXXMPN.exe – be sure to enter the "*Default Directory to Save Files*" under "options" menu to record data). Press calculate and then log to record the mpn/100 ml and the upper and lower boundaries of the 95% confidence interval. *Record QA sample results (including zero) the same as routine results (MPN expressed as cfu/100 ml and 95% upper and lower confidence limits).*

14. Transfer IDEXX log file to the Bacteria Database Upload Form (attachment to this SOP) and enter additional data, as recorded on the Bacteria Record Sheet. Confirm that there are no data QA issues and then convert data file to csv for upload to NMEDAS database. Additional instructions and details are provided on the Bacteria Database Upload Form.

Processing IDEXX Samples in the Field

A field kit has been assembled for IDEXX sample processing in the field. Procedures are the same as for laboratory processing, however a 12 volt incubator and adaptors and voltage converters to operate the sealer off the electrical system of a vehicle are available. There is a checkout sheet for the field kit in the SWQB public folders: SWQB PUBLIC\Sonde&Equipment Checkout. Use this sheet when taking the kit from the building.

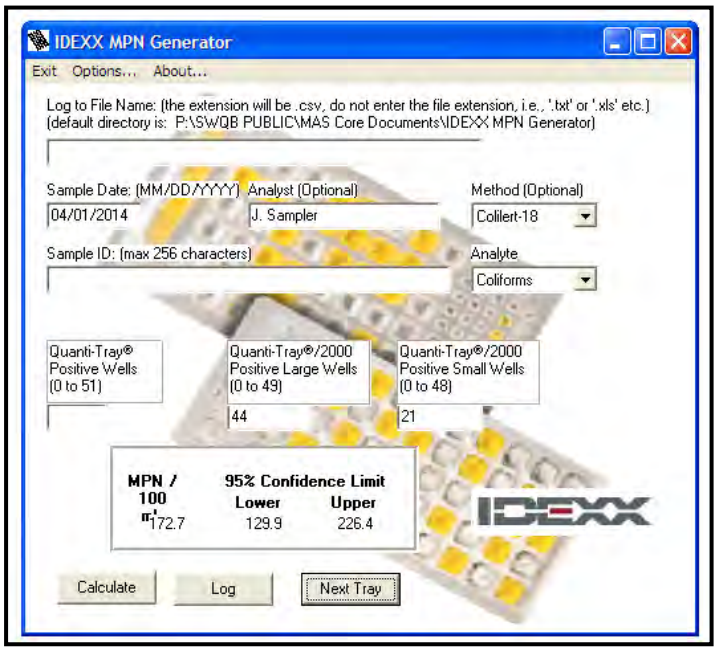


Figure 2. IDEXX MPN Generator Screenshot

Before leaving on a sampling run, it is advisable to test all field kit components to ensure that they are all working and present.

If it If it is necessary to seal Quanti-Trays in the field, the IDEXX sealer may be powered by the vehicle alternator.
To operate the 110V sealer, power must be converted from the 12V supplied by the vehicle alternator, and the vehicle must be running to supply sufficient amperage. A DC/AC 800 watt converter is used for this power conversion. To use the 110V sealer:

1. Clamp the alligator clips on the leads to the converter to the respective terminals on the vehicle battery.

2. Plug the sealer into the converter.

3. Start the vehicle.

4. Turn the converter on.

5. Turn the sealer on.

Follow procedures 1-8 under SWQB laboratory processing.

Use the portable DC incubators for field incubation. These are supplied with a power cord that allows them to be plugged into a vehicle's cigarette lighter. However, they draw sufficient amperage to drain the vehicle battery if the vehicle is not running. There is also an AC/DC converter that converts 110V AC to 12V DC allowing the incubator to be powered by a standard home 3-prong electric outlet for use in a hotel room or field office. The power cord for the Thermotote® model can be inserted two ways: one orientation will heat the incubator (red lamp lights) and the other will cool it (green lamp lights).

To incubate the samples, follow steps 9-11 under SWQB laboratory processing.

It may be necessary to enumerate Quanti-Trays in the field. Follow steps 12 and 13 under SWQB laboratory processing. The biggest challenge to this task is finding a dark place to discern which cells fluoresce. Service station washrooms, WWTP laboratories and SWQB field offices are often used for this purpose.

Storage and Disposal of Used Quanti-Trays

Following the sample incubation period, Quanti-Trays are considered a bacteriological biohazard and must be handled and disposed of accordingly. Trays collected in the field must be stored in a red or

orange “biohazard” bag inside a rigid container (e.g., small cooler, cardboard box, etc) and returned to the Runnels Building laboratory for storage. Used Quanti-Trays that are generated in the field or in the lab must be stored in an appropriate containment area until they can be properly disposed. A red bagged storage container is kept in the Runnels laboratory walk-in cooler and has been designated for staging of all used Quanti-Trays. This container is periodically emptied by Stericycle for proper disposal. Consult the *SWQB Chemical Hygiene Plan* for more information on handling and storage of used Quanti-Trays, as necessary.

Quality Assurance

If the samples are analyzed by SLD, the Laboratory should provide information to verify that the incubation temperature was maintained within the method-specified range, that the incubation began within 2 hours after sample receipt, and that the incubation time was within that required by the method.

If the samples were processed using SWQB equipment, the following sample rejection rules apply:

If both the temperature at the initiation and conclusion of the incubation were within 35 ± 0.5°C, the data is not flagged (i.e. no qualifier code) . If either temperature is less than 34.5 °C, the data is rejected (qualifier code = Er). If either temperature is between 35.5 and 38 °C, the data is flagged and may only be used as supporting evidence for assessments (qualifier code = Ea). If either temperature is greater than 38 °C, the data is rejected (qualifier code = Er).

The sealed trays should be read within the period indicated by the reagent. If trays are read before the nominal incubation time or more than 4 hours after, the data is rejected.

If the blank shows a value greater than the blank validation criteria value, then all of the results since the last in-range sample are rejected. This is summarized below:

	Validation Criteria	Validation Codes and Action	
Blank Sample	<1 cfu/100 mL	<1 cfu/100 mL, no code	≥1 cfu/100mL RB1, reject results

7.0 Related Forms

Bacteria Record Sheet
Bacteria Database Upload Form

8.0 Revision History

Revision 1 – 3/01/2013 – added information on the precautions one should take when working with potentially contaminated water and on the proper storage and disposal of used Quanti-Trays to be consistent with *SWQB’s Chemical Hygiene Plan*.

Original – 3/21/2011.

9.0 References

Oblinger, J.L. and J.A.Koburger (1975) Understanding and Teaching the Most Probable Number Technique. J. Milk Food Technol. 38(9): 540-545**.**

American Public Health Association (2005) Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association

Appendix B

AMIGOS BRAVOS
FIELD DATA SHEET AND CHAIN OF CUSTODY FORM

Site ID Code: _____ **Stream Name:_____**

Sampler Names:_____

Sample Date: _____ **Sample Time:_____**

General description of site and water Conditions:_____

Precipitation in the last 24 hours(check one):

___Storm(heavy rain >2.5 cm) cm) _____Steady rain (.85 cm to 2.4 cm)

___Showers(up to .85cm) _____Overcast _____Clear

Precipitation in the last week: _____

General Notes: _____

_____

Physical Characteristics Assessment (Check all that apply):

Water appearance:
_____Clear _____Cloudy _____Foamy ____Orange scum _____Milky/white
_____Muddy_____Dark brown

Water Conditions:
_____Normal _____Dry ____Flooded _____High _____Low _____Very low

Water Odors:
_____No unusual smell ____Chlorine____Fishy ____Sulfur(rotten eggs)

_____Sewage_____Moldy ____Musty ____Earthy Petroleum ____Other

Sediment:

_____Clear, gravel or sandy bottom _____Muddy, silty _____Algae coating bottom

_____Strands of filamentous algae

DATE_____ **SITE ID CODE**_____

PARAMETERS--Streamside Analysis

Temperature, degrees Ceslius _____

pH, units_____

Dissolved oxygen, mg/L or ppm_____

Electrical Conductivity, microsiemens/cm_____

Flow: Transfer Final Adjusted Stream Flow from "Calculating and Measuring Stream Flow" Data Sheet

_____

Laboratory Analysis Check off tests desired:

____ E. coli _____
___ Microbial Source Tracking (MST) _____

CHAIN OF CUSTODY

Important! Anytime samples change hands, they must be signed for below:

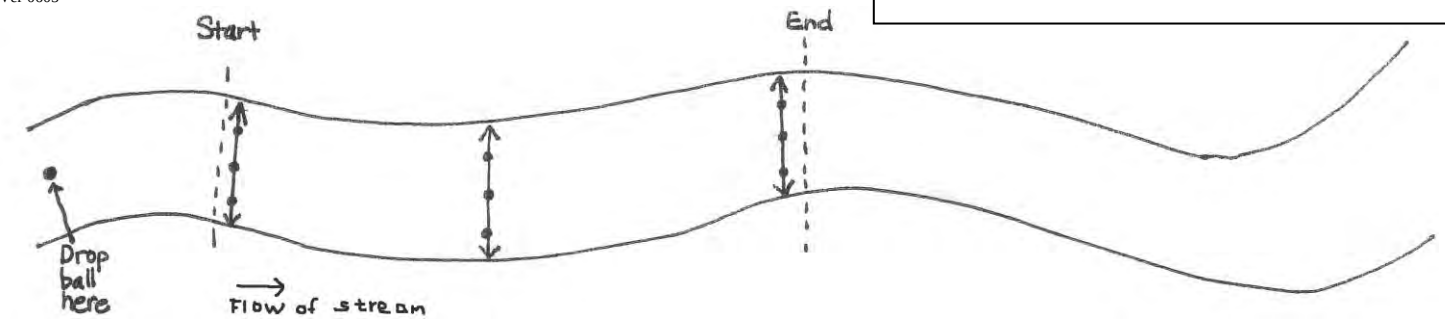
Sample ID Code:	Signature of Receiver:	Relinquished by:	Date and Time:
_____	_____	_____	_____
_____	_____	_____	_____

Appendix C

Date:
Location:
Recorder's name:

CALCULATING AND MEASURING STREAM FLOW

Ver 0605



Measuring

1. Find a fairly straight stretch of river where the water flows fast and the bottom is relatively flat. Measure a 3-meter section, marking where it begins and ends. Record this as the distance in column A.
2. Drop a ping pong ball in the center of the river at least one meter upstream of the starting point. When the ball reaches the start point, begin timing in seconds. When the ball reaches the end point, stop the timer. Record the amount of seconds in column B. Repeat this step two more times. (If the ball gets lodged in a rock or stuck in an eddy, begin the trial over.)
3. Measure the width of the river at the start point, at the end point, and at a point midway between the start and end. Record these measurements in column D. (See lines with arrows, above.)
4. Measure the depth of the river 3 times along each width line for a total of nine depth measurements. Record these measurements in column F. (See dots on lines with arrows, above.)

A	B	C	D	E	F			G
Distance (meters or feet)	Time (sec.)	Average Time (sec.)	Width of River at 3 points	Average Width of River	Depth of River at 9 points			Average Depth of River

Calculating

5. Calculate the averages for columns B, D and F and record the averages in columns C, E and G.
6. Now, divide the distance (column A) by the average time (column C) to obtain the velocity. Record this number in the equation below.
7. Multiply the average width (column E) by the average depth (column G) to obtain the area. Record this number in the equation below.
8. Multiply the velocity by the area to obtain the streamflow and record that in the equation below.

_____	x	_____	=	_____
velocity		area		streamflow

9. To calculate the final adjusted streamflow, you must multiply your result by a factor which takes into consideration the uneven surface bottom of your river or stream. For streams with rocky bottoms use a factor of 0.8, but for smooth bottomed streams, use a factor of 0.9.

_____	x	_____	=	_____
streamflow from		factor of 0.9 or 0.8		Final Adjusted Streamflow

Appendix D

4985 SW 74th Court, Miami, FL 33155 USA
Email: info@sourcemolecular.com
Tel: (1) 786-220-0379, Fax: (1) 786-513-2733

ISO 17025 Accredited Testing Laboratory
Certificate Number: 4126.01

Source Molecular Corporation QA/QC Summary

The QC requirements are summarized below. Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reportable. Analysis are repeated on test samples that do not meet QC requirements and if they do not pass a second time, they are flagged.

QC Requirements for MST qPCR

QC Item/Activity	**Data Quality Indicator and Purpose**	**Frequency**	**Measurement Performance Criteria**
Field blank	Accuracy/Bias - Evaluates contamination during sampling	As required by Client Provided by Client	No detection or detection at least 3 CT units above sample CT values (presence/absence tests)
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	One for every week samples are extracted	No detection or detection at least 3 CT units above sample CT values (presence/absence tests)
Sample duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed	± 1.0 standard deviation unless CT value ≥33
Diluted sample	Accuracy/Bias -Monitors for sample matrix inhibition affects	Every sample analyzed	CT value must be greater than that of un-diluted sample
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction per PCR plate, per test	Detection
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions per PCR plate, per test	No detection or detection at least 3 CT units above sample CT

Standard Curve	Accuracy/Bias, Comparability, Sensitivity -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range for accurate quantification	One curve in duplicate per PCR plate, per test if result is quantifiable and requires quantification	R2: ≥0.98 Efficiency: 80-110% Slope: -3.0 - -4.0 Sample unknown must be within the linear dynamic range limits

QC Requirements for MST ddPCR

QC Item/Activity	**Data Quality Indicator and Purpose**	**Frequency**	**Measurement Performance Criteria**
Field blank	Accuracy/Bias - Evaluates contamination during sampling	As required by Client Provided by Client	No detection
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	One for every week samples are extracted	No detection
Sample duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed	30% CV
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction per PCR plate, per test	Detection
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions per PCR plate, per test	No detection or insignificant detection in portion of replicates

Special Training/Certification

Individuals appointed to MST projects hold at minimum a Bachelor's degree and have a sound knowledge in genetics and molecular biology. Individuals must have had 1 year of previous hands-on qPCR experience at another laboratory. Trainees undergo supervised hands-on

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training by the Laboratory Manager, which typically lasts 1-6 months depending on experience. An initial demonstration of technical capability, including cross comparison trials, is required before personnel are permitted to work independently on client projects.

Handling of Test Samples

Source Molecular SOP's outline the procedures for the transportation, receipt, handling, protection, storage, retention and/or disposal of test items, including all provisions necessary to protect the integrity of the test item, and the interests of the laboratory and the customer. Samples, reagents, and standards are stored so as to ensure their integrity by preventing deterioration, contamination, and loss of identity. All policies are properly and accurately documented and adhered to be personnel.

Samples are unpacked immediately by personnel when they are received at the laboratory. Anyone coming into contact with samples must wear disposable gloves at all times. Personnel accepting the samples perform a series of checks and inspections to assure all necessary sampling and preservation requirements have been met, sample integrity has been maintained during transit, sample labels match samples written on the Chain of Custody sheet, all samples identified on the Chain of Custody are present in the cooler, and all necessary information has been supplied. Upon successful completion of this system of checks, samples are logged, handled and protected to avoid contamination.

Test samples are systematically identified as they arrive at the laboratory. The identification is retained throughout the life of the item in the laboratory. The system is designed and operated so as to ensure that items cannot be confused physically or when referred to in records or other documents. When abnormalities in sample condition are identified, the abnormality is recorded on the Chain of Custody sheet and the client is notified immediately via telephone and/or email for further instruction.

Instrument/Equipment Testing, Inspection, and Maintenance

Access to laboratory equipment is controlled to ensure that only authorized personnel use the equipment. Instructions on the use and maintenance of equipment are readily accessible by authorized personnel. Generally, the handling, transport, storage, use and maintenance of equipment are outlined in the manufacturer's manual. Manuals are located in the laboratory at all times. Specific requirements, if any, are outlined in the test method standard operating procedures.

Routine test work is completely discontinued on equipment that shows minor non-conformances. Not only do we do this for ethical reasons in support of our customer, but minor non-conformances are often indicative of major breakdowns in expensive equipment. These

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breakdowns need to be avoided wherever possible. Out of service equipment is clearly marked with an “out of service” label. All measurement and test equipment having an effect on the accuracy or validity of tests are calibrated and/or verified before being put into service. Calibration records for these equipment, including calibration dates and due dates, are maintained in the Source Molecular web-based storage system. Equipment may be calibrated internally or externally. External calibration services must be conducted by a calibration laboratory that demonstrates competence by being accredited and demonstrating measurement capability and traceability. The frequency of calibration depends on the accuracy requirements of the test, the stability of the instrument and manufacturer recommendations. It is crucial that calibration measurements are traceable to the International System of Units (SI) whenever possible.

Inspection/Acceptance of Supplies and Consumables

For all test methods, only services and supplies of the required quality and grade are used. If the specified reagent or material is discontinued by the manufacturer, an alternative from a different manufacturer may be purchased as long as the grade and specifications are identical to the discontinued item. The Laboratory Manager verifies and approves the alternate items and the change is made in the appropriate SOP.

Shipments are received at the receiving area and brought to the laboratory. The Laboratory Manager or other authorized personnel is responsible for checking shipments for accuracy. Packing slips are checked against package content labels and matched with the electronic order list. Certificates of analysis (COA) are verified (when applicable) to ensure the received item meets minimum specifications. All standards, reagents, filters, and other consumable supplies are purchased from manufacturers with performance guarantees and industry recognition, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality. Reagents are marked with the “date received”.

All supplies will be stored as per manufacturer labeling and discarded past expiration date. Nucleic acids shall be stored long term at 80˚C. Whenever possible, consumables and reagents that come into contact with test samples are received pre-sterilized and disposable (e.g. filtering funnels). They are used once and not reused. Specific information of supply and consumable vendors are specified in individual Test Method SOPs’ materials list.