**Quality Assurance Project Plan**
**for**
**San Juan Human Bacteria Sampling and Investigation**

Prepared by
San Juan Soil & Water Conservation District
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Prepared for
USEPA Region 6, Water Quality Protection Division
Dallas, TX 75202

## Group A. Project Management

### A1. Title and Approval Page

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Program Manager, MASS, SWQB

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Heidi Henderson
604(b) Program Coordinator, TMDL and Assessment Supervisor, SWQB

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Nelly Smith
Chief, State and Tribal Programs Section, WDAS, EPA Region 6

## A2. Table of Contents

## Acronyms

| | |
|---|---|
| AU | Assessment Unit |
| BMP | Best Management Practice |
| EPA | United States Environmental Protection Agency |
| LAWBP | Lower Animas Watershed Based Plan |
| LU | LuminUltra Technologies, LTD |
| MASS | Monitoring Assessment and Standards Section, Surface Water Quality Bureau |
| MST | Microbial Source Tracking |
| NMED | New Mexico Environment Department |
| PQAPP | Project Quality Assurance Project Plan |
| QA | Quality Assurance |
| QAO | Quality Assurance Officer |
| QAPP | Quality Assurance Project Plan |
| QC | Quality Control |
| qPCR | Quantitative Polymerase Chain Reaction |
| SJSWCD | San Juan Soil and Water Conservation District |
| SJWG | San Juan Watershed Group |
| SOP | Standard Operating Procedures |
| SWQB | New Mexico Environment Department Surface Water Quality Bureau |
| USGS | United States Geological Survey |
| WPS | Watershed Protection Section, Surface Water Quality Bureau |
| WQPD | Water Quality Protection Division |
| WWTP | Wastewater Treatment Plant or Water Reclamation Facility (used interchangeably here) |

**Introduction**

**The purpose of this Quality Assurance Project Plan (QAPP)** is to document the necessary quality assurance (QA), quality control (QC), and other technical activities that will be implemented to ensure that the results of the work performed for the *San Juan Human Bacteria Sampling and Investigation* project will satisfy the stated performance criteria. Additional tasks described within this document will also describe the acquisition of environmental data or information from direct measurement activities, existing data, or generated by models to be used throughout this study.

This QAPP will cover *E.coli* and *Bacteroides dorei* water sample collection efforts described in the workplan attached to NMED 604(b) sub-grant agreement 667-469-1. The project will analyze the extent of exceedances of state water quality standards for *E.coli,* compare these results to microbial source tracking (MST) data for *Bacteroides dorei* human fecal markers and analyze geographic trends in the data to make inferences on sources of bacteria pollution.

This QAPP is a companion document to the Bureau QAPP (Surface Water Quality Bureau Quality Assurance Project Plan for Water Quality Management Programs, NMED/SWQB 2018 or most current version). The Bureau's SOPs (Standard Operating Procedures) are incorporated in the Bureau QAPP by reference. Unless otherwise specified, all policies and procedures in the Bureau QAPP will be followed.

When changes affect the scope, implementation or assessment of the outcome, this QAPP will be revised to keep project information current. The SWQB 604(b) Project Coordinator, with the assistance of the QA Officer, will determine the impact of any changes on the technical and quality objectives of the project. This QA Project Plan will be reviewed annually by the SJSWCD Project Coordinator (Project Coordinator) to determine the need for revision.

A1 Title and Approval Page (EPA QA/R-5 A1) - See page 1.

A2 Table of Contents (EPA QA/R-5 A2) - See page 3.

## A3. Distribution List (EPA QA/R-5 A3)

Table 1. Distribution List and Project Roles and Responsibilities

| | | | | |
|---|---|---|---|---|
| Kristopher Barrios | SWQB | MASS Program Manager | Reviewing and approving QAPP | 505-946-8713 kristopher.barrios@state.nm.us |
| Miguel Montoya | SWQB | QA Officer | Reviewing and approving QAPP | 505-819-9882 miguel.montoya@state.nm.us |
| Heidi Henderson | SWQB | 604(b) Project Coordinator | Reviewing and approving QAPP; managing project personnel and resources | (505) 819-9986 heidi.henderson@state.nm.us |
| Kyla Chandler | EPA | Environmental Protection Specialist | Review and approve QAPP | (214) 665-2166 chandler.kyla@epa.gov |
| Nelly Smith | EPA | Chief, State and Tribal Programs Section | Review and approve QAPP | (214) 665-7109 Smith.nelly@epa.gov |
| Melissa May* | SJSWCD | Project Manager | Manage and oversee grant finances and contracts, training on proper sample technique, data analysis, verification and validation of data, and reporting | (505) 234-6040 x 1 melissa.may@sanjuanswcd.com |
| Alyssa Richmond* | SJSWCD | Project Coordinator, Lead Sampler | Prepare QAPP, collect data, data entry and analysis, oversee volunteer training, prepare final report | (505) 234-6040 x 3 alyssa.richmond@sanjuanswcd.com |
| Nicholas King | Jacobs | Laboratory Lead | Analyze and report *E.coli* data | (505) 634-8699 Nicholas.King@jacobs.com |
| James Herrin | LuminUltra | Laboratory Lead | Analyze and report MST data | (786) 220-4651 jherrin@luminultra.com |

The persons listed above will receive a copy of the QAPP. The Project Coordinator will require those marked with an asterisk to read this QAPP and sign the QAPP Acknowledgement Statement (Appendix A).

## A4. Project Task/Organization

Grant/Project Manager: Melissa May has served as the District Manager for the San Juan Soil & Water Conservation District since 2016, was the lead sampler for the 2013-2014 Microbial Source Tracking Study, and was the lead author of the Lower Animas Watershed Based Plan. She holds a BS in Environmental Resource Management and an MS in wildlife and fisheries science from Penn State University's Watershed Stewardship program. Ms. May will be responsible for all contractual and financial elements of the project, ensure that the

project budget is adhered to, train other staff and volunteers on proper sampling techniques, and work closely with the Project Coordinator on data analysis and all components of the project discussed above. She will communicate with the SWQB Project Coordinator on work accomplished in this plan and any problems or deviations that need to be resolved and/or approved.

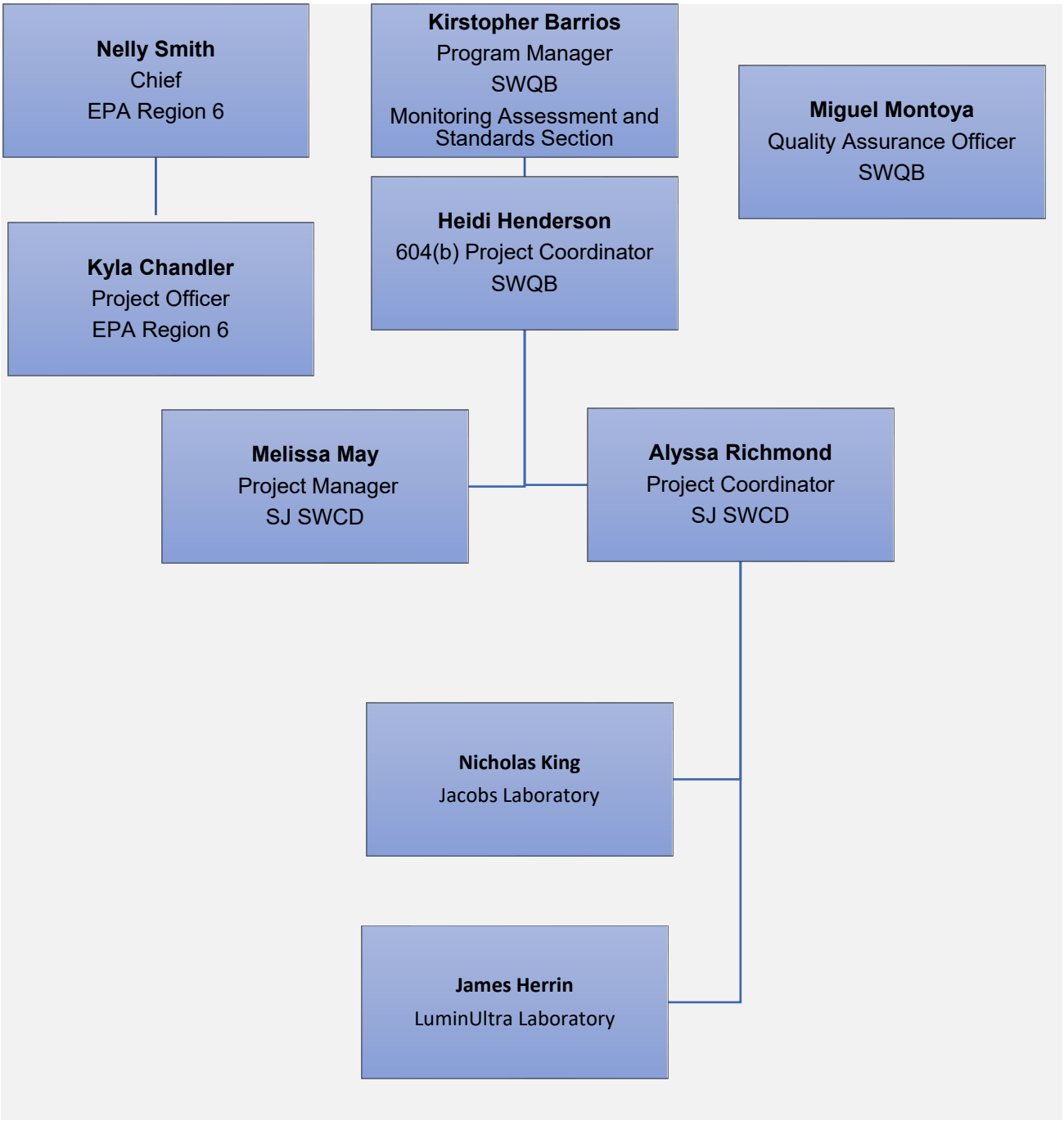
Project Coordinator and Sampling Lead: Alyssa Richmond has served as the Coordinator of the San Juan Watershed Group and employee of the San Juan Soil & Water Conservation District since 2019. She has participated in multiple water quality sampling events through her work with the National Park Service and has successfully implemented two 319(h) Best Management Practice (BMP) grants following the Lower Animas Watershed Based Plan and a BOR WaterSMART San Juan Watershed Planning grant. Ms. Richmond will serve as the lead sampler for this study and will be responsible for ensuring sample collection follows time schedule and standard operating procedures, coordinate with participating laboratory contractors, coordinate stakeholder engagement and outreach meetings, oversee volunteer training and data entry with assistance from Ms. May, and compile the final report. She will ensure data results reported by Jacobs Laboratory are provided in both portable document format (pdf.) and Microsoft Excel (or equivalent).

Laboratory and Data Analysis Leads:

James Herrin will be the contact person and responsible for assigning appropriate laboratory staff from LuminUltra (Miami, FL) to perform the MST qPCR analysis for human fecal markers specified in this plan. He will assist with lab data analysis.

Nicholas King will be the contact person for Jacobs laboratory in Farmington, NM and will oversee all analysis and laboratory QA/QC for *E.coli* quantification analysis.

Figure 1. Organization Chart



## A5. Problem Definition/Background

Bacteria pollution is an ongoing concern within the San Juan Watershed and poses serious health risks for communities along the San Juan River and its tributaries. The stretch of the San Juan River from the Navajo Nation Boundary at the Hogback to the Animas River Confluence (NM-2401_10) has been on the impaired 303(d) list for *E.coli* since 2005, with the stretch from the confluence of the Animas River to Canon Largo (NM-2401_00) listed as impaired for *E.coli* since 2006. Total Maximum Daily Loads (TMDLs) were established for these Assessment Units (AU) in 2005. The San Juan River from the Animas River Confluence to the Canyon Largo Confluence (NM-2401_00) was delisted in the most recent assessment cycle, but maintains its *E.coli* load reduction goals as outlined in the TMDL. As of the 2020-2022 CWA Section 305(b)/303(d) Impairment Listing by NMED SWQB, additional AUs in the Upper and Middle San Juan Basin are impaired for *E.coli*, including Gallegos Canyon (NM-9000.A_060), La Plata River (NM-2402.A_01 and NM-2402.A_00), Shumway Arroyo (NM-9000.A_021), and Stevens Arroyo (NM-2401_11).

Table 1.5 - Assessment Unit and TMDL Determinations for the Upper and Middle San Juan Basin

| Assessment Unit | Previous Impairments (2004-2016) | 2020 Impairments | TMDL Complete |
|---|---|---|---|
| San Juan River Animas River to Canon Largo (NM-2401_00) | *E.coli* | | Yes |
| | | Sedimentation/Siltation | Yes |
| | Turbidity | | No |
| San Juan River Navajo Bdn at Hogback to Animas River (NM-2401_10) | *E.coli* | *E.coli* | Yes |
| | | Sedimentation/Siltation | No |
| | Turbidity | | No |
| Gallegos Canyon - San Juan River to Navajo Bdn (NM-9000.A_060) | | *E.coli* | No |
| | Selenium (Total Recoverable) | Selenium (Total Recoverable) | Yes |
| | | Temperature | No |
| Animas River - San Juan River to Estes Arroyo (NM-2403.A_00) | *E.coli* | | Yes |
| | Sedimentation/Siltation | | No |
| | Total Phosphorus | | Yes |
| | Total Nitrogen | | Yes |
| | Turbidity | | No |
| | | Temperature | Yes |
| La Plata River - San Juan River to McDermott Arroyo (NM-2402.A_00) | *E.coli* | *E.coli* | Yes |
| | Dissolved Oxygen | Dissolved Oxygen | No |
| | Sedimentation/Siltation | Sedimentation/Siltation | Yes |
| Stevens Arroyo - Perennial Prts San Juan River to Headwaters (NM-2401_11) | | *E.coli* | No |
| Shumway Arroyo - San Juan River to Ute Mtn Ute Bdn (NM-9000.A_021) | | *E.coli* | No |

In coordination with the New Mexico Environment Department (NMED) and a previous 604(b) project, the San Juan Watershed Group (SJWG) and the San Juan Soil & Water Conservation District (SJSWCD) conducted a Microbial Source Tracking (MST) study in 2013-2014 to assist NMED in impairment determinations, quantify *E.coli* and nutrient concentrations, and identify animal sources of bacteria at 5 sites along the Animas and San Juan Rivers. MST bacteria sampling is an alternative bacterial sampling methodology that allows bacteria from a water sample to be traced back to its host organism using molecular qPCR analysis. Many bacterial populations have become adapted to the unique environment of their animal 'host' species and differ genetically from bacteria in different 'host' species. A human-source specific DNA marker for *Bacteroides dorei* has been confirmed through comprehensive, multi-laboratory MST evaluation studies to be the most accurate for quantification based on sensitivity and specificity in comparison to other bacterial species tested. Comparing this with *E.coli* concentrations allows a comparison of source-specific data with Clean Water Act water quality criteria. The 2013-2014 study found that 46% of San Juan River *E.coli* samples exceeded the single sample standard for primary contact. Primary Contact means any recreational or other water use in which there is prolonged and intimate human contact with the water, such as swimming and water skiing, involving considerable risk of ingesting water in quantities sufficient to pose a significant health hazard. Primary contact also means any use of surface waters of the state for cultural, religious or ceremonial purposes in which there is intimate human contact with the water, including but not limited to ingestion or immersion, that could pose a significant health hazard.

Through concurrent testing for presence/absence of various source categories of bacteria, the MST study also found that 94% of samples tested positive for human source bacteria and 90% positive for ruminant source bacteria. The majority (79%) of all samples were quantifiable for human source bacteria and were analyzed for magnitude of concentrations, revealing an order of magnitude increase in human source bacteria on the San Juan River between Farmington and the Hogback (Navajo Nation jurisdictional boundary). While ruminant source bacteria was expected due to livestock production in the river corridor (cattle, sheep) and the presence of wildlife (deer, elk), the near-constant presence of human sewage in the river was unexpected and alarming. Regional stakeholders have identified potential sources of human fecal bacteria to include failing or improperly installed septic systems, illegal dumping of septage waste (by RVs or waste disposal companies), leaking sewer infrastructure, legal (permitted) discharges from wastewater treatment facilities, and/or outdoor defecation. While the MST study provided a baseline for determining the presence of human fecal bacteria, the low number of sampling sites left critical data gaps is identifying potential "hotspots" that contribute to these elevated concentrations of human bacteria within the San Juan Watershed. Further sampling adapting the inverse approach (i.e. a higher density of sampling sites and fewer sampling events) is required to better characterize the geographic distribution of potential nonpoint and point sources of human fecal bacteria, prioritize short and long term water quality improvement and outreach strategies, and implement solutions on a watershed scale. Since the MST study was designed under the assumption that treated wastewater from permitted point source discharges from Waste Water Treatment Plants (WWTPs) would not lead to detections of human source bacteria, this is an additional data gap that needs to be better characterized. While these facilities operate in accordance with their National Pollution Discharge Elimination System (NPDES) permits, the SJWG and SJSWCD identified the need to conduct additional targeted MST monitoring to determine what portion (if any) of human fecal bacteria detected in the river could be coming from permitted discharges. While culture dependent *E.coli* samples measure the quantity of surviving *E.coli* bacteria that grow and reproduce within the sample, the cultureless qPCR method used in MST detects and quantifies the presence of host specific DNA markers, which can theoretically include both living and recently dead bacteria cells. Hence the importance of quantifying whether successfully treated wastewater still has a detectable signal.

Given these data gaps and the water quality monitoring conducted by NMED SWQB, the goals for this project are as follows:

1. Follow NMED SWQB sampling protocols to provide additional *E.coli* data to assist in evaluating 303(d) impairment listings and TMDL goals.
2. Sample surface water at sites of interest for analysis of both *E.coli* and *Bacteroides dorei* human fecal marker that bracket potential human bacteria sources, especially in reaches where high human bacteria concentrations were detected in past studies.
3. Increased public knowledge of human fecal bacteria sources, extent, relationships, and solutions.
4. Utilize this data in the San Juan Restoration Plan to effectively prioritize mitigation measures to remediate sources of human bacteria along the San Juan River and its tributaries.

## A6. Project/Task Description

**Project Description:** To meet the goals of the San Juan Human Bacteria Sampling and Investigation Project, as provided in Section A5, the SJWG and SJSWCD will lead the sampling effort to monitor 17 sites (see Table 1.6.1 and Figure 1.6) on the San Juan River, Animas River, La Plata River, Stevens Arroyo, and Shumway Arroyo. Water samples will be collected and analyzed for *E.coli* and MST human fecal marker and will be collected concurrently once a month for three months between July and September (refer to Table 1.6.2) at all 17 sites. An additional sampling date in October will be conducted and the sample will only be analyzed for *E.coli*. E.*coli* IDEXX quantification will be completed by Jacobs Laboratory in Farmington, while *Bacteroides dorei* qPCR analysis will be conducted through LuminUltra Technologies, LTD. in Miami, Florida. Samples at six sites will be collected via raft through a local river guide company, while the remaining nine sites will be collected from the bank at sites accessed via vehicle travel and two samples are collected from WWTP outflows. All sampling locations were selected with expertise from federal, state, nonprofit, and local stakeholders throughout the watershed to bracket potential sources, including WWTPs, housing developments with known failing/improperly installed septic tanks, and tributaries with *E.coli* impairment determinations. Further details on the methods used for designing the study is provided in Section B1.

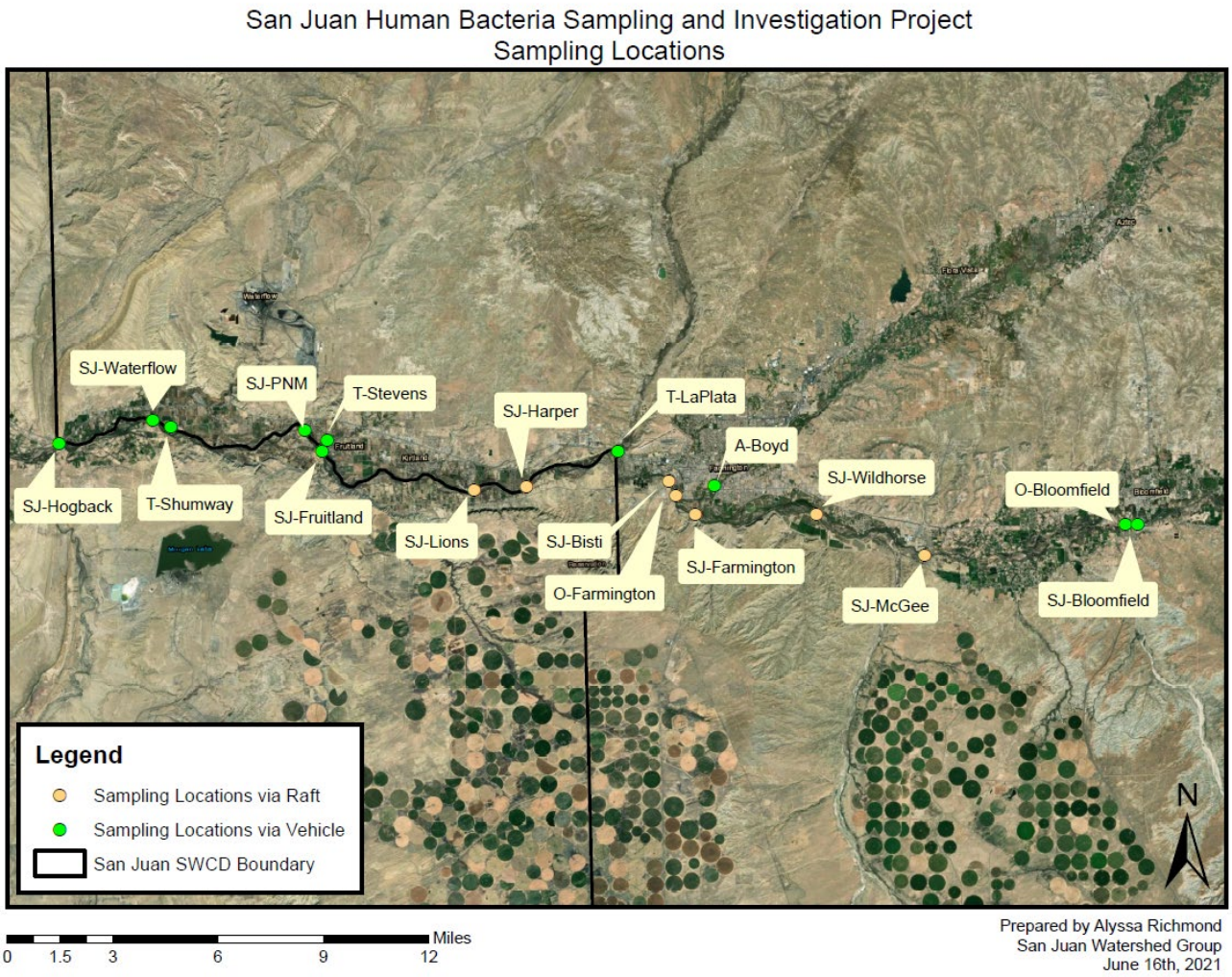
Sampling location ID's and coordinates. All sites highlighted in yellow will be collected via raft. Remaining sites indicated in white will be accessed via vehicle travel.

**Table 1.6.2 Sample Locations**

| Sample Site # | **Assessment Unit** | Sample ID | Sample Rationale | Latitude (Decimal Deg.) | Longitude (Decimal Deg.) |
|---|---|---|---|---|---|
| 1 | NM-2401_00 | SJ-Bloomfield | Upstream of permitted discharge | 36.69968796 | -107.9861755 |
| 2 | NM-2401_00 | O-Bloomflield | Point discharge | 36.6999054 | -107.992218 |
| 3 | NM-2401_00 | SJ-McGee | Upstream of residential, Downstream of permitted discharge | 36.68959808 | -108.0957336 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | NM-2401_00 | SJ-WildHorse | Upstream and downstream of residential areas | 36.70754242 | -108.1505737 |
| 5 | NM-2401_00 | SJ-Farmington | Long term sampling site, Upstream of NM2403.A_00 | 36.70911407 | -108.2126617 |
| 6 | NM-2402.A_00 | A-Boyd | Long term sampling site, Previous 303(d) *E.coli* Listing for NM2403.A_00 | 36.72075272 | -108.202446 |
| 7 | NM-2401_10 | O-Farmington | Point discharge | 36.71711731 | -108.2223206 |
| 8 | NM-2401_10 | SJ-Bisti | Downstream of point discharge and NM2403.A_00, Upstream of NM-2402.A00 | 36.72299957 | -108.2256088 |
| 9 | NM-2402.A_00 | T-LaPlata | 303(d) *E.coli* Listing for NM-2402.A_00 | 36.7363739 | -108.2513046 |
| 10 | NM-2401_10 | SJ-Harper | Long term sampling site, downstream of NM-2402.A_00 and NM2403.A_00. Upstream of decommissioned Harper Valley WWTP | 36.72254181 | -108.2989273 |
| 11 | NM-2401_10 | SJ-Lions | Upstream of residential, Downstream of former Harper Valley WWTP | 36.7220459 | -108.3256531 |
| 12 | NM-2401_10 | SJ-Fruitland | Downstream of residential | 36.73980331 | -108.4029999 |
| 13 | NM-2401_11 | T-Stevens | 303(d) *E.coli* listing of NM-2401_11 | 36.74411392 | -108.4003143 |
| 14 | NM-2401_10 | SJ-PNM | Upstream of residential | 36.74819946 | -108.4116287 |
| 15 | NM-9000.A_021 | T-Shumway | 303(d) *E.coli* Listing of NM-9000.A_021 | 36.75128555 | -108.4801636 |
| 16 | NM-2401_10 | SJ-Waterflow | Downstream of NM-9000.A_021, Downstream of residential | 36.7543335 | -108.4892807 |
| 17 | NM-2401_10 | SJ-Hogback | Long term sampling site, downstream of residential | 36.74546432 | -108.5378494 |

**Figure 1.6 Sampling Location Map**




Day 1 Sampling Locations Each Month: SJ-Bloomfield, O-Bloomfield, SJ-McGee, SJ-Wildhorse, SJ-Farmington, O-Farmington, SJ-Bisti, SJ-Harper, SJ-Lions
Day 2 Sampling Locations Each Month: A-Boyd, T-LaPlata, SJ-Fruitland, T-Stevens, SJ-PNM, T-Shumway, SJ-Waterflow, SJ-Hogback

Other data not directly generated under this funded project but intended to be used in conjunction with collected data include flow data obtained from USGS gaging stations to evaluate correlation between flow and bacteria concentrations (See Non-direct Measurements Section of this QAPP). Additional data to be collected by the SJWG and SJWCD during sampling will include optical brighteners and turbidity measurements ascertained through a field fluorometer. This data collection may be limited by available field equipment and is intended for internal use as ancillary data by SJWG and SJSWCD; it will not be included in the data provided to NMED SWQB and will not be used in final report to correlate or drawn conclusion from *E. coli* or MST data collected under this project. Optical brighteners and turbidity measurements will be collected by volunteers for educational purposes only, to encourage public participation, and will not be funded under the current 604(b) grant.

Data collected from this study will be compiled to provide a final report, an *E.coli* database configured for 303(d) analysis by NMED, and made publicly accessible through select online databases. The final report will include *E.coli* standard exceedances, statistical interpretations of the data, comparison and analysis of trends between bacteria sample methods, and evaluation of geographic and temporal variation. Analysis of likely bacteria source activities will be discussed, along with recommendations on outreach and BMPs to further assess for effectiveness of controlling and/or reducing source activities. Results from this study will also be used in conjunction with data from past synoptic studies to inform the San Juan Restoration Plan.

**Project Schedule**

Bacteria sampling will be conducted monthly from July through October 2021, with each sampling "event" taking place over a span of two sampling days. Due to budget constraints, one of the sampling events (i.e. October) will collect samples for *E.coli* only and not *Bacteroides dorei*; the three paired sampling events are planned for the summer months (i.e., July, August, September) when river recreation (and thus risk of primary contact with river water containing bacteria) is more likely. In the event sampling needs to deviate from the proposed schedule (i.e. inclement weather, laboratory availability constraints, etc.), sampling days will be rescheduled to ensure that at least 68 samples are analyzed for *E.coli* and 51 for *Bacteroides dorei* human fecal marker.

Samples being analyzed for bacteria (*E. coli*) will be delivered to the lab within 22 hours after collection, with *E.coli* samples dropped off in person to be analyzed at Jacobs Laboratory in Farmington. *Bacteroides dorei* human fecal marker samples will be shipped overnight on ice to LU in Miami, Florida.

Data analysis will be conducted by SJWG and SJSWCD staff (e.g., Project Manager and Project Coordinator) from October to December and submitted to NMED for review in January 2022. Data from this project and legacy bacteria data collected from the 2013-2014 MST study and other SJWG monitoring projects will be made available through online databases for public accessibility.

Table 1.6.2 – Sampling Schedule for San Juan Human Bacteria Sampling and Investigation

*E.coli* bacteria and *Bacteroides dorei* bacteria human fecal marker 2021 monitoring will be conducted on the following schedule.

**Table 1.6.2 Sampling Schedule**

| Month | Date | qPCR Samples[1] (per day) | *E.coli* Samples[1] (per day) |
|---|---|---|---|
| July | 28 | 10 | 10 |
| July | 29 | 9 | 9 |
| August | 25 | 10 | 10 |
| August | 26 | 9 | 9 |
| September | 29 | 10 | 10 |
| September | 30 | 9 | 9 |
| October | 27 | 0 | 10 |

| | | | |
|---|---|---|---|
| October | 28 | 0 | 9 |
| TOTAL | | 57 | 76 |

[1]Includes one blank per sampling trip (batch)

## A7. Quality Objectives and Criteria for Measurement Data

**Objectives and Project Decisions:** The baseline data collection of the San Juan Human Bacteria Sampling and Investigation Project, in extension of the 2013-2014 MST Study, is intended to answer the following questions:

1. Are the AUs within the project reach meeting water quality standards for *E.coli*? Do the data support recent impairment listings/delistings?
2. Do geographic trends in *E.coli* and *Bacteroides dorei* concentrations point to any potential human fecal bacteria source "hotspots" in the project reach?
3. Is there detectable *Bacteroides dorei* signal coming from permitted WWTP discharges?
4. From this information, what are the most effective projects and outreach strategies that could reduce bacteria loads?

To answer these questions, *E.coli* concentrations and quantification of *Bacteroides dorei* human fecal marker will be compared among and between sites, and will be evaluated against flow data and relevant water quality criteria. Moreover, sites have been selected to bracket reaches with hypothesized sources identified by multijurisdictional stakeholders throughout the watershed, to ultimately inform land managers and said stakeholders for future restoration efforts. This information will be compiled into a Needs Based San Juan Restoration Plan for the New Mexico stretch of the San Juan Basin that addresses this analysis and recommendations, while incorporating other aspects of restoration to prioritize, implement, and monitor water quality improvement methods.

**Action Limits/Levels:** Quantitation limits are the minimum concentrations that can be identified and quantified above the detection limit within some known limits of precision and accuracy/bias. It is recommended that the quantitation limit is supported by the analysis of a standard of equivalent concentration (typically, the lowest calibration standard). Detection limits are the minimum concentration that can be detected above background or baseline/signal noise of an instrument.

The quality of the data following Jacob Laboratory's FARLAB-007 *E.coli* IDEXX Quantification SOP and LuminUltra Technology's 2021 Quality Assurance Manual will be adequate to provide a high level of confidence for development of reach-specific and impairment-specific restoration measures, and to be used in impairment assessments by SWQB staff.

**Table1.7.1 Analytical Parameters and Target Limits**

| Analytical Parameter | Laboratory Limits[1] (applicable units) | |
|---|---|---|
| | **Quantification Limits** | **Detection Limits** |
| *E.coli* | 1.0 cfu/100mL | 1.0 cfu/100mL |
| MST | 10 copies/reaction | 3 copies/reaction |

[1]Laboratory quantitation limits and detection limits are those that an individual laboratory or organization is able to achieve for a given analysis on a routine basis.

**Measurement Performance Criteria/Acceptance Criteria:** The measurement quality indicators discussed in Table 1.7.2 will be sufficient to achieve the Data Quality Objectives and will be in conformance with those listed in the Bureau QAPP and Bacteriological Sampling and Analysis SOP 9.1.

Sampling locations, methods, and analytical methods (discussed more in Section B4) have been designed to match the sample procedures used during the 2013-2014 MST Study to ensure comparability and representativeness.

**Table 1.7.2 Data Quality Indicators**

| Data Quality Indicator | Data Acquisition |
|---|---|
| Precision | Precision will be ensured by consistently assigning the same people the responsibilities of collecting, recording and analyzing data. If resources allow replicate field data may be obtained. |
| Accuracy | Accuracy will be determined by the effective use of methods provided in this QAPP and referenced SOPs. |
| Bias | Bias will be reduced by using professional and experienced staff to collect and analyze data. |
| Representativeness | Sample location distribution is representative of the entire project area. |
| Comparability | Sampling locations and methods from the 2013-2014 MST Study were included in this project to ensure comparability, and new sites were co-located with other agencies' sampling locations where feasible. Methods for data collection and analysis have been selected to be standardized and reproducible. |
| Completeness | Sampling will be conducted as described in this QAPP. If sampling frequency deviates from schedule the SWQB 604(b) Project Coordinator will be notified to determine effects on the project. |

## A8. Special Training Requirements/Certification

This project will be implemented by contractors (lab analysis), SJSWCD staff (data analysis), SJWG volunteers (ancillary data collection), and SWQB (data analysis and report review). The lead sampler for SJSWCD will be Alyssa Richmond, San Juan Watershed Group Coordinator, with guidance and assistance from Melissa May, M.S., and will be responsible for data collection and for following the requirements of the Bureau QAPP and the methodologies outlined in this QAPP.

Ms. May received her M.S. in Wildlife and Fisheries Science from Pennsylvania State University in 2011 and completed her master's thesis on the assessment of nutrient pollution using periphyton indicators in freshwater streams. Ms. May has over five years' experience with sample collection, lab analysis, and data analysis through her positions as research assistant in the Penn State Aquatic Ecology Lab (2008-2011), water quality technician at the Prince William Sound Science Center (summer 2011), and lead sampler for the MST Study (2013-2014).

Ms. Richmond received her B.S. in Environmental Science with minors in Sustainability Studies and Geospatial Analysis from Davis & Elkins College in 2017. She has participated in multiple water quality sampling projects through her work with the National Park Service at New River Gorge National River and designed data collection and database management methods for the 2019-2021 Groundwater Spring Inventory at Chaco Culture National Historical Park.

With Ms. Richmond and Ms. May as lead samplers, a briefing on sampling methods will be conducted by the applicable lead sampler at the beginning of each sampling day. Volunteers participating in sampling event will be required to sign a Volunteer Protocol and Consent Form that indicates they have received, read, and understand sampling protocols and their role in the sampling effort. Sample collection crews will be composed of one SJSWCD lead sampler and up to four volunteers. Use of volunteers will be in accordance with this QAPP, *E. coli* and MST samples will not be collected by volunteers, all volunteers will be directly supervised by lead samplers. Volunteer participation is for educational purposes and to encourage public participation in work conducted in the watershed. Volunteers will only conduct data collection for ancillary data (optical brighteners and turbidity measurements). No further specialized training is required.

## A9. Documents and Records

The Project Coordinator will make copies of this QAPP and any subsequent revisions available to all individuals on the distribution list and volunteers via email communication.

Project and report documents will include this QAPP, field sampling forms (collected via both electronic devices and on paper), lab chain of custody forms for all samples, downloaded USGS sonde/gauge data, an Excel database of analytical data in electronic form, and final report.

All documentation will be maintained in a consolidated virtual database managed and backed up via Google Drive by the SJSWCD and SJWG, with paper records stored at the SJSWCD office. All data collected will be stored and utilized internally by the SJSWCD and SJWG indefinitely. Volunteer participation records and signed forms will be filed and stored at the SJSWCD office. Documentation and data directly correlated with *E.coli* samples will be compiled in a NMED approved Bacteria Database for review, analysis, and utilization in impairment determinations. Data, data analysis, and final report will be transferred to the NMED SWQB and will be

maintained in accordance with the requirements of the Bureau QAPP. The data, data analysis, and final report will be stored on the SWQB network drive in accordance with 1.21.2 NMAC, Retention and Disposition of Public Records (see table A9.1)

**Table A9. Reporting Format and Storage**

| Monitoring Technique | Reporting Format | Storage Location And Time | Field Sheet Used |
|---|---|---|---|
| Direct measurements: | | | |
| MST: *Bacteroides dorei* Human fecal Marker qPCR | Chain of custody sheets, Excel spreadsheet of results from LuminUltra | Paper copies in project file, electronic copies on hard drive and Google Drive at LuminUltra and SJSWCD. | Chain of Custody form and Field Notebook |
| *E.coli* IDEXX Quantification | Chain of custody sheets, Excel spreadsheet of results from Jacobs Lab. | Paper copies in project file, electronic copies on hard drive and Google Drive at Jacobs Lab, SWQB, and SJSWCD. | Chain of Custody form |
| Non-direct measurements: | | | |
| USGS stream gages – records of instantaneous flow | Flows downloaded from online flow gauge, transferred to Excel spreadsheets, double checked later when final data released | Electronic copies on hard drive and Google Drive at SWQB and SJSWCD. | Not applicable |
| WWTP Outflow *E.coli* Quantification | Excel spreadsheet of results from Farmington WWTP and Bloomfield WWTP | Electronic copies on hard drive and Google Drive at SWQB and SJSWCD. | No Applicable |

**Table A9.1. SWQB Document Storage Location**

| Document | Type of Form | Storage Location | Field Sheet Used |
|---|---|---|---|
| **QAPP** | Electronic (.doc) | SWQB File depot QAQC Documents Folder | EPA Requirements for Quality Assurance Project Plan. EPA QA/R-5. |
| **Project Data, Data analysis, and Final Report** | Electronic (.doc) & Hard Copy | SWQB File Depot Project Folder | NA |

All field and laboratory data will be collated in an Excel spreadsheet and will be submitted as an Appendix to the final report.

## Group B. Data Generation and Acquisition

### B1. Sampling Design (Experimental Design)

As discussed in Section A7, the project was designed to answer the following questions:

1. Are the AUs within the project reach meeting water quality standards for *E.coli*? Do the data support recent impairment listings/delistings?
2. Do geographic trends in *E.coli* and *Bacteroides dorei* concentrations point to any potential human fecal bacteria source "hotspots" in the project reach?
3. Is there detectable *Bacteroides dorei* signal coming from permitted WWTP discharges?
4. From this information, what are the most effective projects and outreach strategies that could reduce bacteria loads?

Stakeholder coordination by the SJWG and SJSWCD has continued since the results of the 2013-2014 MST Study to identify sampling locations that will yield the most effective information to answer these questions. At the same time, sampling locations were selected for accessibility and to mirror existing sampling locations utilized by other agencies, including the previous SJWG and SJSWCD water quality sampling locations, NMED SWQB Water Quality Stations, Navajo Nation EPA Water Quality Program, and 2021 USGS Sediment and Source Metal Tracking sampling locations. New sampling locations were determined through relationships with local landowners and WWTP facilities. Before the lead sampler (Project Coordinator) guides volunteers into the field for sample collection, land access permission from private landowners will be acquired and all sampling locations will be visited via vehicle or raft travel as deemed appropriate by the lead samplers. See Figure 1.6 for a map of sampling locations.

*E.coli* and MST human fecal marker samples will be collected concurrently once a month for three months between July and September (refer to Table 1.6.2 and Figure 1.6) at all 17 sites. An additional sampling date for only *E.coli* analysis will be collected in October. Samples will be either hand-delivered or shipped overnight to their respective lab for analysis to meet the 24-hour max holding time. This sampling design was determined with the following criteria in mind: (1) sampling months were chosen to reflect times of high river recreation and higher likelihood of water ingestion, (2) once per month to balance SJSWCD staff sampling and lab analysis capacity given the high number of sites, (3) late summer and fall sampling months to increase chances that a sampling event could capture changes in river flows caused by stormwater events, and (4) *E.coli* samples were prioritized for an additional sampling event to increase the amount of data that will be usable for NMED SWQB impairment assessments.

If sampling locations cannot be accessed and/or sampling cannot be conducted on the dates depicted in this QAPP, due diligence by the Project Coordinator will be done to ensure an alternative sampling site is located (based on approval by the 604b Project Coordinator) and/or alternative sampling days are conducted.

### B2. Sampling Methods

The procedures provided below will be followed for all sampling events and locations included in this study. The Project Coordinator will be responsible to ensure that all sampling procedures are followed. Any corrective action taken during sampling will be documented on the paper and/or electronic field forms.

Water Sample Collection:
Jacobs laboratory will provide a one-time use sampling vessel (500mL sterile bottle) for each sampling station and event. Approximately 250mL of sample water will be decanted from the one-time use sampling vessel, at sample location, to each individual sterile sample containers for E.coli and MST. 250mL is more than adequate for laboratory testing purposes.

When sampling from the bank samples will be collected using the one-time use sampling vessel (500 mL sterile bottle), provided by Jacobs laboratory, and will be attached to the sampling pole extended to ~10 ft to capture a sample in a steady flowing run of the river; Samplers will avoid pools or eddies, and excessive sediment/debris. During sampling, the sampling vessel will be aimed upstream and held ~3 inches below the water surface until filled. Surface scums will be avoided during sample collection.

***E.coli* Procedure:** Most up to date NMED SWQB Bacteriological Sampling and Analysis SOP 9.1 (https://www.env.nm.gov/surface-water-quality/sop/)
Sanitize hands using hand sanitizer before handling sampling bottles, and between each sampling site.
Using the 250mL sample bottles for *E. coli* provided by Jacobs Lab, label the bottle, record additional information in printed/virtual field form, and chain of custody sheet.

SJ/A/T/O-Site Name
2021-06-28 (YYYY-MM-DD)
13:45 (military time)
M.May (sampler's first initial and last name)

Decant 250mL of sample water into the sterile labeled *E. coli* bottle to the marked shoulder. Cap when filled. During transit, samples must be held at less than 10 $^{o}$C. Samples must be delivered to Jacobs Lab by 3pm on day of sampling for analysis to be conducted within the 24-hour max holding time.

**MST Human DNA Marker Procedure:** LuminUltra 2021 MST SOP
Sanitize hands using hand sanitizer before handling sampling bottles, and between each sampling site.
Label the 250mL collection bottle provided by LuminUltra, record additional information in the either print or virtual field form, and chain of custody sheet

SJ/A/T/O-Site Name
2021-06-28 (YYYY-MM-DD)
08:45 (military time)
M.May (sampler's first initial and last name)

Decant 250mL of sample water into the labeled LuminUltra bottle. If contamination occurs (touching the threads of the bottle, etc), get a new bottle. Replace cap (being careful not to touch threads) and seal sample bottle in individual Whirlpak or Ziploc bag for shipment. Keep sample out of sun and store immediately in cooler on ice. Pack sample bottles and ice packs in shipping container with chain of custody sheet, ship overnight to LuminUltra Technologies, LTD. in Miami, FL.

WWTP Outflow Samples:

MST samples collected from the WWTP outflows in Farmington and Bloomfield will be collected as duplicate samples alongside the WWTP's regular *E.coli* samples collected as part of their NPDES permit required monitoring (covered under their separate QAPP/SOP). WWTP staff will be provided with a sterile, labeled MST sample bottle to fill in accordance with instructions above on the sampling day. Sample will be kept on ice and picked up by the sampling team and shipped alongside river water samples. WWTP staff will provide a copy of *E.coli* sample chain of custody, sample collection time, and analysis results to SJSWCD to analyze alongside MST results. (Note: in the event WWTP staff are not able to collect/analyze outflow samples, SJSWCD sampling team will collect outflow sample in accordance with all sampling instructions above).

## B3. Sample Handling and Custody

**Table 2.3 Analytical Method, Containers, Preservation, and Holding Times Requirements**

Matrix/Media:

| Analytical Parameter | Analytical Method Number | Containers ( Size/volume) | Preservation Requirements (chemical, temperature, light protection) | Maximum Holding Times[1] |
|---|---|---|---|---|
| *E.coli* | FARLAB_007 Rev. 7 (Attachment B) | 250mL | Ice, dark cooler | 24 hrs to lab |
| MST | LuminaUlta Technologies, Ltd.* | 250mL | Ice, dark cooler | 24 hrs to lab |

[1] Maximum holding times include all pertinent holding times for each analytical parameter (e.g., from sample collection to sample preparation, from sample preparation to analysis, from sample collection to analysis, etc.).
* See *Sample Filtration, Extraction and Human DNA Marker Bacteroides Dorei qPCR Procedures* (Attachment C)

## B4. Analytical Methods

No field measurements or analyses will be required for completion of this study.

Laboratory Methods

***E.coli* IDEXX Quantification:** All *E.coli* samples will be analyzed via the IDEXX QuantiTray method at Jacobs Lab in Farmington, NM (FARLAB-007 SOP, Revision No. 7, Attachment B).

**MST Human Fecal Marker Analysis:** qPCR of human fecal marker for *Bacteroides dorei* for all 57 samples will be

conducted by LU via their 2021 qPCR Methods and Quality Control Plan (Attachment C). The human-associated qPCR tests targeted DNA from anaerobic bacteria frequently shed from the gastrointestinal tract with human feces worldwide and is widely distributed in the USA. The human-associated marker DNA sequence is located on the 16S rRNA gene of *Bacteroides dorei*. The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its sensitivity and specificity. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested.

## B5. Quality Control Requirements

Measurement data quality will be monitored using the quantitative and qualitative data quality indicators described in Section A5 of this QAPP.

***E.coli*:** One field blank (NMED/SWQB 2018) will be prepared per sampling day with a goal of reaching 10% of the total number of samples collected for *E.coli* as specified in the NMED Bacteriological Sampling and Analysis SOP 9.1 (NMED/SWQB 2020). Jacobs will provide autoclaved sterilized deionized water which will be decanted into *E.coli* sample bottles in the field. These will be delivered as blanks alongside samples bottles when they are picked up from the lab. Field blanks will be held in sampling coolers to serve as trip blanks and handled with the same procedures as all collected samples.

**MST Human Fecal Marker Analysis:** The 51 samples will be analyzed in six qPCR runs (i.e. qPCR plates, one per sampling trip day), each with a 5-point standard curve. Each sample will be analyzed in duplicate qPCR reactions to ensure data quality control. One field blank will be prepared per sampling day with a goal of reaching 10% of the total number of samples collected for MST samples. Jacobs will provide autoclaved sterilized deionized water which will be decanted into MST sample bottles in the field. Approximately six field blanks (one per sample trip/batch) will be shipped to the lab; these will be frozen by the lab to preserve sample integrity and will only be analyzed if deemed necessary (i.e.: if *E.coli* blank has a positive result).

If any field blank show signs of contamination the results will be compared with measured sample concentrations to determine data usability.

## B6. Instrument/Equipment Testing, Inspection, and Maintenance

No field instruments will be required for this project.

Laboratories will follow all standard QA/QC processes and SOPs necessary under their EPA certification for equipment testing, inspection, and maintenance. See Table 2.3 for analytical method numbers.

## B7. Instrument/Equipment Calibration and Frequency

No field instruments will be required for this project.

Laboratories will follow all standard QA/QC processes and SOPs necessary under their EPA certification for equipment calibration. See Table 2.3 for analytical method numbers.

## B8. Inspection/Acceptance Requirements for Supplies and Consumables

Sampling vessels provided by Jacobs Laboratory for initial sample collection before decanting, will be single-use and sterile. All sampling bottles are all single-use only, and will be discarded if contamination occurs. MST Human Fecal Marker 250mL bottles (provided by LuminUltra Technologies, LTD.) are new and sterile, and caps are stored in a new sterile whirlpak while sample collection occurs. The 250mL bottles for *E.coli* analysis (provided by Jacobs Lab) will be sterile. If the lead sampler touches the threads of the bottle or cap during sample collection, or otherwise suspects sample bottle contamination, the bottle will be discarded.

Laboratories will follow all standard QA/QC processes and SOPs necessary under their EPA certification. See Table 2.3 for analytical method numbers.

## B9. Data Acquisition Requirements (Non-Direct Measurements)

**Flow Measurements:** Flow gauge data will be downloaded from the USGS data website and compared to results for all sampling locations. According to the SWQB QAPP "gage data collected by USGS are considered to meet the SWQB's QA requirements and may be used for most purposes" (NMED/SWQB 2018).

**WWTP Outflow *E.coli* Analysis:** As discussed in section B2 above, *E.coli* samples collected from the WWTP outflows in Farmington and Bloomfield will be collected as part of their regularly scheduled NPDES permit required monitoring (covered under their separate QAPP/SOP). WWTP staff will provide a copy of *E.coli* sample chain of custody, sample collection time, and analysis results to SJSWCD to analyze alongside MST results. (Note: in the event WWTP staff are not able to collect/analyze outflow samples, or NPDES sampling time/date does not align with sample collection dates, SJSWCD sampling team will collect outflow sample in accordance with all sampling instructions above).

## B10. Data Management

See Table A9 and A9.1 for details. All samples collected will be recorded in the field data sheet (paper and electronic) and on the chain of custody sheets for their respective labs. Copies of chain of custody sheets will be made and retained at the SJSWCD office before delivering samples. All field data sheets will be copied to an Excel spreadsheet in the SJSWCD Google Drive; SJSWCD files are backed up daily on a remote server.

Data, data analysis and final report will be sent to the SWQB Project Officer by the end of Winter 2022 by Project Coordinator. Upon receiving data, data analysis and final report the SWQB 604(b) Project Coordinator will store data on SWQB network drive in project specific folder. The SWQB network drive is backed up daily and maintained by the NMED Office of Information Technology. All files will be stored on the SWQB network drive in accordance with 1.21.2 NMAC, Retention and Disposition of Public Records

# Group C. Assessment and Oversight

## C1. Assessments/Oversight and Response Actions

The SWQB 604(b) Project Coordinator will provide project oversight by periodically assisting with and/or reviewing data collection efforts during the life of the project. Quarterly reports will describe the progress of the

project tasks and any potential problems with task implementation or schedule. This process includes justification for adjusting the task, or the task schedule, and making adjustments to the timeline if applicable. The SWQB 604(b) Project Coordinator will be responsible for approving any changes and ensuring changes are implemented by the designated party. All problems and adjustments to the project plan will be documented in the project file and included in the final report.

### C2. Reports to Management

Quarterly reports are submitted by the contractor to the SWQB Project Coordinator and include progress of project implementation and any available data. Printouts, status reports or special reports for SWQB or EPA will be prepared upon request. Separate annual monitoring reports will also be provided and included in the final report. The SWQB Project Coordinator will be responsible for maintaining project progress in the EPA Grants Reporting and Tracking System and the final report, and all other required project deliverables to be submitted to the EPA under this grant.

## Group D. Data Validation and Usability

### D1. Data Review, Verification, and Validation Requirements

Data will be considered usable if there is reasonable evidence that the requirements of this QAPP were followed. Results that appear incomplete or questionable for specific analysis will be flagged for review. Flagged data will be discussed with the SWQB 604(b) Project Coordinator and applicable SWQB staff to determine the potential cause and usability before used in data analysis. If a reasonable justification for use of the data cannot be attained, those data will be not used for any environmental decision making activity listed under this QAPP unless the data can be recollected and assessed for usability.

### D2. Verification and Validation Methods

The Project Manager will ensure that valid and representative data are acquired. Verification of field sampling and analytical results will occur in the review of data, performed by the Project Manager. *E.coli* data will be verified and validated according to applicable section of SWQB SOP 15.0 Data Verification and Validation. Data will then be provided to the SWQB 604(b) Project Coordinator for their review, upon request. In the event questionable data are found, the SWQB 604(b) Project Coordinator will consult with project personnel to determine the validity of the data. Results of the verification process will be included in the final reports.

### D3. Reconciliation with User Requirements

The user requirement is a restatement of the data quality objective: The information gathered as part of the San Juan Human Bacteria Sampling and Investigation will provide baseline information that will enable development of reach-specific and impairment-specific restoration measures.

If project results do not meet this requirement, then additional monitoring may be necessary to fill in data gaps and it may be necessary to extend the monitoring period to collect additional information.

## 5.0 REFERENCES


LuminUltra Technologies 2021. Sample Filtration, Extraction, and Human DNA Marker Bacteroides Dorei qPCR Procedures. Accessed privately. Accessed on June 11, 2021.

Jacobs Laboratory 2020. Quantification of Total Coliforms and *E.coli*. Accessed privately. Accessed on June 2, 2021.

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NMED/SWQB 2005. Total Maximum Daily Load (TMDL) for the San Juan River Watershed Part One Report. Accessed at https://www.env.nm.gov/surface-water-quality/wp-content/uploads/sites/25/2017/07/SJR_Pt1TMDLs.pdf. Accessed on: June 14, 2021.

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NMED/SWQB 2020. Bacteriological Sampling and Analysis Standard Operation Procedure 9.1 Rev. 4. Accessed at https://www.env.nm.gov/surface-water-quality/sop/. Accessed on May 16, 2021.

SJWG 2019. San Juan Watershed Project Site-Level Analysis. Accessed privately. Accessed on January 2, 2019.

## Appendix A. Acknowledgement Statement

New Mexico Environment Department Surface Water Quality Bureau

### San Juan Human Bacteria Sampling and Investigation
**Quality Assurance Project Plan Acknowledgement Statement**

This is to acknowledge that I have received a copy (in hard copy or electronic format) of the "San Juan Human Bacteria Sampling and Investigation" Quality Assurance Project Plan.

As indicated by my signature below, I understand and acknowledge that it is my responsibility to read, understand, become familiar with and comply with the information provided in the document to the best of my ability.

_____
Signature or Electronic Signature (e-certified accepted)

_____
Name (Please Print)

_____
Date
***Return to SWQB 604(b) Project Coordinator***

**Attachment B. FARLAB-007 SOP, Revision No. 7**

**Attachment C. Sample Filtration, Extraction and Human DNA Marker Bacteroides Dorei qPCR Procedures**