UC Davis - US Forest Service Grazing Allotment *E. coli* Monitoring Quality Assurance Project Plan (QAPP)

Submitted by:
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Background/Objective

There continues to be great concern that microbial pollution by grazing livestock degrades water quality on multiple-use rangelands. Given the importance of clean water on these shared landscapes, there has been growing stakeholder interest in additional water quality research across a range of common resource use activities at a high sampling frequency. During the 2016 summer grazing-recreation season, we will conduct a cross-sectional survey of microbial water quality conditions associated with livestock grazing, recreation, and residential use on three multiple-use watersheds in the central Sierra Nevada and southern Cascade ranges of California. Our study objectives are to 1) quantify fecal indicator bacteria (FIB; fecal coliform and E. coli) concentrations in surface waters; 2) compare results to water quality regulatory benchmarks, and 3) examine relationships between water quality, environmental conditions, and primary land use.

Water Sample Collection

Sample collection will be conducted by UC Rangelands staff and/or US Forest Service staff. All participants will be trained in sample collection (e.g., bottle labeling, sample handling, data recording) to assure consistency and data quality. A standard data collection sheet will be used.

On the day of sampling, at each sample site planned for that day, a single 500 mL water sample will be collected with a sterile plastic bottle in the morning and delivered to the appropriate laboratory location within 6 hours of collection time.

UC Laboratory Locations

- UC Rangelands Laboratory, UC Davis, Room 1223, Plant and Environmental Sciences Building (PES), University of California, Davis, (530) 754-8766
- InnovationLab, 101 Hospital Road, Sonora, California 95370, (209) 989-4058
- 234 North Buckeye Drive, Bridgeport, California 93517
- Lassen County Cooperative Extension, 707 Nevada Street, #2, Susanville, California 96130, (530) 251-8133

Field Water Sample Collection Protocol

1. Travel to the farthest sample site (in terms of time) from the laboratory location and work back from there.
2. At each site:
    a. Label 250 mL sample bottle with sample site ID, and date and time of sample collection.
    b. Record sample site ID, date and time of collection, and any notable observations at time of collection on the chain of custody form.
    c. Collect sample using methods described in section 7.1 Fecal Indicator Bacteria of the National Field Manual for the Collection of Water Quality Data (https://water.usgs.gov/owq/FieldManual/Chapter7/7.1_ver2.1.pdf). Refer to the point-sampling methods section on page 26 which describes how to collect a hand-dipped sample.
        i. Collect sample from a well-mixed area of the main reach of the stream.
        ii. Minimize sediment disturbance during collection.
        iii. Fill the 250 mL sample bottle allowing 2 to 3 cm of headspace.
        iv. Tightly recap sample bottle.
        v. Place bottle on ice in cooler.
        vi. Note any relevant observations on chain of custody form (e.g., high stream flow, water turbidity, livestock present, or presence of recreation).

vii. If you have to sample stagnant, non-flowing pools (if the stream has stopped flowing at the sample site), take the sample and note that the site is stagnant on the chain of custody form.

3. If any site is not collected for any reason, note that it was not collected and a detailed reason (e.g., vehicle emergency, road was impassible, site dry, etc.) on the chain of custody form.
4. Deliver sample to laboratory location within 6 hours of collection time.
5. Document sample handoffs on the chain of custody form.

Laboratory *E. coli* Concentration Enumeration

E. coli concentration for each sample will be determined by direct membrane filtration through a 0.45 µm membrane and direct membrane incubation on a selective agar (CHROMagar *E. coli*). This is Standard Method 9222 "Membrane Filter Technique for Members of the Coliform Group" as detailed in "Standard Methods for the Examination of Water and Wastewater" (eds.) Eaton, Clesceri, and Greenberg. APHA, AWWA, and WEF.

E. coli Concentration Enumeration Protocol

1. Materials required:
    a. Millipore 47mm diameter, 0.45 µm pore size, sterile, gridded membrane filters
    b. Full ethanol dipping container
    c. Matches/Lighter (flame)
    d. Candle
    e. Forceps
    f. Type II water squirt bottle
    g. *E. coli* plates prepared with CHROMagar *E. coli*
    h. Disposable centrifuge tubes (1 for each sample plate)
    i. Type II water in liter glass bottle
    j. Plate racks
    k. 0.1% Iodine solution
    l. Gauze roll
    m. Discard H_2O container for vacuum trap
    n. Hand counters
    o. Magnification lamp
    p. Data sheet
    q. Pen/pencil
2. Equipment required:
    a. Refrigerator
    b. Incubator set at 44.5 ^oC
    c. Vacuum filter housing manifold
    d. Vacuum pump
    e. Double vacuum trap
    f. UV vacuum filter housing sterilizer
    g. Vacuum housings
3. Methods:
    a. Receive water samples
        i. Check chain of custody form for completeness and accuracy.
        ii. Inquire about possible additional notes.
        iii. Sign back of chain of custody form and take formal possession of sample.
    b. Prepare lab space
        i. Confirm that incubator is working and set at 44.5°C.
        ii. Using Iodine solution and gauze roll, disinfect counter work space.
        iii. Remove agar plates from refrigerator.
        iv. Label top and bottom of each agar plate with sample name and volume to be filtered and set out in front of sample bottles.

v. Place filter housings in UV sterilizer for at least three minutes to sterilize.
vi. Assemble vacuum pump, vacuum traps, and vacuum filter housing manifold.

c. Filter water samples
i. Set up all filter housings on the vacuum filter housing manifold.
ii. Place water samples and labeled plates in front of corresponding vacuum filter housings.
iii. Place a 50 mL disposable tube in front of each sample bottle.
iv. Sterilize forceps with ethanol and flame.
v. Open Millipore 47mm wrapper without touching filter. Use sterile forceps to place filter, grid-side up, on filter housing. Place one filter on each of the filter housings.
vi. Place top of filter housing back on base, making sure halves are lined-up properly.
vii. Turn on vacuum pump.
viii. Turn on the vacuum to the filter housing you will run water through first.
ix. Shake up water sample and pour into 50 mL disposable tube.
x. Pour sample from disposable tube into filter housing.
xi. Run decided volume of water sample through each filter.
xii. Wait for samples to be completely filtered, sterilize forceps, place filters grid-side up onto labeled agar plates, making sure no bubbles form between filter and agar.
xiii. Rinse each filter housing well with type II water and let filter housing sit with vacuum on to remove any residual water.
xiv. Rinse filter housings with type II water and place in UV sterilizer for at least three minutes.
xv. Record the time in the time processed column on the chain of custody form for each sample.
xvi. Repeat steps A through P for all water samples.
xvii. Filter a blank control with 50 mL of type II water.
xviii. Place all plates onto incubator rack, place in incubator set to 44.5°C, record time and note time plates must be removed from incubator (24 hours).
xix. Place unfiltered portion of water samples back into refrigerator and retain until plates have been counted and results are clear.

d. Count colony forming units (cfu)
i. Remove agar plates from incubator 20-24 hours after filtering.
ii. Place sample under magnification lamp (if colonies are small).
iii. *E. coli* colonies will be blue and shiny in appearance, do not count colonies that are different colors.
iv. Use hand clicker to count each distinct *E. coli* colony.
v. Count colonies one grid row at a time (within area exposed to water by vacuum housing).
vi. Generally, counts less than or equal to 400 are reliable, if much higher, need to re-run water sample at a lower volume.
vii. Discard recorded plate into trash bag for return to UC Davis laboratory for autoclaving.

Laboratory *E. coli* Agar Preparation Protocol utilized by the UC Rangelands is as follows:

1. Materials required:
   a. dehydrated CHROMagar E.coli
   b. type II water
   c. 1000 mL glass beaker
   d. weighing paper and plastic weigh boat
   e. measuring tools: scoopula, digital scale
   f. 100 60mm petri dishes
   g. 25 mL glass pipettes tips
   h. Pipette
   i. 0.1% Iodine solution
   j. cotton gauze
2. Methods:
   a. Make liquid agar solution

i. Weigh out 18.65g dehydrated CHROMagar *E.coli.*
ii. Suspend the powder in a 1000 mL beaker containing 500 mL type II water and mix thoroughly.
iii. Bring the agar to a boil in the microwave by short, repeated heatings (30 sec). When solution begins to foam and rise, remove from microwave, swirl/stir until foam disappears and reheat. Continue until agar is completely dissolved and solution is clear (not cloudy).
iv. Disinfect hood fume counter with cotton and 0.1% iodine solution.
v. Remove liquid agar solution from microwave and place in fume hood.

b. Pour agar solution into plates
i. Allow liquid agar to cool to approximately 55°C (can barely hold with bare hand).
ii. Attach glass pipette tip to pipette and fill with 25 mL of liquid agar solution.
iii. Dispense 4-5 mL of warm solution into plate. Swirl plate to evenly distribute agar.
iv. Allow plates to cool until agar is firm (approximately 1 hour).
v. Agar in plates should be whitish-yellow in color with no large bubbles.
vi. Store plates in original plastic sleeves, secure with tape, write date on tape.
vii. Store in refrigerator for up to 4 weeks.

Laboratory Quality Control

Precision describes how well repeated measurements agree, assuming that the constituent of concern is uniformly distributed between the duplicate samples. Precision for *E. coli* concentration enumeration will be determined by having the same analyst complete the procedure described above for field samples that are split in the laboratory. This is a preferable approach to measure precision (compared to duplicate samples) due to the fact that bacteria are often highly variable in the water column (duplicate samples), due in part to bacterial colonies adhering to suspended particulate matter. At a minimum we will run duplicates on 5% of samples. The results of the duplicates should be within the 95% confidence limit of its pair based on plate counts as described in Standard Methods 9222 Table 9222.II and Section 6.c. **Detection Limit** is the lowest possible concentration the laboratory method can detect. **Sensitivity** is the ability of the method to detect one concentration from the next. Using the standard operating procedures described above, the detection limit for *E. coli* is concentration enumeration is 1 cfu/100 ml, and the sensitivity is 1 cfu/100 ml.

Training Requirements

All UC and USFS staff will be fully trained on all aspect of their duties associated with sample collection and laboratory analysis. Trainings will occur on-site in the field and laboratory in a hands-on approach. Project staff will conduct multiple collections and analyses and meet the data quality objectives described above. Field and laboratory safety training will also be conducted during each QA session (http://ehs.ucdavis.edu).

Documentation and Records

Documents and records we expect to generate from this project include: field data sheets, a laboratory notebook (raw laboratory data, duplicate results, etc.), and chain of custody forms, and final data spreadsheets. Hard copies of all documentation and records will be stored in Room 1231 Plant and Environmental Sciences Building, UC Davis Campus, Davis, CA. All electronic copies of documentation and records, including data, will be stored on nightly backed up computers in 1231 Plant and Environmental Sciences Building. All documents and records will be made available for review by NCRWQCB and United States Forest Service representatives upon request, and in a reporting format appropriate to address the request. In general, data will be reported in tabular and graphical format with accompanying interpretive text.

All field data will be recorded at the time of collection using field data sheets. Field data sheets will be reviewed for outliers and omissions before leaving the field site. Field data sheets will again be reviewed and finally approved upon entry into the local database or spreadsheet. Upon collection, each water sample is assigned a unique identification number, which allows us to track it through the various stages of handling, preparation, analysis, data correction, and reporting. Analytical procedures and results will be recorded in a laboratory notebook along with records of all quality control samples. Results from individual analytical runs will be recorded in a laboratory notebook and entered in a spreadsheet (M.S. Excel). Field data sheets and laboratory notebooks are archived for 10 years. If data entry is ever performed at another location, duplicate data sheets will be used, with the originals

remaining at the headquarters site. A chain of custody form will accompany each set of samples collected by an individual field data collector on each collection date, and will be stored in the laboratory notebook.

Instrument/Equipment Testing, Inspection and Maintenance

A maintenance log is kept for each instrument used in the study. The log details the dates of instrument and sampling gear inspection, calibrations performed in the laboratory, battery replacement, and any problems noted with instruments. Crucial spare parts for all equipment will be maintained at the appropriate work site (laboratory or field kit). Any problems identified with equipment will be corrected by the field data collectors, or laboratory analysts. If the problem cannot be corrected, the item will be returned to the manufacturer.

Inspection/Acceptance Requirements

All required reagents, equipment, or other supplies required for this project will be purchased new from reputable commercial sources (e.g., Fisher Scientific, Inc.). Upon receipt, reagents will be inspected by the project staff for leaks or broken seals, and will compare the age of each to the manufacturer's recommended shelf-life. All other sampling equipment will be inspected for broken or missing parts, and will be tested to ensure proper operation. Field data collectors are responsible for field equipment and standards, while laboratory analysts are responsible for laboratory items.

Data Management

All data and measurements for this project will be made directly by project staff following the project monitoring plans for each National Forest, and will meet the requirements detailed in this QAPP. Field data sheets are checked in the field by the field sample collection staff. Sample identification information and the chain-of-custody forms will be reviewed by project staff. Working with field and laboratory staff, the data manager will identify any results where sample holding times (8 hours) have been exceeded, sample identification information is incorrect, samples were inappropriately handled, or data quality objectives were not met. The data manager will bring such data to the attention of project leader for review, and will be "flagged" upon entry into the project spreadsheet.

The data manager will oversee the entering of data with the supervision of the project leader. Upon entering the data the data manager will archive the field data sheets. Data will be entered into a spreadsheet (MS Excel) compatible with CEDEN data reporting requirements. All electronic files will be stored on a computer which is automatically backed-up nightly. Following initial data entry the data manager will review electronic data, compare to the original field data sheets/laboratory notebooks and correct entry errors. After performing data checks and ensuring that data quality objectives have been met, data analysis will be performed to achieve the objectives of the project.

Assessment and Response Actions

Review of all field, laboratory, and data management activities is the responsibility of the project leader and the laboratory manager. The project leader and laboratory manager have authority to stop work if problems are found and implement corrective actions as required. All assessment information as well as corrective actions implemented will be reported to the project leader and laboratory manager. Training (as described above) will be utilized to correct any problems with data quality attributable to staff's implementation of procedures described above. Retraining will be scheduled as frequently as required to meet data quality objectives. All field and laboratory activities, field data sheets, laboratory notebooks, as well as maintenance logs may be reviewed as requested.

The project leaders are always actively engaged in the daily management of project staff, thus providing a continuum of oversight and assessment throughout the project. Field staff oversight will be the direct responsibility of the project leaders. Oversight and assessment of field staff performance and resulting data quality will occur in the field during at least 5% of the sample collection events conducted during the project. Field staff in need of performance improvement will be retrained on-site. The laboratory manager will be directly responsible for oversight and assessment of laboratory analysts. Oversight and assessment of laboratory staff performance and resulting data quality will occur in the laboratory during at least 5% of the sample analysis events conducted during the project.

Data Review, Validation and Verification

Data review, validation, and verification for this project will follow the guidelines provided by USEPA (2002, “Guidance on Environmental Data Verification and Data Validation”, EPA QA/G-8, http://www.epa.gov/quality/qs-docs/g8-final.pdf). The project staff will collaborate on 2 data review, validation, and verification sessions per year. The project leader has final authority on data acceptance. They will also evaluate compliance with the data quality objectives as described above.