Quality Assurance Project Plan

Sierra Nevada Aquatic Research Laboratory
Center for Eastern Sierra Aquatic Microbial Ecology
(SNARL – CESAME)

Version 1.0
October 2012

A GROUP A: PROJECT MANAGEMENT

A.1 Approval Page

Document Title: Quality Assurance Project Plan for Lahontan Region Bacteria Monitoring

Revision History

Version #	Effective Date	Changes made
1.0	1 October 2012	First version of document

Approvals:

_____ **Date** _____
Roland A. Knapp, Project Director

_____ **Date** _____
Craig E. Nelson, Project Microbiologist

_____ **Date** _____
Thomas J. Suk, Lahontan Regional Water Board

A.2 Table of Contents

APPENDICES

TABLES

A.3 Distribution List

Copies of this Quality Assurance Project Plan (QAPP) will be distributed to the following individuals

Table 1. QAPP distribution list

Title:	Name
SNARL-CESAME Project Director, Research Biologist, Marine Science Institute, UC Santa Barbara	Roland A. Knapp
SNARL-CESAME Project Microbiologist, Associate Specialist, Marine Science Institute, UC Santa Barbara	Craig E. Nelson
Senior Environmental Scientist, California Regional Water Quality Control Board, Lahontan Region	Thomas J. Suk

A.4 Project/Task Organization

A bacteria monitoring program is being implemented by staff of the Sierra Nevada Aquatic Research Laboratory – Center for Eastern Sierra Aquatic Microbial Ecology (SNARL-CESAME). The CESAME staff listed in Table 1 will be involved with oversight and implementation of this QAPP in conjunction with the Lahontan Regional Office of the California State Water Resources Control Board and will be responsible for all review and assessment with the provisions of this QAPP. In addition to the staff listed in Table 1, other CESAME staff, interns, and SNARL-CESAME contractors will conduct sample collection, handling, analysis, and data review and management.

A.4.1 Involved Parties and Roles

The Project Director will have the following responsibilities:

- Oversee work of the staff and interns associated with SNARL-CESAME
- Review project needs, requests, and problems
- Provide training to personnel working in the SNARL-CESAME Lab
- Manage and oversee work of personnel performing bacteria analyses
- Ensure all lab equipment is maintained and operating properly
- Work closely with staff, interns, and SWAMP contractors to ensure proper implementation of the QAPP
- Assist with scheduling and coordination of sample collection and transport

- Manage contracting with contractor laboratories
- Receive and review deliverables from contractor laboratories
- Update QAPP as needed
- Manage filing and record keeping for project

The Project Microbiologist will have the following responsibilities:

- Provide technical expertise and training in bacteria analyses
- Review and assess performance against established procedures and the provisions of this QAPP for bacteria analyses
- Provide assistance with corrective action implementation
- Provide information and training about quality assurance and quality control concepts and practices
- Provide review of QAPP to assure adherence the State Water Resources Control Board (State Water Board) Quality Management Plan
- Oversee production of annual summary report

A.4.2 Persons Responsible for QAPP Update and Maintenance

The Project Director shall be the custodian of the current and any prior versions of this QAPP. Plan modifications, revisions or updates may be suggested by the any personnel involved with the project. SNARL-CESAME staff may call for the revision of this QAPP in order for it to meet changes in procedures or permit conditions. The Project Director will convene meetings and solicit professional opinions when revising this QAPP and shall be responsible for the drafting and finalizing the revised QAPP.

A.5 Problem Definition/Background

A.5.1 Problem statement

Bacterial contamination can occur from many types of land uses and can cause significant public health threats. The Water Quality Control Plan for the Lahontan Region (Basin Plan) contains water quality objectives for bacteria that apply to surface waters in the region.

A.5.2 Decisions or outcomes

The main goal of the study is to assess surface waters for compliance with the bacteria objectives contained in the Basin Plan. Fecal coliform bacteria will be the primary constituent analyzed in the study because the Basin Plan contains numeric criteria for it. Further, because the State Water Board is considering the adoption of new (statewide) bacteria objectives for *Escherichia coli* (*E. coli*), an additional goal of this study is to also (where feasible, given resource constraints) collect synoptic *E. coli* data, in order to assess attainment of current non-regulatory thresholds for *E. coli*, and compliance with any future regulatory standards for *E. coli*.

The specific monitoring questions are as follows: 1. Do fecal coliform concentrations at selected sites comply with the Basin Plan's objectives? 2. Where feasible given resource constraints, do *E. coli* concentrations at selected sites meet USEPA recommendations, and other relevant non-regulatory assessment thresholds? 3. Where data from duplicate samples are available, how do fecal coliform concentrations at selected surface water sites compare to *E. coli* concentrations?

A.5.3 Water quality or regulatory criteria

This project will yield bacterial count data. These data may be used by the SNARL-CESAME for status reporting (305b), comparison to Basin Plan water quality objectives (and 303d listing or de-listing), and watershed assessments.

A.5.4 Work statement and produced products

The project will include monitoring of individual analytes in water. Station types sampled will include: rivers, streams, and/or creeks. Sampling and measurements will be made during either wet or dry weather.

Sample locations will be distributed throughout the southern portion of the Lahontan Region, from Bridgeport Valley south to the vicinity of Lone Pine. At a minimum, sample locations will be documented with a station code, latitude and longitude coordinates and photographs.

Analytical data will be entered into the California Environmental Data Exchange Network (CEDEN) database. The project will provide an annual summary report. The annual report will include maps, photographs, and a written description of each sample location. Maps and photographs showing sample locations will be included in the annual report. At a minimum, photographs will be labeled with water body name and orientation. At a minimum, maps will include the sample location, water body, nearby roads, towns, and orientation.

A.5.5 Constituents to be monitored and measurement techniques

Monitoring consists of field sampling and laboratory analyses for fecal coliform (at all locations) and *E.coli* (where resources permit). Bacteria samples will be collected according to the procedures detailed in Appendix A, Standard Operating Procedures for Bacteria Sample Collection. Sampling techniques will include direct filling of containers.

Field parameters are not required for this project. If field parameters are collected, they will meet the requirements of the SWAMP Quality Assurance Program Plan (SWAMP QAPrP, 2008).

A.5.6 Monitoring schedule

Initial sample locations will be determined during summer 2012, with additional locations added as needed in future years. Samples will be collected at varying frequencies depending on the location. The goal will be to collect multiple samples per month, however this may not be possible at all locations given the large geographic range of the Lahontan Region, limited access to laboratories, and a short holding time for bacteria samples.

A.5.7 Geographical setting

Sample locations will be distributed throughout the southern portion of the Lahontan Region. The Lahontan Region is over 600 miles long, stretching along the crest of the Cascade and Sierra Nevada mountain ranges from the California/Oregon border at the north to the Mojave Desert in the south and includes most surface waters draining to the east.

A.5.8 Constraints

Extreme wet weather could affect sampling because of significant dilution or mobilization of the constituents being measured. Extreme dry weather could also limit or prevent representative sampling due to low flow or no flow at a location. Freezing weather could cause conditions that adversely affect the parameters being measured. It also could prevent access to some of the areas where sampling is needed.

If some areas that are planned to be monitored are not accessible because of legal restrictions then there will be some gaps that could affect some of the conclusions drawn from the data. If there are some unexpected topographical features that make it impractical to monitor some planned areas then other sampling locations will be located, if possible.

Budget constraints, availability of qualified laboratories, or unanticipated analytical difficulties (such as interferences requiring selection of other methods, accepting higher detection levels, or requiring additional clean up of samples prior to their analysis) could result in fewer locations or samples.

A.6 Quality Objectives and Criteria for Measurement Data

This project will include laboratory measurement of fecal coliform and *E. coli* by multiple methods. Laboratory quality control samples will adhere to those specified by the method being used. There are no field quality control samples collected for this project.

Table 2. Test Methods and Minimum Values for Fecal Coliform and *E.coli*

Parameter	Test Method	Reporting Limit*	Units
Fecal Coliform	SM 9222D (membrane filtration)	1	CFU/100mL
E. coli	SM9222G (two-step membrane filtration)	1	CFU/100mL

* Reporting Limit may vary depending on sample dilution.

A.7 Special Training Needs/Certification

Personnel involved with this project will receive training in the collection of samples for fecal coliform and *E. coli*. Personnel collecting samples will be familiar with the contents of this QAPP prior to conducting sampling. Training will be conducted by the SNARL-CESAME Project Director, Project Microbiologist, or other qualified staff persons. Training shall be in the following areas.

- Equipment (GPS unit) operation, calibration, and maintenance
- Sample collection and handling procedures
- Basic quality assurance/quality control

Personnel working in the SNARL-CESAME lab will be trained by the SNARL-CESAME Project Director, Project Microbiologist, or other qualified staff persons. Training shall be in the following areas:

- General laboratory safety
- Analytical methods for fecal coliform and e.coli analyses
- Sample handling procedures
- Basic quality assurance/quality control

A.8 Documents and Records

The Project Director will be responsible for final records management as described in this QAPP. Records to be retained include field notes and observations, the results of laboratory or water quality sample analyses, laboratory reports, chain of custody forms, this QAPP and any revisions, and annual reports. Records will be housed at the SNARL-CESAME office.

Sample collection activities will be documented in bound field books and chain of custody (COC) forms. Any errors in field book entries or COC forms will be corrected by a single line through the text being revised, and all such corrections initialed and dated. The following information will be recorded in the bound field books:

- Initials or names of samplers
- Sample date and time of collection
- Station location code (referenced to GIS database)
- Sample ID number
- Any unusual weather events
- Visual observations

COC forms will be filled out by personnel collecting samples and delivered to the laboratory with the samples.

B GROUP B: DATA GENERATION AND ACQUISITION

B.1 Sampling Process Design

Sample locations and frequency of collection will vary throughout the region. Sample locations may be selected to assess potential impacts from different land uses.

B.2 Sampling Methods

Sampling methods for bacteria samples consist of grab samples collected at each locations. Bacteria samples will typically be collected in disposable sterile containers. Sample collection procedures are described in Appendix A, Standard Operating Procedures for Bacteria Sample Collection.

B.3 Sample Handling and Custody

Samples collected are to be handled as in Table 9. Additional sample handling procedures are described in Appendix A, Standard Operating Procedures for Bacteria Sample Collection.

Sample locations will each have a unique location code. If the station is an existing SWAMP station, the SWAMP station code will be used. For newly identified non-SWAMP locations, the location code will consist of the following: a 3 letter creek code and a 2 digit location code (generally beginning with 01 for the most upstream location).

Each sample collected will also have a unique sample identification number. Samples collected at SWAMP sites may have a SWAMP specific sample ID (per SWAMP QAPrP, 2008). Other sites will use a SNARL-CESAME sample ID which consists of the following:

Unique Sample Identifiers: ABC.##.ddmmmyy.hhmm

- ABC is 3-letter catchment/stream code

- ## is two digit site numeric code (generally increasing downslope)
- Date is in form 25JUL12
- Time is rounded to nearest 10 minute interval and is optional in unique ID

Table 3. Containers, sample preservation, and sample holding times

Parameter	Container	Preservation	Minimum Sample Volume	Holding Time
Fecal coliform	Autoclaved polypropylene or glass containers or Whirlpak bags	Cool < 10°C	100mL	8 hours
E. coli	Autoclaved polypropylene or glass containers or Whirlpak bags	Cool < 10°C	100mL	8 hours

B.4 Analytical Methods

The analytical methods listed in Table 10 will be used for analysis. Additional methods, such as those listed in the Code of Federal Regulations 40 CFR 136.3, may also be used.

Table 4. Analytical methods

Parameter	Method reference
Fecal Coliform	SM 9222D (Membrane Filtration)
E.coli	SM9222G (two-step Membrane Filtration)

B.5 Quality Control

Quality control processes will follow those described in the method being used by the laboratory. There are no field quality control samples collected for this project.

B.5.1 Corrective Actions

If there are failures to meet the above Quality Control criteria then SNARL-CESAME staff will initiate Corrective Action procedures, or direct the organization responsible for failures to do so. The Project Director will document the effort and effectiveness of any actions taken. The basic plan for Corrective Actions is as follows.

- Investigate and determine if a cause can be discerned. Examine the actual procedures, equipment performance, equipment maintenance records, condition of solutions and standards, interview staff conducting tests, and any other actions that are deemed appropriate to gain an understanding of the cause(s).

- Take action and correct the discerned problem. Amend written procedures, purchase repair and replacement materials, retrain staff, alter maintenance or restocking schedules, and any other change(s) deemed necessary that will prevent a repetition of the problem.

- Follow up and be sure changes were made and actions effective. Take additional actions as necessary.

B.6 Instrument/Equipment Testing, Inspection, and Maintenance

No meters or equipment are needed for bacteria sample collection.

If field parameters are added to the monitoring program all meters and devices used to measure pH, temperature, or other parameters will be tested, inspected, and serviced in accordance within all of the manufacturer's specifications.

Laboratory equipment will be tested, inspected, and serviced in accordance within all of the manufacturer's specifications and with laboratory SOPs or QA manuals.

B.7 Instrument/Equipment Calibration and Frequency

No meters or equipment are needed for bacteria sample collection. Laboratory equipment will be calibrated in accordance within all of the manufacturer's specifications, method required specifications, or laboratory SOPs or QA manuals.

B.8 Inspection/Acceptance of supplies and Consumables

We will inspect upon receipt, all shipments of supplies and consumables used for bacteria monitoring and analysis for obvious physical damage or loss. Any damage or other issue with the supplies and consumables will be reported to the supplier and the affected material set aside and not used.

Supplies and consumables received in good order will be stored as directed by the manufacturer.

B.9 Non-Direct Measurements (Existing Data)

Non-direct measurements are either those that are generated by another agency or organization outside of our monitoring program; or those created by calculation from values generated by direct measurement. No data generated outside of our monitoring program will be used for this project.

B.10 Data Management

Data collected for this project will be entered into the CEDEN database which was designed to facilitate integration and sharing of data collected by many different participants. CEDEN's mission is to simplify and improve access to California's water resource monitoring data by providing services that integrate, standardize, and display data from a diverse array of monitoring and data management efforts. Data for SWAMP sites will be entered into the SWAMP database using SWAMP protocols. CEDEN serves as the venue through which SWAMP data are made available to the public. CEDEN can be accessed at http://ceden.org/ .

C GROUP C: ASSESSMENT AND OVERSIGHT

C.1 Assessments & Response Actions

The Project Director or SNARL-CESAME Project Microbiologist will review and assess compliance with all sample collection and analytical procedures for bacteria monitoring at least twice each calendar year. Any failures to follow the written sampling and analytical procedures of this QAPP are to be followed up with all appropriate responses including refresher training.

The Project Director or SNARL-CESAME Project Microbiologist will review all reports generated by the contract laboratories and call for responses and corrective action if they are needed.

C.2 Reports to Management

The Project Director will send periodic reports to the SNARL-CESAME office management regarding compliance with the provisions of this QAPP and any issues and resolutions arising from review and assessment by SNARL-CESAME staff.

D GROUP D: DATA REVIEW AND USABILITY

D.1 Data Review

Data review is the in-house examination to ensure that the data have been recorded, transmitted, and processed correctly. Data will be reviewed for data entry errors, transcription errors, transformation errors, calculation errors, and errors of data omission. Entries in field books and COC forms will be checked against laboratory data reports for consistency.

D.2 Verification and Validation Methods

Data from the SNARL-CESAME facility and contractor laboratories will be obtained in electronic format or hand entered into electronic spreadsheets. These spreadsheets will be verified and checked for entry errors, transcription errors, transformation errors, calculation errors, and errors of data omission by a second person. The person conducting the verification will compare the entries in the spreadsheets to the field book, COCs, and lab repots or lab data sheets.

D.3 Reconciliation with User Requirements

The Project Director will be responsible for reporting project reconciliation, including how well the project objectives were met. The data will be compared with bacteria objectives contained in the Basin Plan. Explanations will be provided for any data determined to be unacceptable for use or flagged for quality concerns.

E APPENDIX

SNARL-CESAME Microbiology Standard Operating Procedure

(Version 1.0, updated 26 Jul 2012)

Data Management:

Sampling Chain of Custody: Time of collection, Sampler, Sampling Site, Unique Sample ID, Date/Timestamp, Livestock present/absent, Recommended Dilution, Additional Notes
Laboratory Chain of Custody: Unique Sample ID, Time of arrival at lab, Volume of sample filtered, time of placement on m-FC media, Fecal coliform colonies, *E. coli* colonies
Unique Sample Identifiers: ABC.##.ddmmmyy.hhmm

- ABC is 3-letter catchment/stream code
- ## is two digit site numeric code (generally increasing downslope)
- Date is in form 25JUL12
- Time is rounded to nearest 10 minute interval and is optional in unique ID

Lab Organization:

Autoclave Instructions:

- Ensure sufficient water in top-loading reservoir (above top of coil, below release valve)
- Switch on autoclave (front power and/or rear-emergency power red switch)
- With door open, turn control knob to "Fill Water" until water reaches front groove
- Load autoclave, ensuring liquid goods are either in solid-bottom metal tray or PP autoclave pan
- Tighten door, and turn control knob to "Sterilize". Set Temperature to 121degC.
- Activate timer to 30 minutes, which will provide 15m to pressurize and 15m to sterilize
- When timer rings, allow 10 minutes for pressure to decrease to absolute zero
- Turn knob to "Exhaust/Dry" and carefully open door a crack
- Dry goods (filter towers, sample bottles, tweezers/needles, sterivex caps, beakers, etc.)
    - Turn timer to 15 minutes to dry goods completely
    - When timer rings, turn control knob to "0" and remove goods
- Liquid goods (agar media, squirt bottles with water, other liquids to be sterilized)
    - Turn control knob to "0" and remove goods; do not set timer on Exhaust/Dry cycle
- If autoclave is to be decommissioned (>1 wk), turn off, drain reservoir and chamber, close

Before sample collection: Ensure that adequate stocks of all items exist; all culturing materials are either sterile packed and disposable or autoclaved before use:

- *Disposable*:
    - Petri dishes with pads (47mm; Fisher PD20047S5)
    - Petri dishes without pads (47mm; Fisher PD2004705)
    - m-FC media ampules with Rosolic Acid (2ml; Fisher MHA000P2F)
    - Serological Pipets (50ml; Fisher 1367610R)
    - Filters (47mm 0.45um; Fisher HAWG047S6)
    - qPCR bead tubes (MoBio Powerwater, VWR 101449-430)
    - Sterivex cartridges (0.22um, Fisher SVGP01050)
    - Cryovials (1ml; Fisher 2912710)

- *Autoclaved*:
  - Rinse water (DI) and squirt bottles (PP; Fisher 0340914A) – autoclave liq. cycle
  - Sample collection bottles (PP; Fisher 033112E)
  - Filter towers & support discs (o-rings (PSF; Fisher 0974023D) – autoclave in foil
  - Agar media (NA-MUG; Fisher DF0023156) – autoclave liquid cycle
  - tweezers and needles (Fisher XX6200006P) – autoclave pouches (Fisher 181254)
  - Sterivex caps (Nylon; Value Plastics LP34-3) – autoclave in pouches
  - Syringes (60ml; Fisher 136898) – after rinsing/drying, autoclave in pouches
- *Other prep*:
  - Ensure adequate stocks of PFA aliquots in fridge (10% paraformaldehyde)
    - Reorder from Electron Microscopy Sciences (Cat. 157-8)
  - Pour NA-MUG plates
    - Weigh powder in front room with our scoopula and weigh-boats
    - Add DI water to mix - 23.1g/L, 5ml agar per plate (100ml/2.31g is good)
    - Add stirbar (1-2cm) and stir with vortex and heat (level 3) 5-10 min
    - Autoclave on liquid cycle in polypropylene autoclave tray
    - Let cool until neck is hot but tolerable
    - Pour approx 5ml per plate (fill plate 2/3 then swirl to distribute)
    - Let cool on benchtop until solidified, 2-24h
    - Store in refrigerator in tupperwater or ziplock up to 2 weeks
  - Freeze ice blocks (Fisher 353154)
  - Print chain-of-custody and filtration record forms
  - Organize Field Cooler
    - Trash bags (used ziplocks are ideal)
    - Gloves (Fisher 19048134)
    - Field notebook
    - Chain-of-Custody forms on clipboard
    - Ballpoint Pen and/or pencil
    - Label Tape
    - Sharpies
    - GPS
    - Camera
    - Sterivex
    - Sterivex Caps
    - 60ml Syringes
    - Sample Bottles (Autoclaved)
    - Ice Blocks (Frozen)

Sample Collection

1) Wrap label tape around sample bottle completely to meet on far side
2) Label sample bottle with Site Code and Timestamp (Unique ID as described above)

3) Photograph site and record GPS coordinates if a new site
4) Record Ancillary sample collection Data: weather, cattle/human impacts, sampler name, notes
5) Don fresh gloves, rinsed 3X in streamwater before opening sample bottle
6) Submerge sample bottle 3cm beneath water surface facing upstream of investigator
7) Ensure 1cm neck headspace in sample bottle – collection volume should be >1L
8) Using sample bottle pour 4 X 65ml into sterile syringe and push through Sterivex filter
9) Cap Sterivex with sterile caps, leaving capsule full of water
10) Transport samples with icepacks in small collapsible cooler to larger cooler in auto

Sample Processing

- All samples must arrive at lab facility within 6h of collection
- All sample processing must be completed within 8h of collection
- If more than 30 minutes lapses between sample administration to filter towers, re-autoclave
- All samples should be stored in cooler or refrigerators during processing
- Ethanol-wash all benchtops in preparation for sample handling

1) Sterivex Freezing: remove caps and dry-flush each sterivex (used syringe), replace caps, frz -40C
2) Culturing Membrane Filtration:
    a. Organize Filtration plan (sample order, volumes, and blanks) and record in logbook
    b. Place sterile support disks on receiving reservoirs and connect reservoirs to vacpump
    c. Place sterile membrane on support disk, squirt sterile rinsewater to saturate membrane
    d. Seat filter tower on membrane, tighten collar (a quick reverse spin to avoid misthreads)
    e. Shake sample bottle vigorously 25X to mix
    f. Using a new sterile serological administer 100ml and/or 30ml of sample to towers
    g. Place sample bottle in front of respective towers to avoid confusion
    h. Label petri dishes with pads with unique ID on bottom (Millipore etching is bottom)
    i. Add complete ampule m-FC/Rosolic media to each plate with pad and cover to absorb
    j. Run pump until filters are dry (recommend lowest vacuum pressure of <10 cm Hg
    k. While pump is running, squirt-rinse each tower with 20-50ml autoclaved DI water (20s)
    l. Turn off pump and lift-remove filter funnels vertically and invert on benchtop
    m. Using sterile tweezers place each filter face-up on m-FC-soaked pad, close & invert dish
    n. NOTE: avoid bubbles between filter and pad/agar by rolling filter onto surface
    o. NOTE: Run 100ml autoclaved DI Blank at start, end, and after every ten filters
    p. NOTE: after 3-4 filters per tower empty reservoirs into sink (after Blank 2 is a good time)
    q. Place stacks of 5-6 plates inverted into snack packs + water bath 44.5C 22-26h
    r. After 22-26 hour incubation, transfer filter face-up to NA-MUG plate, incubate 35C 4h
3) qPCR Membrane Filtration
    a. As above steps a-g, but filter 2 rounds of 400ml per filter, emptying reservoir between
    b. Rinse after second round of 400ml as above step k
    c. NOTE: Blanks continuous with previous filters through this filter set (ie every 10 filters)
    d. NOTE: Measure volumes using scale on filter tower, not serological pipettes
    e. NOTE: Vacuum may need to be increased on second round – 25 cm Hg maximum
    f. NOTE: if one sample is slow, hook directly to pump and remove others (process filters)

   g. Roll filter (tweezers/needle), sample inside, and freeze -40C labeled bead tube (MoBio)
4) Flow cytometry cryovial sampling
   a. AFTER all sample filtration, aliquot 1ml each sample into labeled cryovials
   b. In fume hood, add 50 µL paraformaldehyde (PFA, in 1ml aliquots in fridge)
   c. Invert tube 5-10 times to mix, freeze -40C in tube storage box
5) Count plates and record counts for all plates (use long-wavelength UV for NA-MUG agar plates)
   a. Select the highest volume dilution
   b. When colonies adjoin, they are counted separately when clear "pinching" is evident
   c. Fluorescence of *E. coli* on MUG agar is best differentiated from the plate bottom
6) Cleanup and reset
   a. Wash all sample bottles in sink (rinse - no soap or scrub) – autoclave before next use
   b. Wash all syringes and leave on benchtop to dry – autoclave in pouches before use
   c. Glassware should be washed with soap (VersaClean 1:50) and scrubbed (brush)
   d. Rinse used freezer ziplocks and dry and label for use as field trash bags
   e. Dry and ethanol-wash all benchtops (ethanol and bleach are in hazardous cabinets)
   f. Check inventory of all consumables
   g. Autoclave Petri waste (autoclave bags S503971)
   h. Autoclave tweezers/needles, filter towers and support disks, squirt bottles with DI
   i. Clean and check water baths
   j. Decommission Water baths and Autoclave if not planning work for > 1 week

Calibration with cultured standards (Microbiologics EZ-FPC pellets):

1) Autoclave 500ml water in 1L polypropylene bottle and cool to 35degC
2) Warm pellets to room temp
3) Place one pellet in bottle; calculate CFU/mL
4) Incubate 35degC 30min then vigorously shake; Filter within 30 min of end of incubation period
5) Filter two dilutions equivalent to 30 and 100 CFU/filter for direct culturing
   a. For highly concentrated pellets, add just a few mL to a larger volume of autoclaved DI
   b. For less concentrated pellets filter at least 30ml…if need to filter less add DI to mix
6) Carry out membrane filtration incubations as described above
7) Filter two dilutions equivalent to 100-300 and 1000+ CFU/filter for qPCR
   a. For concentrated pellets, this might be 80ml and the remaining 400+ ml in the bottle
   b. For less concentrated pellets perhaps just one filter of 300-800 CFU/filter would work
8) Store filters for qPCR as described above
9) Notes on sterility in this procedure:
   a. All serologicals must be disposed of in autoclave waste
   b. Use a new pair of tweezers/needles for each species of bacteria
   c. Rinse towers thoroughly
   d. Return all filtrate to original sample bottle – add water to 750ml and autoclave
   e. Bleach (10%, 15min) filter funnels, support disks, and tygon tubing; rinse thoroughly
   f. Autoclave filter funnels, support disks, tygon tubing, and reservoirs
   g. Sterilize all benchtops with 5% bleach followed by 70% ethanol wipes

h. Change gloves frequently and place in autoclaved waste
i. Autoclave waste all incubation plates and anything else that contacts the cultures