AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY

STANDARD OPERATING PROCEDURES FOR MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS (ENTEROCOCCI ENUMERATION *AND BACTEROIDALES* HUMAN SPECIFIC MARKER HF183– SOP #024W

1. Enterococci enumeration

1.1. Cleaning of bottles to be used for sampling

1. Wash each sample bottle and cap with a brush and phosphate-free detergent.
2. Rinse three times with distilled water.
3. Immerse bottles for 10 min in 10% HCL.
4. Rinse bottles three times with deionized water.
5. Leave bottles to dry and cap after drying.
6. Seal bottles across the cap and the bottle with sterile indicator tape.
7. Autoclave bottles (American Sterilizer Corp) at 121^{o}C, 15 psi for 15 min. Do not remove "sterile" indicator tape after autoclave.
8. After autoclave, place bottles in plastic box or container for storage.

1.2. Grab sampling[1]

1.2.1 Laboratory

1. Identify bottles to be used for sampling during the incursion.
2. Adhere identification labels on bottles.
3. Fill out labels with all of the required information.

1.2.2. Field

1. Put on disposable, powder-less gloves.
2. Select bottle to be used for sampling at the particular site and fill out any other missing information required in the label.
3. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches; fill the sample bottle about one-quarter full, cap bottle, shake gently.
4. Discard rinse water by swirling the solution out of the bottle.
5. Repeat the procedure (steps 3 and 4)
6. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches, fill the sample bottle to the top and cap.
7. Place bottles in a cooler with ice, shielding from direct sunlight.
8. Transfer samples to UPRM-BNF laboratory
9. Process samples for fecal indicator bacteria within the allowed time limit. 6 hours is allowed for samples to reach the lab with an additional 2 hours is allowed for the analysis. The analysis must be <u>completed within 8 hours of collection</u>.

[1] See Appendix 2 (APPENDIX 2. STREAM WATER SAMPLING FOR NUTRIENTS AND MICROBIAL INDICATORS IN WATER, SOP #019W), Section 3.0. for details.

1.3. Analysis fecal enterococci[2]

1. Permit water sample in each sample bottle to reach room temperature
2. Transfer a 100 mL water-sample aliquot to sterile 100-mL manufacturer supplied polystyrene bottle; this will be the undiluted sample
3. Transfer 10 mL of water sample to a 90 mL sterile dilution tube (this will be the diluted samples and must be labeled as 10^{-1} D); if further dilution is needed the 10-1 D sample must be used and the procedure repeated.
4. Transfer the 10-1 D sample to 120-mL polystyrene bottles
5. Mix each sample with manufacturer-supplied growth medium until dissolved.
6. Pour the contents of each bottle into sterile Quanti-Tray® panel containing 97 wells and heat-seal.
7. Incubate Quanti-Tray® panels for fecal enterococci enumeration at 41 ± 0.5°C for 24 to 28 hours after sealing.
8. Determine the presence of fecal enterococci wells by detection of fluorescence with UV light at 365 nm.
9. Use a manufacturer-supplied table to convert the number of positive wells to most probable number (MPN) values. As needed use the proper dilution used in each subsample to quantify final MPN values.

2. *Bacteroidales* human specific marker HF183

2.1. Cleaning of bottles to be used for sampling – Follow procedures as in section 1.1.

2.2. Grab sampling – Follow procedures as in section 1.2.

2.3. Sample Processing

1. Each sample will be filtered twice and labeled as A and B.
2. Label tubes (MoBio DNA extraction).
3. Set up vacuum filtration unit. Unit consists of side-arm vacuum flask (500 – 1000 mL) fitted with a filter holder (for 25 mm filters) with capacity for > 100 mL water sample. Filter units must be washed, scrubbed, and well-rinsed prior to use.
4. Rinse filtration unit thoroughly (without filter) with 70% methanol and vacuum dry.
5. Place filter membrane (nitrocellulose, 0.22-µm-pore-size GSWP, Millipore, Cat. # GSWP04700) on filter unit using flame-sterilized forceps. Do not touch filter with your hands/fingers. Preferably wear gloves.
6. Filter 100 mL of water sample. You may use less water if the sample is cloudy/high in suspended solids and refuses to go thru the filter, but you need to note how much water sample is filtered.
7. Remove filter membrane from unit using flame-sterilized forceps. Using a second set of flame-sterilized forceps, roll filter loosely and place it in labeled tube. The procedure is show in the following video: http://www.mobio.com/water-dna-isolation/powerwater-dna-isolation-kit.html . Try not to place lid of tube down on bench while doing this as you want it to remain sterile.

[2] Enumeratation of fecal enterococci with the Enterolert™ system (IDEXX Laboratories, Westbrook, ME).

8. If you are going to reuse filter units, you need to wash, scrub, and rinse them before reusing them.
9. Run a blank extraction using ~100 ml of the purest water available (de-ionized/ distilled water or better quality). The blank should show the absence of human specific marker HF183 - be sure you use a clean/methanol-rinsed filtration unit and sterile forceps!
10. Freeze tubes at -20°C once filtration is finished.
11. Place tubes in a cooler with ice packs and send by overnight courier to GSU.

Notes:

- Aseptic techniques must be followed at all times.
- Manage all samples, glassware and materials in accordance with Good Laboratory Practices.
- Dispose and wash all materials in accord with laboratory SOPs and Good Laboratory Practices.