STANDARD OPERATING PROCEDURES FOR
Bacteriological Monitoring

Mobile Baykeeper, Inc.

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Approved by:

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STANDARD OPERATING PROCEDURES (SOP)

FOR BACTERIOLOGICAL MONITORING

I. OBJECTIVE

Mobile Baykeeper is committed to ensuring citizens in the Mobile Bay Watershed and coastal communities have clean water, clean air, and healthy communities. Mobile Baykeeper has several programs that utilize water quality testing to inform citizens about where and when it is safe to swim, fish, paddle, and enjoy. The water quality testing conducted looks for a bacterium known as enterococci, an indicator for fecal contamination of the waterway.

II. SCOPE

The following document provides the standard procedures for sample collection, analysis, quality control, and all activities related to bacteriological monitoring conducted and overseen by Mobile Baykeeper program staff.

III. OVERVIEW OF METHODOLOGY

Mobile Baykeeper is grateful to have acquired an IDEXX system to use the Enterolert Test to detect enterococci through an U.S. Environmental Protection Agency (EPA) Environmental Education grant. The Defined Substrate Technology (DST) nutrient indicator fluoresces when metabolized by enterococci. This test is approved by the EPA as an accurate method for enterococci detection in ambient waters: including fresh, marine, or estuarine waters used for recreation, industry, navigation, as a source for drinking water facilities, and also for groundwater and wastewater tests.

IV. DEFINITIONS

- *Collector* : shall be the individual who collects the water sample and handles the sampling vessel.
- *Processor* : is the individual who processes the sample in the laboratory and reads the sample after incubation.

V. SAMPLE COLLECTION PROTOCOL

A. Staff Responsibilities and Qualifications

a. All individuals who plan to collect samples must first review Mobile Baykeeper's SOP for Bacteriological Monitoring.

b. All individuals must be approved by the Program Director prior to sampling by demonstrating a clear understanding of the protocols and the completion of a bacteriological training course offered by the Program Director and/or Program Coordinator.

B. *Equipment*

a. List of Equipment : Sterile NASCO Whirl-Pak or other sterile bottle with sodium thiosulfate, cooler with ice (if longer trip is planned, place ice in ziploc bags to prevent melted water touching samples), Chain of Custody (COC) Form, Disposable sterile gloves, Antibacterial gel (with at least 70% ethyl alcohol), Permanent markers, and additional items as needed.

b. All equipment are stored in the Laboratory section of the office in a clean, dust-free, and dry environment within their original packaging containers not to be opened until use.

c. Equipment that is considered disposable will be disposed immediately after use is completed in a designated trash receptacle so as not to contaminate samples or unused sterile equipment.

d. Coolers will be washed with warm water and mild detergent and dried/wiped down with paper towels. Coolers are to be washed every time sampling occurs once all samples have been processed.

C. *Sampling Collection Technique*

a. Create a sample blank at the laboratory and place in the cooler with ice prior to departure (see Section V - E. for more detail).

b. Once at the site location note the following in the Field Notebook:

i. Weather conditions

ii. Stream conditions (current, flow, any smells or discoloration)

iii. Any difficulty in the sampling process

c. Place a pair of clean disposable gloves on and check for tears or holes.

d. Take a clean, sterile NASCO Whirl Pak and label the following information with a permanent marker:

i. Site ID (as described in Section VI)

ii. Sample ID (as described in Section VI)

iii. Collector Initials (First letter in first name and first letter in last name, collectors with duplicate initials will receive a numeric definer).

e. Collect surface water using a clean, sterile NASCO Whirl Pak or other appropriately sized, clean, sterile bottle treated with sodium thiosulfate and preserved on ice for transport to the laboratory.

*Safety Note: Sodium thiosulfate has a low toxicity but can cause eye irritation, please use caution.

f. Whirl-Paks and bottles (also referred to as sampling vessel) should never be pre-rinsed to remain sterile before collection.

g. Tear the top from the Whirl-Pak or remove the casing from the bottle. Holding near the bottom, submerge the sampling vessel into the water with the opening faced down, taking care to avoid surface scums. The surface film is often enriched with particles and bacteria that are not representative of the water mass as a whole. To

avoid contamination, position the mouth of the sampling vessel away from the collector's hand.

h. Sampling depth should be approximately 6 inches below the water surface. After reaching the sampling depth, tip the sampling vessel up. Pull apart the Whirl-Pak tabs keeping glove hands behind the opening of the bag to avoid contamination.

i. Tightly seal Whirl Paks with a minimum of three revolutions and twist metal ties together at least three times. If using a bottle, tightly close the bottle cap. Once properly sealed, immediately place in the cooler of ice.

j. Dispose of gloves, Whirl Pak/bottle plastic seals and sanitize hands with the antibacterial gel.

k. Only samples can be stored in the sampling coolers. Food and beverages must be stored in a separate, designated cooler - never to placed in the sampling cooler.

l. Site Specific Sampling

- *i.* Coastal Beach/Shoreline- in a location like this, it is often not possible or beneficial to retrieve a "mid-stream" sample. To grab a sample from this environment, wade out into the waters until you reach a water depth of at least 3 feet. Be careful not to disturb the sediment, if sediment becomes disturbed, wait until the sediment settles in the area before sampling.
- *ii.* Wadeable Stream - many locations can be sampled using waders, hip waders, or high boots. To grab a sample, ensure that you wade into the stream downstream of where you intend on taking the sample, so as not to disturb the bottom substrate.
- *iii.* Boat/Kayak based - in some locations, the sample desired may only be accessible by boat, kayak, or other floating device. In this case, ensure that the bow of the vessel is positioned into the current and turn off the engine/stop paddling. Grab the sample off the bow, away from any wake and avoid contact with the vessel.
- *iv.* From a Bridge - Access rights and overgrowth may prevent accessibility of waterways. The collector should take precautions when bridge sampling, particularly when minimal shoulders exist on the road. It is advised to park before the bridge on the shoulder with vehicular hazard lights turned on. Safety neon vests are available for collectors to wear when bridge sampling. To grab a sample from the bridge, use the Whirl Pak Bridge Sampler (shown in image below) and lower to the water (downstream of where you intend to collect) without creating any slack in the line. Once in the water, pull the line upstream and then rapidly out of the water, avoiding any contact with the bridge or nearby objects (tree limbs, etc.)
- *v.* From a Dock or Pier - when sampling off of a dock or pier, walk out to the end of the platform and position body so that the current is facing you and begin collecting the sample away from the pier.

vi. Sanitary Sewage Overflow (SSO) - extra care should be taken by the collector with SSO sampling. To grab the sample, select the highest flow section of the SSO and tilt the sampling vessel towards the flow so as to fill the vessel (like one would fill a water bottle in a fountain). Make sure the sampling vessel does not touch the manhole etc. to prevent contamination.

vii. During Inclement Weather - sampling may be conducted during heavy rain and wind as long as the conditions are safe for the collector to follow typical protocol. It is the Program Director's decision whether or not to head out but it is the collector's discretion in the field whether or not the conditions are conducive for sampling.

D. Sample Handling & Preservation

a. Samples are transported on ice in a cooler held below 10° C (50° F). They are never to be frozen or submerged in water.

b. Samples must be transferred to the Mobile Baykeeper Laboratory within 6 hours after sample collection time.

c. A Chain of Custody (COC) form must be filled out and maintained for each sample collection. The COC form will reflect any transfer of samples from one individual to another and when the collector exchanges the samples with the designated staff processor at the laboratory.

d. Every sample and its collector/processor can be traced and accounted for through the COC, field notes, and staff time sheet tracking.

E. Field Quality Control & Quality Assurance

a. Field blanks will be used as a quality assurance, therefore a field blank should be included in the every sampling cooler prior to leaving to collect samples. If a field blank returns a bacteria count greater than 1, the corresponding samples from that day should be excluded from public reporting/campaigns.

b. Duplicate samples may be used for field quality control as specified by the Program Director. If the collector feels that the collection of a sample may have been compromised, a duplicate sample must be taken.

VI. SITE ID, SAMPLE ID, AND CHAIN OF CUSTODY

A. Site ID

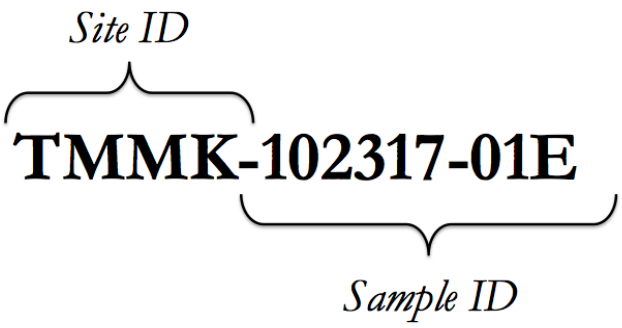
a. All site locations must receive a designated two letter waterway ID and two letter location ID assigned by the Program Director. This ID indicates the waterway and location that the sample was taken and provides streamlined data analysis.

i. Example Three Mile Creek at MLK drive = TMMK

b. The Program Coordinator will maintain the Site Location List containing all site IDs ensuring there are no duplications or undocumented IDs.

B. *Sample ID*

a. The sample ID consists of the DATE in the following format month ##, day ##, and year ## (i.e. 102317 for October 23, 2017), a two-digit SAMPLE NUMBER (i.e. 01 for the first one, 02 for a duplicate, etc.), and a one letter designating the PARAMETER (i.e. E for Enterococci).



C. *Maintaining Sample Custody*

a. The possession of samples shall be traceable from the time samples are collected until the samples have been analyzed and properly disposed of. A sample is under custody if *1)* it is in the individual's physical possession, *2)* it is in the individual's view in a designated secure area.

b. The collector is responsible for the care and custody of the sample until it is transferred to the processor. It is important to have as few people as possible handling the sample.

c. A single COC form may contain multiple records of samples collected just as long as each sample collected has a COC record.

d. If corrections are necessary on the COC, they can be updated by placing a single line through the incorrect information and then provide the correct information next to with the initials and date that correlate to this change.

e. The processor may not accept samples collected that do not have a COC form completed.

D. *Transfer of Custody*

a. To transfer the possession of samples, the individual relinquishing the samples must sign, date, and record the time (military format) that they relinquished the samples. The individual receiving the samples must then verify the number of samples and sign, date, and record the time (military format) that they received the samples. There should be no lapsed in time between the relinquished and received times on the Chain of Custody form.

VII. LABORATORY PROCEDURES

A. Staff Responsibilities & Qualifications

a. All individuals who plan to process samples must first review Mobile Baykeeper's SOP for Bacteriological Monitoring.

b. All individuals must be approved by the Program Director prior to processing samples by demonstrating ability to conduct these techniques and the completion of a bacteriological training course offered by the Program Director and/or Program Coordinator.

B. Laboratory Processing Procedures

a. Turn on the IDEXX Sealer and incubator to 41° C, the incubator can take more than an hour to stabilize in temperature and should be turned on either prior to leaving to sample or by a designated staff member in the office two hours prior to sample incubation.

b. Transcribe information from the COC form onto the datasheet and set to the side.

c. Turn on the Quanti-Tray Sealer Plus and make sure the monitor screen displays 5 yellow rectangles and one green rectangle indicating the sealer is ready.

d. Put gloves on and use Ethanol or Antibacterial gel (with at least 70% alcohol) to sanitize, allow to dry before proceeding.

i. Replace gloves any time throughout the process if you have tears, anything splashes or spills on the gloves.

e. Pull out samples and place Whirl Paks and/or bottles in the Sample Rack located on the laboratory counter.

f. Grab a sterilized IDEXX 120mL disposable bottle treated with sodium thiosulfate from the shelf, this bottle should be sealed, if the seal is compromised do not use and grab another.

g. Label the bottle with the Site and Sample IDs. Remove the seal by twisting counter clockwise to open and set the bottle cap on the counter with the inside facing upward.

h. To dilute the sample, take the fixed volume pipettor and a sterile individually wrapped 1 mL (1000 □ L) pipette tip to dispense the chosen amount of the sample into the bottle. The pipettor is set to 1 mL meaning a 5/100 dilution would be 5 pipes of the sample). Dispose the pipette tip in the designated lab receptacle.

i. The Dilution Table is posted on the laboratory wall and contains all dilutions listed with their corresponding detection ranges for reference.

ii. Sample blanks should never be diluted.

iii. Sample dilutions must be processed within 20 minutes.

i. Fill the remainder of the bottle to the 100 mL line (shown in image below) with distilled water from the designated 500 mL Wash Bottle labelled "Distilled Water".

i. If the sample bottle is filled over the 100 mL line, the process must be restarted using a new, fresh bottle and new sterile pipette tip so as to not

affect the dilution of the sample. Access water can never be removed from the original bottle when using a dilution factor.

j. Reseal the original sample vessel to avoid contamination of other samples.

k. Open either one Colilert/or Colilert-18 packet into the processing bottle and seal. Ensure that when the packet is snapped open, there is no contamination with the neck of the packet opening prior to pouring. Shake vigorously until the mixture is completely dissolved and let sit until any foam subsides.

l. To pour the sample solution from the bottle into an IDEXX Quanti-Tray 2000 follow these specific instructions:

   i. Use one hand to hold the Quanti-Tray upright with the well side facing the palm. Squeeze the upper portion of the tray so that the plastic bends towards the palm. Gently pull the foil by the upper tab to separate the foil from the plastic tray. Do not touch the inside of the foil or tray during this process, if the inside is touched or foil is ripped, start over with a new tray.

   ii. Pour the sample solution into the Quanti-Tray avoiding any contact with the foil tab or tray and angle the pour from the foil side (to avoid introduction of bubbles or foam).

   iii. Slowly release the squeeze on the upper portion of the tray so as to close the tray, and tap or lightly knock out any air bubbles.

m. To seal the Quanti-Tray 2000 in the IDEXX Sealer 2X use the following steps:

   i. Carefully place the Quanti-Tray on the rubber insert so that the tray is properly aligned with each well of the tray within its corresponding rubber hole.

   ii. Slide the tray and rubber insert into the Sealer (with the tray facing upward) until the rollers grab the rubber insert and begins to draw it into the Sealer slot without assistance.

   iii. The tray will run through the Sealer to the opposite side (after approximately 5 seconds) where it will be ejected. Remove the rubber insert and tray and place on the counter.

   iv. If the tray becomes misaligned or needs to be removed for any reason, press the "Reverse" button. This button is not to be used if the rubber insert has been fully drawn into the Sealer slot. If the tray becomes ripped or stuck, do not use the sample tray, begin full process over.

n. On the back of the sealed Quanti-Tray 2000 record the Site ID, Sample ID, and incubation start time (when all samples have been processed and are ready to be incubated). Labelling should always be done after tray is sealed, never before.

o. Place sealed and labelled Quanti-Trays into the incubator (that must read 41° C consistently) on one of the racks. There can be up to 6 trays stacked on one another and no more than 36 trays can be in the incubator at one time. Once all sample trays are placed in the incubator, close the incubator door ensuring the door seals completely.

p. Record the incubation start date and time, the dilution used, and the processors first and last name on the "Bacteriological Monitoring Datasheet".

q. Turn off the Quanti-Tray Sealer Plus when finished and dispose of any used bottles and gloves. Wipe down the lab counter and wash hands thoroughly.

C. *Procedure for Reading Results*

a. The sample Quanti-Tray should remain in the incubator for the appropriate incubation time (18-22 hours for Colilert-18 and 24-28 hours for Colilert). After this point, remove the sample from the incubator and record the incubation end date and time in the "Bacteriological Monitoring Datasheet".

b. All tray counts must be completed within 30 minutes of the incubation end time.

c. To detect fluorescence, turn on the Spectroline UV Light Emitter and place sample tray under the UV light.

d. To count total coliform bacteria, take a permanent marker and place a vertical line on the positive wells (wells that fluoresce under the UV light). Start with the large wells (including the large rectangular well at the top of the tray) and then move on to the small wells at the bottom.

e. Once all positive wells have been marked, count the number of marks and use the IDEXX Quanti-Tray 2000 MPN Table (located on the laboratory wall) to find the Most Probable Number (MPN). Take the number located in the intersection between the number of large wells and the number of small wells found in your sample and multiply by the appropriate number from your dilution factor.

f. This number will be your final MPN Enterococcus CFU/100 mL, record this in the "Bacteriological Monitoring Datasheet" in the column labelled "Final Coliform Count".

g. Once all trays have been read, turn off the incubator and UV light emitter. *(maybe add data logging for temperature accuracy verification of the incubator)

h. All sample trays must be digitally documented and stored on the Mobile Baykeeper server. Scan the sample wells by selecting a 2-sided scan, placing the each tray with the Site and Sample ID facedown on the scanner 1st and marked wells facedown 2nd.

i. Send this scan to program director, Cade Kistler's scan folder where it will be renamed as Site ID_Sample ID_Count (as ##CFU)(Example: TMMK_102317_01E_104CFU) and relocated in Dropbox folder Water Quality > IDEXX > Testing > IDEXX Well Scans.

D. *Laboratory Safety and Sterility*

a. Safety is of the utmost importance, staff members and any trained individuals must understand all procedures laid out in SOPs, following all instructions carefully to ensure the overall sterility and safety of those working in the laboratory.

b. All processors must be diligent in their use of antibacterial gel/ethyl alcohol to maintain sterility and protect themselves against harmful bacteria.

c. The laboratory space must always be organized, clean, and utilized for sample processing only.

d. Food and drink must not be consumed on the laboratory counter or at any time during sampling or lab processing.

e. Laboratory countertops and areas will be wiped down with disinfectant wipes before and after every use. Clean aluminum foil may also be used to lay over the counter.

f. In the event of a spill (sample or anything else), immediately disinfect and cleanup the area. Notify the Program Director of the spill and describe the cleanup process used.

g. Once per month the Quanti-Tray Sealer must be cleaned. To clean, first ensure the power is off and unplugged. Let cool for at least 90 minutes. Next remove the tray shelf and loosen all four quarter turn fasteners and remove panel. Using a mild detergent or sufficient substitute (diluted bleach, isopropyl alcohol, etc.) clean all surfaces including inside the sealer and roller spaces. Do not use abrasive materials or caustic cleaners. Dry surfaces and reattach panel and the tray shelf.

E. Laboratory Quality Control and Quality Assurance

a. Logbooks and field notebooks will be periodically reviewed by the Program Coordinator and/or Program Director for accuracy.

b. A second read on well counts by another trained individual will be conducted for approximately once a month on positive samples. Well counts by both individuals must agree within 5%.

VIII. DATA MANAGEMENT

a. The Program Coordinator and Program Director are responsible for ensuring all data are recorded and archived properly.

b. All data will be reviewed by the Program Coordinator and/or Program Director before they are distributed to the public or used to inform program campaigns.