Abalone Coast Analytical Laboratory
Quality Assurance Manual

This manual has been reviewed and determined to be appropriate for the scope, volume and range of testing activities conducted at Abalone Coast Analytical Laboratory. The procedures outlined in this Quality Assurance Manual are to be implemented and followed at all times.

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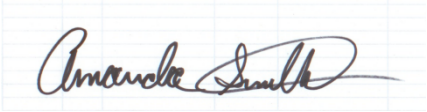
Prepared By:_  _____10/19/16_____
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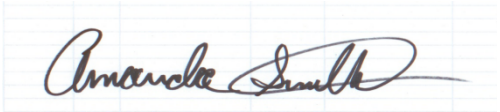
Approved By:_  ___ __10/19/1_____
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Abalone Coast Analytical

Quality Assurance Manual

(Standard Methods for the Examination of Water and Wastewater 9020)

Laboratory Organization/Responsibilities

Abalone Coast Analytical Owner and Lab Director, Amanda Smith is responsible for all daily operations in the laboratory. Amanda Smith graduated from UC Riverside with a Masters of Soil Chemistry degree and a Bachelor of Science (Suma Cum Laude) degree in Environmental Sciences. She was a senior chemist for the State of Idaho Food Quality Assurance Laboratory and a semi-volatile organic chemistry assistant manager at Zymax Envirotechnology Laboratories in San Luis Obispo.
The Laboratory Director will be responsible for contacting clients to report compliance failures, plan projects or contract-related services, including, specifications of work to be accomplished and estimates for that work.

Caitlin (Galloway) Kennedy is the Project Manager and undertakes much of the responsibility client contacts, scheduling the crew, maintaining inventory, marketing, human resources and running company meetings. Her duties sometimes include sample receiving, data entry and reviewing final reports for errors and omissions before final signature by lab director.

Quality Assurance Objectives

- All samples are stored and testing initiated within guidelines (CA DHS-ELAP).
- All aspects of testing are under control guidelines established in each protocol.
- All raw data is reviewed for accuracy before publishing.
- All Custody and Sample Receiving documentation is complete and accurate.
- Microbiological data reproducibility (grab samples) within ranges of 95% Confidence Interval.
- Implement protocol per SM.9020

Sampling Procedures

Sampling for bacteriology is undertaken with sterile 120-mL sample containers with sodium thiosulfate added to neutralize chlorine present in the sample. These containers are distributed to clients for their sampling needs by the laboratory. Non-sterile containers and samples with excess chlorine are not acceptable for microbiology. When the laboratory is doing the sampling, staff will use the following procedure:

1. Wipe faucets clean of debris, remove screens and sterilize with flame or bleach.
2. Run the sample station faucet for at least 3 minutes or until at least three volumes of the pipeline is flushed. During this time write pertinent information on sample jar.
3. Obtain a steady flow of water and remove the cap of the bacteriology jar. Without touching the cap or the inside of the jar, allow sample to flow into the jar to the 100-mL mark. Carefully replace the lid. Never pre-rinse or over fill as thiosulfate preservative may be lost.
4. Immediately transfer sample to a cooler maintained at 4 degrees Celsius and transfer to the laboratory.
5. Upon arrival at the laboratory check the condition and temperature of the sample jar and record with other client and sampling information on the Chain of Custody.
6. Write the unique Lab ID number on the body of the bacteriology jar and cap and transfer to holding refrigerator until testing is initiated.
7. If sample container is compromised or chlorine is still present at a concentration that cannot be neutralized by the sodium thiosulfate, inform the Laboratory Director immediately so the client can be notified and the sample location can be resampled.

Custody, Holding, Sample Disposal

- The Chain of Custody will contain the following elements:

1. Name and address of client
2. Signature and/or printed name of sample collector
3. Sample Number (client & lab)
4. Number of containers
5. Sample collection site
6. Sample collection date and time

7. Sample type/matrix
8. Unique sample ID number (by lab)
9. Sample container type and size
10. Required test/ analysis
11. "Relinquished by" signature, date, time
12. Several "Received by" fields with signature, date, time
13. Method of shipment
14. Preservation of sample throughout shipment (i.e.: temperature/ + sodium thiosulfate)
15. Condition of sample upon arrival at lab (cracks, temperature, residue)

- The Lab will hold bacteriology samples only until they out date and all other samples for one month. Acid preserved samples are not accepted unless being held for transit to another lab. Obviously toxic samples will not be handled without special arrangements with clients for disposal. Toxic waste must be appropriately stored and labeled for transport to a hazardous waste site if not returned to client.
- The lab is generally dealing with biological waste that must be disinfected before disposal. Autoclaving @121 C for 30 minutes, chlorination or dry heat evaporation above 180 degrees Celsius will accomplish this task. If bleach is used for disinfecting caution must be exercised in disposing lab waste to small biological waste systems such as septic tanks or small waste treatment plants.

Equipment, Facility and Supply Quality Control Procedures

- **Refrigerators**. Refrigerators must be maintained between 1-4 degree C (recorded once daily).
- **Balance**. For weighing samples and media use a balance with a sensitivity of 0.1g per 150g load. Check accuracy at least monthly. Maintain and clean per manufacturer instructions.
- **Incubators**. Incubators for coliform and plate counts should be kept at a temperature of 35 +/- 0.5 degrees Celsius. Compare incubator thermometer to NBS traceable thermometer at least twice annually to establish true temperature range. Temperature should be documented twice daily at least 4 hours apart.
- **Water bath**. Water baths for fecal coliform tests should be kept at a temperature of 44.5 +/- 0.2 degrees Celsius. Compare water bath thermometer to NBS traceable thermometer at least twice annually to establish true temperature range. Temperature readings should be documented twice daily at least 4 hours apart.
- **pH meter**. Run a two or three point calibration daily (pH 7,4,10) before use of the pH meter. Additionally, run a second pH 7 standard from a different buffer lot or manufacturer. Document results. Tolerance limit is +/- 0.1 pH units vs. standard.
- **Microscopes**. Field of view diameters for each objective should be calibrated initially and with any replacement of objectives. Maintain and clean the instrument per manufacturer instructions. Document calibrations and maintenance schedules.
- **Autoclave**: Test and document autoclave effectiveness at least monthly with spore ampules of *Bacillus stearothermophilus*. Check and document autoclave timer

accuracy vs. an accurate timer at least monthly. Each autoclave run will be documented including date, run time, time-in & time out; total time elapsed from start through batch removal and identification of the batch. Each batch should have sterility strip to indicate a sterile run and a temperature reading from an autoclave thermometer.

- **Thermometers**. Check each thermometer twice annually against an NIST traceable thermometer in the range at which the thermometer will be utilized. The "Correction factor" for NIST Thermometer is determined by a qualified Certification company. Use NIST thermometer results to make corrections in the operating ranges of equipment thermometers and document these ranges on temperature data sheets accompanying each piece of equipment. The "Correction factor" for the thermometer being calibrated is calculated by subtracting its observed temperature from true temperature of NIST Thermometer. True temperature of Thermometer being Calibrated = Observed Temp. + correction factor for that thermometer
- **Bacteriology sample containers**. Each new lot of bacteriological sample jars will be examined for presence of preservative and sterility. Sterility is tested by adding 20-mL tryptic soy broth to a random sample container from the new batch. Incubate 24 hours at 35 degree Celsius and check for turbidity visually. No turbidity indicates a sterile batch of sample containers.
- **Plastic Sterile Pipets**. Check each new lot of pipets delivering volumes of 10mL and 1mL for accuracy within 2.5%. Additionally check and verify 2 drop delivery (0.1mL) for accuracy within 2.5%. Record and maintain documentation for each.
- **Air Quality Monitoring**. Air quality will be monitored at least monthly in the microbiology area. Expose Plate Count Agar petri plate to open air and another unopened control plate for 15 minutes. Close lid and incubate as plate count protocol dictates. Examine at 48 hours and document colony count per 15 minutes. Air colony counts exceeding 15 cfu/15min indicate questionable air quality.
- **Buffered dilution water**. Each new lot of buffered dilution water acquired from a commercial source will be examined for sterility. Sterility is tested by adding 10-mL aliquots of dilution water from a random container to each of five tubes of TSB, or equivalent non-selective media. Incubate 24 hours at 35 degree Celsius and check for turbidity visually. No turbidity indicates a sterile batch.
- **Test for Inhibitory Residue on glassware.** This test is performed before using new glassware detergent or when a new lot of old product is purchased. An experienced microbiologist usually undertakes this test. (SM.20th.9020B.4.a.2.)
- **Test for Bacteriological Quality of Reagent water**. ASTM Type I, II water is exempt from this requirement. De-ionized water will not be produced on site and all reagent water will be purchased as certified reagent grade water ASTM Type I, II.

  Test reagent water for pH and conductivity daily, check monthly for Total Organic Carbon, Organic Nitrogen (TKN) and Ammonia and annually for Pb, Cd, Cr, Cu, Ni, Zn and total heavy metals.

Overview QC Procedures for Microbiology

Abalone Coast Analytical focuses on three fields of Microbiology. Any single field may include more than one method but in general the three fields of interest are:

1. Drinking Water Microbiology
2. Waste Water Microbiology
3. Recreational, Marine Water Microbiology

All Standard Operating Procedures (SOP) for these three fields of study and the procedure for sample receiving are kept together in plain view for the technician's use and available for inspection. The Method Update Rule 2012 (MUR) is included in each chemical protocol listed below in addition to the basic QC requirements included on previous versions.

Rapid Analysis For Total Coliform In Drinking Water MMO-MUG
SM 9223. B.
Sample hold time: 30 hrs.

- Each batch of new Colilert™ (MMO-MUG) is examined for unusual discoloration and stored according to manufacturer's instructions. A control blank and Positive/Negative combinations are run for each new batch of Colilert™ reagent The combinations are as follows:

PRESENT/PRESENT: E.coli
PRESENT/ABSENT: Klebsiella pneumonia
ABSENT/ABSENT: Psudeomonas aereginosa

- For instructions and discussion see Colilert™ booklet included with each box.
- Participate in regular water studies as required by Environmental Laboratory Accreditation Program.

Quality Control Heterotrophic Plate Counts
SM 9215. B.
Sample holding tine: 8 hours

- Each dilution may be run in at least duplicate plates. Historical samples may be run with only one dilution. Sterility blanks are run for media and dilution water (if needed) for each batch of plate counts. Air Quality analysis should be run concurrently with test and at least monthly for compliance with QA/QC (SM).
- Each technician doing this analysis shall be able to duplicate bacterial counts within 90-110% of any others doing the same analysis on the same plates.
- Commercially prepared buffered dilution water shall be tested for sterility before use.
- Each batch of new Plate Count Agar (Standard Methods Agar) is subjected to "USE TEST". ASTM Type I, II water exempt from this requirement
- Participate in regular water studies as required by Environmental Laboratory Accreditation Program

MultipleTube Fermentation: MTF -Total Coliform (Waste/ Recreational Water)
SM. 9221. B.
Holding time: 6, 8 hours, respectively

- Media prepared in-house is subjected to tests which meet criteria listed in Standard Methods
- Reagent water is subjected to a Quarterly "USE TEST" to show suitability of source. ASTM Type I, II water exempt from this requirement
- If using commercially prepared Lauyrl Tryptose Broth (LT) and Brilliant Green Bile Broth (BGBB), test each batch with appropriate control organisms (positive control *E.col;* negative control Pseudomonas aereginosa) and for sterility with a blank tube under test conditions.
- Check for optimum pH range before use.
- Commercially prepared buffered dilution water shall be tested for sterility before use.
- Participate in regular water studies as required by Environmental Laboratory Accreditation Program

Quality Control Multiple Tube Fermentation: MTF –
Fecal Coliform (Waste/ Recreational Water)
SM 9221. E.and E.2.

- Any media prepared in-house is subjected to tests, which meet criteria listed in Standard Methods.
- Reagent water is subjected to a Quarterly "USE TEST" to show suitability of source. ASTM Type I, II water exempt from this requirement.
- Commercially prepared buffered dilution water shall be tested for sterility before use.
- Inoculate each new batch of prepared EC and/or A1 media with appropriate control organisms (positive control *E.col ;* negative control *E. aerogenes*) and for sterility with a blank tube under test conditions.
- Check for optimum pH range before use.
- Run a positive and a negative control (*above*) in EC and/or A1 media with each batch of samples sharing the same warm water bath. Invalidate results if controls are not typical.
- Participate in regular water studies as required by Environmental Laboratory Accreditation Program

Acquisition, Reduction, Validation, Reporting Data

The analyst documents microbiological data on the raw data sheet. Upon test completion the raw data is reviewed /validated before these results are published in a final report. Each raw data worksheet for the various bacteriology tests will minimally require:

- Unique ID number assigned by the laboratory
- Method of Analysis
- Media type with traceable lot numbers
- Date and time samples prepared with analyst initial
- Date and time samples placed in the incubator with analyst initial
- Date and time samples removed from the incubator with analyst initial (repeat above if needed)
- Results (all math, 15-tube positive combinations and/or assumptions clearly visible)
- Analyst signature or initial and Reviewers signature or initial

To ensure legal defensibility of the results the final report will include the following:

- Name and address of our laboratory Abalone Coast Analytical, Inc. on letterhead
- Unique ID number assigned by the laboratory
- Method of Analysis
- Results of Analysis
- Analysis completion date
- Signature of Laboratory Director (Amanda Smith)
- Date of report preparation.
- Records of all analyses kept by Abalone Coast for a minimum of 5 years.

Internal Quality Control Checks

All media and supplies are subjected to performance criteria listed in Standard Methods 9020.
Test each batch of rapid test products; such as Colilert, with appropriate control organisms.
All media is checked for optimum pH range before use (See: Quality Control SOP).
The lab technicians will demonstrate competency per test with at least one week of training and duplicate samples.

(Method Update Rule: June 2012)

The Method update format for chemical analysis (12-point) is included in each of the SOP's for general chemistry. The format is included in the SOP's for microbiology for future upgrades of those QC plans.

Performance & System Audits

The lab will participate in PT water studies regularly.
The Lab Director shall audit random Lab numbers from sample receiving through final report for each quarter. All data and calculations are reviewed on a continuous basis to assure that data is reported properly. To ensure legal defensibility of the results all aspects of the paper flow should clearly identify successive steps and QA/QC performed on each component of that test.

Preventative Maintenance

All laboratory equipment and devices shall be maintained per manufacturer's instructions and a log of all work, cleaning and part changes for that equipment shall be kept available for inspection.

Assessment of Precision & Accuracy

The lab microbiologists and technicians will demonstrate accuracy per method with ERA check samples and PT water and wastewater studies. Additionally all analysts should each demonstrate proficiency and precision by duplicate counts of the heterotrophic plate counts on drinking water studies and/or ERA check samples. Proficiency and precision should be demonstrated at least quarterly. Counts should agree within 10%.

Corrective Action

Because of short holding times and limited sample size the microbiology must be performed correctly each and every time. When any aspect of the testing or QA/QC falls outside limits set in each method protocol, the test is considered invalid and resampling should be requested as soon as possible. Any failure shall be documented with corrective action narrative included. The final report shall include narrative addressing this failure and validity of the results.

Quality Assurance Reports

Quality assurance reports and certificates from suppliers for each of the various products utilized should be kept for each batch and compared to in-house QA/QC analysis of the same parameters (if required). Other in-house studies such as internal audits, detection limits and performance evaluations (PT Studies) shall also be kept available for inspection.