**SOLDOTNA WWTP LABORATORY**

**Soldotna, Alaska**

**Standard Operating Procedure**

**For**

**Fecal Coliform by Membrane Filtration**

**SOP Number: 002r05**

**Revision Date: 03-31-20**

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Table of Contents

[**1.0** **SCOPE AND APPLICATION:** 3](#_Toc132000129)

[**2.0** **DEFINITIONS:** 3](#_Toc132000130)

[**3.0** **HEALTH AND SAFETY:** 3](#_Toc132000131)

[**4.0** **CAUTIONS:** 4](#_Toc132000132)

[**5.0** **INTERFERENCES:** 4](#_Toc132000133)

[**6.0** **PERSONNEL QUALIFICATIONS:** 4](#_Toc132000134)

[**7.0** **SPECIAL APPARATUS AND MATERIALS:** 4](#_Toc132000135)

[**8.0** **INSTRUMENT OR METHOD CALIBRATION:** 5](#_Toc132000136)

[**9.0** **SAMPLE HANDLING AND STORAGE:** 5](#_Toc132000137)

[**10.0** **PROCEDURE AND ANALYSIS:** 5](#_Toc132000138)

[**11.0** **DATA ANALYSIS/CALCULATIONS:** 7](#_Toc132000139)

[**12.0** **RECORDS MANAGEMENT:** 7](#_Toc132000140)

[**13.0** **QUALITY CONTROL:** 8](#_Toc132000141)

[**14.0** **CORRECTIVE ACTION:** 8](#_Toc132000142)

[**15.0** **REFERENCES:** 8](#_Toc132000143)

[**16.0** **FORMS AND DATA SHEETS:** 9](#_Toc132000144)

1. **SCOPE AND APPLICATION:**
   1. This SOP applies to the determination of Fecal Coliform bacteria by Membrane Filtration (SM 9222D).
   2. This method is applicable for determination of Fecal Coliform bacteria counts in our effluent discharge.
   3. In addition the Fecal Coliform counts are reported to the EPA through our DMR. The parameters reported can be found in our NPDES permit, # AK-002003-6.
2. **DEFINITIONS:**
   1. DMR – Discharge Monitoring Report
   2. NPDES – National Pollution Discharge Elimination System
   3. EPA – Environmental Protection Agency
   4. FC – Fecal Coliform Bacteria
   5. Fecal Coliform Bacteria - are defined as facultative anaerobic, gram-negative, non-spore-forming, rod shaped bacteria that develop blue colonies within 24 h at 44.5 oC using m-FC media.
   6. TNTC – To Numerous To Count.
   7. RPD – Relative Percent Difference
   8. Dilution/Rinse Water – Phosphate-buffered rinse water that has been autoclaved to insure sterility.
3. **HEALTH AND SAFETY:** 
   1. The microorganisms of interest are considered to be a bio-hazard. The microorganisms can, under certain circumstances, cause disease in humans.
   2. Treat all samples as potentially hazardous.
   3. Wear protective equipment when working with samples. Eye protection, lab coat and gloves are a must when working with samples.
   4. With the use of methanol for sterilization be sure to use caution due to the flammability of the solvent. **Do not** use around an open flame.

1. **CAUTIONS:** None
2. **INTERFERENCES:** 
   1. If a sample has high turbidity caused by algae, particulates, or other interfering material an alternate method will have to be employed to obtain usable results. The membrane will become plugged not allowing sufficient sample volume to be used.
   2. Samples containing large amounts of non-coliforms or toxic substances may yield low Coliform estimates.
3. **PERSONNEL QUALIFICATIONS:**
   1. Personnel are required to be knowledgeable with the procedures in this SOP.
   2. The laboratory personnel using this method must be trained experienced and demonstrate proficiency in processing, maintaining, storing and disposing of biohazard material.
   3. Analyst must past proficiency samples annually. This can be satisfied by completing the annual DMR QA required by the EPA to maintain our NPDES permit.
4. **SPECIAL APPARATUS AND MATERIALS:** 
   1. m-FC Broth: Commercially prepared media (HACH or equivalent)
   2. Dilution/Rinse Water (SOP 009).
   3. Filtration Apparatus: Calibrated glass or plastic filtration apparatus capable of sterilization.
   4. Membrane Filters: Nitrocellulose, 0.45um, white 47mm girded, sterile (PALL or equivalent).
   5. Millipore Petri dishes with pad, 47mm.
   6. Forceps: Smooth flat forceps, with out corrugations on the inner sides of tips.
   7. Water Bath Incubator: Capable of maintaining 44.5 + 0.2oC.
   8. Microscope and light source.
   9. Quart size Zip Lock Bags
   10. 1 – 100 mL Class A graduated cylinder
   11. 1000 mL Beaker
   12. 400 mL Beaker
   13. Vacuum manifold
   14. Vacuum pump
5. **INSTRUMENT OR METHOD CALIBRATION:** None
6. **SAMPLE HANDLING AND STORAGE:**
   1. To collect samples remove the 1000 mL and the 400 mL beakers from the autoclave (see section 10.1).
   2. Proceed to the effluent weir just after the UV basin. This is located just outside the building on the east side.
   3. Take a sample using the 1000 mL beaker just as the effluent passes over the weir. To insure sample is not contaminated, don’t touch the inside of the container or the lip.
   4. Proceed next to the polishing clarifier and take a sample using the 400 mL beaker just as the water passes over the weir.
   5. The last sample is a portion of the influent for the control positive sample. You can use any container you would like to use for collecting the sample.
   6. If samples can not be processed immediately the sample must be kept at 4.0 + 2.0oC until analysis.
   7. Sample must be processed within 6 hours of collection.
7. **PROCEDURE AND ANALYSIS:**
   1. Place one filter funnel and base, one 1000 mL and one 400 mL beaker, and one 100 mL graduated cylinder into the autoclave. Autoclave the contents using the unwrapped cycle (SOP 006).
   2. When preparing to run the samples wipe down the bench top with methanol to insure the working area is sterile.
   3. Be sure to wash hands and use gloves while handling glassware and samples.
   4. Remove six petri dishes from the package and label them one through six. Number one and three will be for the blanks, two and four will be for the effluent samples, five will be the polishing tank (Clarifier #2) sample, and six will be for raw influent (Control Positive).
   5. Add the entire contents of the m-FC broth ampule to the petri dishes with the absorbent pad. The pad must be saturated with the medium (about 2 mL). Decant the remaining media by tipping the bottom portion of the Petri dish over the sink and allow the excess media to flow out, **do not shake the dish**.
   6. Remove the filtration apparatus from the autoclaved and place on the vacuum manifold. Be sure to note that the autoclave tape is showing the appropriate markings (see SOP 006).
   7. Connect the vacuum manifold tube to the filter flask beside the vacuum manifold and saturate the filter on the base with Dilution/Rinse Water.
   8. Turn on the vacuum pump using the light switch located on the wall directly behind the analytical balance. Be sure to close all of the manifold valves by making sure the blue knobs are in the horizontal position.
   9. Sterilize forceps by dipping in methanol and flaming.
   10. Using the sterilized forceps remove the membrane filter from packaging, making sure not to touch with your hands, and place on the filtration base grid-side up.
   11. Put the funnel top on to the filtration base. At this point you are ready to run samples. Proceed to run samples in order from one to six using the following procedures for each:
       1. For blank samples put 20-30 mL of Dilution/Rinse water into the 100 mL sterilized graduated cylinder making sure to rinse the entire capacity of the cylinder. Pour this into the filter apparatus. Be sure to rinse the walls of the filter funnel all the way from the base to the 100 mL mark. At this point filter the blank.
       2. For both effluent samples make sure to agitate the sample by swirling the 1000 mL beaker gently. Pour 100 mL of the sample into the graduated cylinder. Pour the measured sample into the filter funnel and rinse graduated cylinder twice with about 10-20 mL of dilution/rinse water. At this point filter the samples.
       3. For the polishing tank sample, place approximately 20-30 mL of Dilution/Rinse water into the filter funnel. Using a sterile pipet transfer 2 mL of sample into the filter funnel and filter sample.
       4. For positive sample, again place approximately 20-30 mL of Dilution/Rinse water into the filter funnel. Using a sterile pipet transfer about 0.5 mL of sample into the filter funnel and filter sample. To obtain sample for positive use the sample within the Inhofe cone.
   12. Just as the sample volume reaches the narrow neck of the filter apparatus rinse the apparatus with two consecutive 10 mL portions of the Dilution/Rinse water. Be sure to rinse the entire volume of the filter flask.
   13. Once the sample has been filtered and rinsed, remove the funnel top with one hand while the manifold valve is still on. Using sterilized forceps remove the membrane filter and replace the funnel top directly onto the filter base. Now close the vacuum manifold valve.
   14. Remove the lid of the appropriately numbered prepared petri dish. Place the membrane filter into the petri dish containing the media soaked pad. This is accomplished by slowly dragging a small portion of the edge of the membrane filter over the lip of the petri dish and allowing it to fall onto the absorbent pad. Be sure that there are no air bubbles between the absorbent pad and the membrane filter.
   15. Repeat section 10.9 to 10.14 for each additional samples listed in 10.10 of this SOP.
   16. Once all samples have been processed, use electrical tape to tape the petri dishes closed and place inside a quart size zip lock bag taking care to remove as much air as possible.
   17. Invert the zip lock bag so the petri dishes are upside down, submerge in fecal bath holding the bag on the bottom using rocks, and incubate for 24 + 2 hours at 44.5 + 0.2oC.
   18. Enter the information onto the appropriate data sheet (see section 16 of this SOP).
       1. Date and time
       2. Temperature of fecal bath water referencing both the digital readout as well as the thermometer in the rear of the fecal bath
       3. “m-FC Exp. Date” and “Lot #” can be found on the bag containing the m-FC broth amplues
8. **DATA ANALYSIS/CALCULATIONS:**
   1. Upon completion of incubation period remove the petri dishes from the water bath and count the colonies. Using the grid lines count colonies moving in an S-shaped pattern. For colonies on the line count them either on the bottom of the grid or the top. Which ever way you choose to count the sample, be consistent throughout.
   2. If sample has confluent growth or has > 200 colonies report sample as TNTC.
   3. All data must be recorded into the appropriate data sheet (see section 16 of this SOP).
9. **RECORDS MANAGEMENT:** 
   1. The NPDES permit for the City of Soldotna Wastewater Treatment Plant has a limit of 200 CFU/100 mL per day. If this limit is violated notify the Utilities Manager immediately.
   2. Record all data using indelible blue or black ink.
   3. White out is strictly prohibited for use on any data sheets within the lab.
   4. Log books must be kept for 5 years and are filed by the Utilities Manager.
10. **QUALITY CONTROL:**
    1. The laboratory will perform positive sample every time a sample is processed. Positive samples are processed using the influent at the Soldotna WWTP. Performance of this test allows the laboratory technician to assess the effectiveness of the media and testing techniques.
    2. Every year the Soldotna WWTP must participate in the DMR QA as part of our NPDES permit. Part of the DMR QA test set is the fecal coliform PE sample. This test must be performed and we must pass the sample to be able to report data for the year.
    3. For all commercially prepared media, the date received, lot number, type of medium and pH verification must be recorded. Media **must** be discarded before the expiration date. New lots of m-FC and all media (commercial or laboratory prepared) should be checked using positive and negative controls.
    4. All media must be stored at 4oC
    5. Perform RPD calculations on the samples using the following equation and record the value:

RPD = #2 CFU - #4 CFU

((#2CFU + #4CFU)/2)

1. **CORRECTIVE ACTION:**
   1. If the blank is contaminated the samples are invalid and must be resampled and reanalyzed. In addition the dilution/rinse water used for the sample should be discarded and a fresh bottle used.
   2. If blanks continue to come up with positive growth, all of the dilution/rinse water should be discarded and a new batch made. In addition the autoclave should be checked to insure proper sterilization is being achieved.
   3. If all of the samples, including the polishing tank and positive sample, fail to show growth the media used should be suspect. The samples will have to be resampled and reanalyzed using different box or lot number of media.
2. **REFERENCES:**
   1. SM 9222D - **“**Standard Methods for the Examination of Water and Wastewater”, 18th edition.
3. **FORMS AND DATA SHEETS:** 
   1. Fecal Coliform Analysis

**Appendix A**

**FECAL COLIFORM ANALYSIS**

Date In: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date Out: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Time: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Time:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Analyst: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Analyst: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Temp. In: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Temp. Out: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

m-FC Exp. Date: Lot #:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dish Number | Sample | mL | Colony Count | Actual Colony Count |
| 1 | Blank | 100 |  | CFU/100 mL |
| 2 | Effluent | 100 |  | CFU/100 mL |
| 3 | Blank | 100 |  | CFU/100 mL |
| 4 | Effluent | 100 |  | CFU/100 mL |
| 5 | Clarifier #2 | 2.0 |  | CFU/100 mL |
| 6 | Control Positive | 0.5 |  | CFU/100 mL |
| 7 |  |  |  |  |
| 8 |  |  |  |  |
| 9 |  |  |  |  |
| 10 |  |  |  |  |
| 11 |  |  |  |  |
| 12 |  |  |  |  |
| 13 |  |  |  |  |
| 14 |  |  |  |  |
| 15 |  |  |  |  |
| 16 |  |  |  |  |
| RPD = % |  |  |  |  |