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STATE OF CONNECTICUT
DEPARTMENT OF AGRICULTURE
Bureau of Aquaculture & Laboratory Services



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PROCEDURE FOR THE COLLECTION OF SEAWATER SAMPLES
FOR BACTERIOLOGICAL EXAMINATION TO BE USED IN THE
CLASSIFICATION and MONITORING OF SHELLFISH GROWING WATERS

INTRODUCTION

The Connecticut Department of Agriculture, Bureau of Aquaculture (DOAG) is the lead agency on shellfish in Connecticut with the authority to classify shellfish growing areas and enact closures. Shellfish are filter feeding organisms that pump large quantities of seawater through their bodies as a part of the normal feeding process. As a result, any microorganisms that may be present in the growing area can become concentrated in shellfish meats by as much as 100 times that found in the water column. Sewage contamination is the main source of human pathogens in shellfish growing waters and the correlation between sewage pollution and disease has been well-demonstrated. In order to ensure the safety of shellfish for human consumption, shellfish growing areas are classified based on evidence of contamination.ⁱ

The DOAG uses the guidelines and standards set forth by the National Shellfish Sanitation Program (NSSP) to classify Connecticut's shellfish growing areas. The NSSP is a Federal/State cooperative program, recognized by the United States Food and Drug Administration (FDA) and the Interstate Shellfish Sanitation Conference (ISSC), for the sanitary control of shellfish produced and sold for human consumption. Growing area classifications are based on evidence of contamination found through the sanitary survey. The sanitary survey includes the identification of actual and potential sources of pollution that may adversely impact the growing area, an evaluation of meteorological and hydrographic factors, and an assessment of water quality. The sanitary survey demonstrates conformance to NSSP Model Ordinance (NSSP-MO) bacteriological criteria and the absence of dangerous levels of poisonous and deleterious substances.ⁱ

The fecal coliform group is one of several fecal bacteria indicators used as an indicator of possible sewage contamination as they are commonly found in the intestinal tracts of warm-blooded animals and humans. Although they are generally not harmful themselves, their presence in streams or seawater suggests that disease-causing, or pathogenic, bacteria and viruses may also be present. Sources of fecal contamination to surface waters include wastewater treatment plants, septic systems, animal waste, and storm water runoff.

The DOAG uses the membrane thermotolerant *Escherichia coli* (mTEC) method for fecal coliform enumeration. The mTEC test was approved by the ISSC in 2003 for use in classifying shellfish growing waters. Individual colonies of fecal coliform bacteria are counted on a membrane filter and reported as the number of colony forming units (CFU) per 100ml of seawater. Test results are available in twenty-four hours after initial inoculation. All state and municipal laboratories in Connecticut that are certified for bacteriological testing of shellfish growing waters use the mTEC method – the CT Bureau of Aquaculture and Laboratory and the CT Department of Public Health are currently the only laboratories approved for testing shellfish growing waters.

EQUIPMENT

1. Use the 125 ml single-use sterilized plastic specimen bottles supplied by the DOAG in Milford or the 125 ml reusable plastic bottles provided by the CT Department of Public Health (DPH) laboratory in Hartford for collecting seawater samples.

Use the sterilized 250 ml potable water bottles containing sodium thiosulfate when sampling sewage treatment plant outfalls in shellfish growing waters in order to neutralize any chlorine residual in the effluent.

Do not clean, boil or sanitize your own bottles for sample collection, as they cannot be accepted by the testing laboratory.

2. Use a water-sampling tool for sample collection. The sampling tool consists of a four (4) foot or longer handle with a clamp or holder at one end to securely hold a sample bottle in a vertical position while minimizing contact with mouth of the bottle and threads. A colored mark on the stick is positioned at 18" above the mouth of the bottle, which marks the depth each sample is collected at.
3. Cooler to hold samples.
4. Ice and frozen ice packs in cooler to cool and maintain the temperature of the samples to 50⁰ F (10⁰ C) or less until refrigerated or delivered to testing laboratory.
5. Tide chart for the area being sampled.
6. Nautical chart of area showing locations of sampling stations approved by DOAG.
7. Shellfish seawater analysis laboratory collection forms and account number supplied by DOAG, DPH Laboratory or private laboratory, respectively.
8. Disposable gloves as needed (recommended when working in areas suspected of being contaminated by sewage).

SAMPLE COLLECTION

The seawater monitoring station number must be written on the label located on the side of the bottle with a pencil or waterproof pen. The monitoring station number will consist of the Town's state tax number followed by a dash (-) and DOAG's assigned station number.

Collection bottles must be properly stored to protect them from contamination. Do not use bottles that have been submerged in water or are visibly soiled. Bottles with loose or missing caps, soap film deposits or any other extraneous particles found in the interior of the bottle, severely deformed (concave) bottles, cracked bottles, or bottles lacking labels should be returned to the Laboratory and not used to collect samples.

Place the capped sample bottle in the sampling tool clamp with the bottle opening facing up, then remove the cap. Take care not to contaminate the interior of the bottle, cap or cap threads at any time when the cap has been removed from the bottle. Using the sampling tool, quickly plunge the bottle below the surface of the water to a depth of at least 12 inches. Avoid collecting the boat wash when sampling from a boat. Collect samples upstream and as far away as possible from any bilge pump discharge. Always collect water that is coming toward you when wading in rivers or tributaries. When wading in to collect samples, avoid disturbing the sediment because this can result in elevated, unrepresentative fecal coliform levels. If the sediment is disturbed, allow adequate time for it to settle.

Remove the bottle from the water and pour out enough water to provide at least a ½ inch air space at the top of DOAG single use sterile bottles. A one-inch air space is needed for Public Health Department laboratory reusable bottles. **This air space is critical for proper mixing of the water sample in the laboratory.** However, at least 100 ml of water is needed for analysis. As a guide, fill DOAG bottles just below the bottom edge of the bottle cap and just below the "neck" of the Health Department reusable bottles. Replace the cap securely (tightly) on the bottle, remove the bottle from the clamp and place the bottle in the cooler with ice and ice packs. Keep bottles in upright position to prevent loss of sample in the event of leakage.

At the first station take two samples, label one with TC (temperature control) and label the other with the station number. Place these samples in the cooler. The "TC" sample will be handled the same as other samples, but will only be used to indicate the temperature of the water samples when they arrive at the laboratory.

Samples should be collected from stations where the water depth is at least three feet. If samples must be collected in less than three feet of water, indicate "shallow water" on laboratory collection form. Always sample water that is flowing toward you, not away from you.

Samples must be collected during a low or ebbing (outgoing) tide which begins 1.5 hours after high tide and continues through 1.5 hours after low tide. Samples collected during high tides will not be used in data analysis for shellfish growing waters.

The level of ice in the cooler should never reach the neck of the sample bottles. Ice melt water should be drained away to prevent possible contamination of sample bottles.

On the shellfish seawater analysis laboratory collection form, indicate the station number, tide and time of collection (military time). Any obvious source of fecal coliforms (i.e. birds, water running out of pipes, suds in water, dredging operations, etc.) that you notice during sampling should be noted on the collection form. Make a copy of the completed collection form for your records. Laboratory collection forms are **not** interchangeable between laboratories. **The laboratory will reject samples if you do not use their collection form.**

The mTEC method has four different dilution options, low, high, x-dilution and xx-dilution. The reporting range for number of fecal coliform colonies per unit are as follows:

Low (100ml) = <1 to > 80 CFU

High (50ml) = < 2 to > 160 CFU

X-Dil (10ml) = < 10 to > 800 CFU

XX-Dil (1ml) = < 100 to > 8000 CFU

The laboratory will run samples in the low range unless otherwise noted on the sample collection form. During routine sampling of Approved and Conditionally Approved shellfish growing areas, the low range should be used. The high range will be run if there is not at least 100ml of water in the collection bottle and could also be used for Restricted areas where you would expect the number of fecal coliform bacteria to be less than 160 units per 100ml of water. The X-dil and XX-dil should be used where you suspect a fecal pollution source. Only DOAG laboratory will run XX dilution samples. Samples with a large amount of sediment or suspended solids may cause some problems with the mTEC method and require a high or extra dilution. Laboratory personnel will make a determination at the time of processing.

HANDLING AND SUBMISSION OF SAMPLES

Who may collect and submit samples to DOAG laboratory:

Only those individuals who have been trained and approved by DOAG Environmental Analyst staff may collect samples for the shellfish program. Local health department personnel or their designees, local shellfish commission members or wardens may collect water and shellfish samples for the shellfish program.

Samples should be submitted to either the DOAG Laboratory in Milford or the DPH Environmental Microbiology Laboratory in Rocky Hill.

Please note: DOAG is not responsible for the cost of analysis of samples conducted at private testing laboratories or for analysis of samples collected at locations not designated by DOAG, nor will it accept data from an unapproved laboratory or collected by someone not approved by DOAG. At the time of the writing of this document, there are no private laboratories certified through the NSSP standardization procedure to process seawater or shellfish samples for the shellfish program.

Water samples must be cooled to a temperature of 50° F (10° C) or lower and held at that temperature until received at the approved laboratory. After the sample is collected, place it immediately into the ice chest with ice and ice packs. Ice packs alone will not adequately lower the water temperature of samples collected in the summer. Mechanical refrigeration is recommended for extended periods of storage. Samples will be analyzed by the laboratory as soon as possible, but must be processed within 30 hours.

Samples will be rejected if they meet any of the following criteria:

- **Samples without a temperature control OR a temperature control over 50° F (10° C)**
- **Samples over 30 hours old OR delivered with an inadequate processing time (samples must be *processed* within 30 hours of collection)**
- **Sample bottles that are cracked**
- **Samples that are frozen or with visible ice crystals**
- **Samples without an adequate air gap**
- **Samples that have leaked *may* be rejected by the laboratory.**

All sampling must be arranged in advanced with a DOAG analyst who manages your area by calling or emailing the analyst. You must contact the DOAG at least 24 hours prior to collection of samples to ensure that media is available and the laboratory is not overloaded with samples. The DOAG analyst will confirm that the samples are being collected under the appropriate conditions, and will let you know if the DOAG laboratory can process the samples.

The DOAG laboratory in Milford will accept samples Monday-Wednesday from 8:00 a.m. to 4:30 p.m. and Thursday from 8:00am to 1:00 pm. No samples will be accepted after 1:00 pm on Thursday.

[SUBMISSION OF SAMPLES TO DEPARTMENT OF PUBLIC HEALTH](#)

If the DOAG laboratory in Milford is unable to process the samples, **DOAG staff** will call the **DPH laboratory supervisor** to confirm that the DPH laboratory is able to process the samples. DOAG staff will then communicate with the shellfish commission contact either by phone or

email. When a state holiday occurs in the week you anticipate sampling, call the DOAG to determine when samples may be submitted to the laboratory. Regardless of which laboratory is used, you must inform the DOAG of your sampling plans 24 hours in advance so that we can confirm that samples will be collected under the correct conditions and arrange for the collection and processing of correlating shellstock samples if necessary.

The DPH laboratory in Rocky Hill will accept seawater samples Monday-Thursday from 8:00 a.m. to 1:00 p.m. No samples will be accepted after 1:00 p.m. on Thursday.

When dropping samples off to the DPH laboratory, please enter through the main entrance and notify security that you are dropping off samples. You will be directed to the receiving room, where staff will call the Environmental Microbiology laboratory to inform them that the samples have arrived.

Please submit both the DPH Sample Submission Form and the DOAG Seawater Collection Form.

Contact Information

Connecticut Department of Agriculture Bureau of Aquaculture

190 Rogers Ave.

Milford, CT 06460

203-874-0696

Scheduling of Samples

Alissa Dragan, Supervising Environmental Analyst

Office: (203) 874-0696 ext 119

Cell: (860) 818-7034

Email: Alissa.Dragan@ct.gov

Emily Marquis, Environmental Analyst II

Office: (203) 874-0696 ext 124

Cell: (860) 929-6414

Email: Emily.Marquis@ct.gov

Laboratory

Joseph DeCrescenzo, Fisheries Biologist II

Office: (203) 874-0696 ext 125

Email: Joseph.DeCrescenzo@ct.gov

Connecticut Department of Public Health
Dr. Katherine A. Kelley Public Health Laboratory
Environmental Microbiology
395 West Street
Rocky Hill, CT 06067

Attachments

DOAG seawater collection form

ⁱ [ISSC] Interstate Shellfish Sanitation Conference. 2019. National Shellfish Sanitation Program: Guide for the Control of Molluscan Shellfish. US Department of Health and Human Services Public Health Service Food and Drug Administration.

Sample Data Entry

Rain Data Entry

**CONNECTICUT DEPARTMENT OF AGRICULTURE
BUREAU OF AQUACULTURE, P.O. BOX 97, 190 ROGERS AVE., MILFORD, CT 06460
SEAWATER SAMPLES FOR FECAL COLIFORMS COLLECTION FORM**

Town: _____ Tax Code: _____ Collector: _____

Date Collected: _____ Rainfall: _____ inches _____ days ago Tide: _____

PLEASE SUBMIT TEMPERATURE CONTROL. ALL SAMPLES MUST BE ICED IMMEDIATELY AFTER COLLECTION.

Date/Time Arrival in Lab/Initials: _____ TC/Initials: _____

Time samples placed into refrigerator/Initials: _____

Date/Time Samples Incubated at 35° C/Initials: _____

Time Samples Incubated at 44.5° C/Initials: _____

CA Area Sampled NAME	Area Status OPEN/CLOSED (O/C)

APC Sample Run?

STA. NO.	TIME COLL.	REMARKS	Station Status (O/C)	*DIL L,H,X,XX	INOCULATED BY/ INITIALS	NUMBER OF POSITIVE COLONIES	PLATES READ: DATE/TIME/ INITIALS	FECAL COLIFORMS: **CFU/100 ML SAMPLE	RESULTS BY/ DATE/ INITIALS	CHECK- DATE/ INITIALS

*Dilution Range: L (100 ml) = <1 - >80 CFU **CFU = Colony Forming Units
H (50 ml) = <2 - >160 CFU Results for fecal coliforms are obtained by using the m-TEC method.
X (10 ml) = <10 - >800 CFU XX (1 ml) = <100 - >8000 CFU