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



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PROCEDURE FOR THE COLLECTION OF SHELLSTOCK SAMPLES  
FOR BACTERIOLOGICAL TSSUE 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.

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. Bacteriological examination of tissues from bivalve molluscan shellstock for fecal coliform bacteria is used in conjunction with seawater examination and sanitary survey information to assess the bacteriological water quality of shellfish growing areas in Connecticut in accordance with the NSSP Model Ordinance<sup>1</sup>. Failure to follow this sampling procedure could result in improper sample identification, inaccurate measurement of the bacteriological burden of shellfish in the growing area, and the exclusion of such results from the database used for growing water classifications and for reopening studies following sewage or rain related closures of growing areas.

All bacteriological analysis of shellfish (clams, mussels, oysters and scallops whole or roe-on) must be performed by a laboratory that is evaluated for compliance with the NSSP. The CT Bureau of Aquaculture and Laboratory is currently the only laboratory approved for testing shellstock samples for growing area classification. Shellfish tissue results analyzed by any laboratory that does not meet the NSSP requirements cannot be accepted by the DOAG.

The DOAG uses the most probable number (MPN) multi-tube dilution method for fecal coliform enumeration in shellstock. A series of tubes are inoculated with twelve shucked and homogenized shellfish. Test results are available in forty-eight hours after initial inoculation.

## EQUIPMENT

1. Heavy-weight plastic bags (food grade) supplied by the DOAG laboratory to collect shellstock samples, or other clean, waterproof container.
2. An appropriate implement (clam rake, etc.) shall be used for collection of the species of interest for shellstock collected from recreational areas.
3. A cooler to hold samples.
4. A water bottle to be used as a temperature control for samples. Any small water bottle may be used for the temperature control.
5. Ice and frozen ice packs in cooler to cool and maintain the temperature of the samples at 50°F (10°C) or less until samples are delivered to the laboratory.
6. Nautical chart or GPS showing location of sampling stations approved by the DOAG.
7. DOAG Shellfish Meats Collection Form (see attached AQ-Lab-02).
8. Disposable gloves are recommended for collection of samples from areas suspected of being polluted with sewage or close to water pollution control facilities.

## SAMPLE COLLECTION

1. **Shellstock samples must be scheduled in advance with an analyst in charge of the area.**  
The shellstock bacteriological examination may take up to 48 hours, and must be appropriately scheduled when the laboratory can accept the sample.
2. The shellstock monitoring station identification number must be written on the bag using a waterproof, permanent marker. The monitoring station number will consist of the Town's state tax number, followed by the DOAG assigned station number or name of lot or growing area.
3. A representative sample of shellstock from the assigned station is collected. 15 individuals *of the same species* are taken in order to obtain a representative sample. With most species, this allows for 200 g of combined liquor and meats. At least 200 g of shellfish tissue are used for analysis; if individuals are smaller, more animals are needed

to meet the necessary weight requirements for examination. Select the shellstock to be examined and place in the bag. Shellstock should be free of excess mud and silt. Clean in original harvest area if necessary. Close top of bag.

4. Place sample in cooler. The sample must be kept above freezing and below 50°F (10°C) until examined. The shellstock must **not** come into direct contact with ice or melted ice water, please use sealed plastic bag and keep sample upright.
5. A temperature control should be collected at the same time, or prior to, meat collection. Any small water bottle may be used for the temperature control. The temperature of the water will be taken when the sample arrives in the laboratory. Any samples that are collected without a temperature control will not be accepted. If temperature control is above 50°F (10°C) when the sample arrives in the lab, the sample will not be examined.
6. Complete a Shellfish Meats Collection Form (AQ-Lab-02). The following information is needed:
  - a. Town, date collected, time collected, collector
  - b. Sample location (station number assigned by DOAG or name of lot/growing area)
  - c. Sample Type (species)
  - d. Date harvested, harvester (for samples collected by a harvest vessel)
  - e. Shellfish relayed from (original harvest area) and date relayed (if applicable)
  - f. Latitude/Longitude coordinates should be recorded under comments if available.
7. Samples of shellstock should be examined within 6 hours after collection, and in no case more than 24 hours after collection.

## 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.

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.

**Shellstock 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 shellstock 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 24 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 24 hours old OR delivered with an inadequate processing time (samples must be *processed* within 24 hours of collection)**
- **A shellstock sample with less than 12 acceptable shellfish**
- **Shellstock that is cracked or gaping (shellfish must be alive and intact)**
- **Shellstock that has been contaminated during collection or transport (e.g. not placed in a clean bag, sitting in ice-melt in the cooler, etc.)**

**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 laboratory can only accept shellstock samples Monday, Tuesday, or Wednesday. Samples must be submitted ***no later than*** 1 pm on Wednesday. In case of an emergency situation, it may be possible to make other arrangements with the approval of the laboratory director.

## 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](mailto:Alissa.Dragan@ct.gov)

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Office: (203) 874-0696 ext 124  
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## Laboratory

Joseph DeCrescenzo, Fisheries Biologist II  
Office: (203) 874-0696 ext 125  
Email: [Joseph.DeCrescenzo@ct.gov](mailto:Joseph.DeCrescenzo@ct.gov)

## Attachments

DOAG shellstock collection form

## References

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<sup>i</sup> [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.

**CONNECTICUT DEPARTMENT OF AGRICULTURE  
BUREAU OF AQUACULTURE, P.O. BOX 97, 190 ROGERS AVE., MILFORD, CT 06460  
SHELLFISH MEATS COLLECTION FORM**

Town: \_\_\_\_\_ Tax Code: \_\_\_\_\_ Collector: \_\_\_\_\_

Date Collected: \_\_\_\_\_ Time Collected: \_\_\_\_\_ Date Harvested: \_\_\_\_\_

Sample Location: \_\_\_\_\_ Depth at Location: \_\_\_\_\_ Harvester: \_\_\_\_\_

Sea Water Surface Temperature at Collection: \_\_\_\_\_ Sea Water Bottom Temperature at Collection: \_\_\_\_\_

Sea Water Salinity Surface at Collection: \_\_\_\_\_ Sea Water Salinity Bottom at Collection: \_\_\_\_\_

Shellfish Relayed from: \_\_\_\_\_ on: \_\_\_\_\_

Sample Type (circle one):    Hard Clam    Oyster    Blue Mussel    other: \_\_\_\_\_

Shellstock (circle one):    in shell    shucked    Collector's Sample No.: \_\_\_\_\_

Analysis Requested (circle one):    Bacteriology    Heavy Metals    Pesticides    PCB's    Other \_\_\_\_\_

Purpose of Analysis:    \_\_\_\_\_ Reopen area    \_\_\_\_\_ Post relay    \_\_\_\_\_ Other

Comments:

(SAMPLES MUST BE AT LEAST 12 ANIMALS AND WEIGH AT LEAST 200 GRAMS. COLLECT SAMPLE IN A CLEAN WATERPROOF CONTAINER. ALL SAMPLES MUST BE ICED IMMEDIATELY AFTER COLLECTION. SUBMIT A WATER FILLED WATER COLLECTION BOTTLE AS A TEMPERATURE CONTROL.)

Date/Time Sample Arrival in Lab/Initials: \_\_\_\_\_ TC/Initials: \_\_\_\_\_

Time sample placed into refrigerator/Initials: \_\_\_\_\_

DIL	0	0	0	0	0	-1	-1	-1	-1	-1	-2	-2	-2	-2	-2	-3	-3	-3	-3
LST 21 HR																			
EC																			

Date/Time LST Inoculated/Initials: \_\_\_\_\_ Date/Time EC Inoculated/Initials: \_\_\_\_\_

Date/Time of EC Results/Initials: \_\_\_\_\_ MPN Value/Date/Initials: \_\_\_\_\_

**FECAL COLIFORM RESULT: \_\_\_\_\_ MPN/100 grams of sample. Date/Initials: \_\_\_\_\_**

MPN Check-Date/Initials: \_\_\_\_\_