



Memorandum

Date: 8 August 2008
From: Consumer Safety Officer, Division of Seafood Safety, HFS-325
Subject: Laboratory Evaluations, Maine Shellfish Sanitation Program
To: Shellfish Specialist, Northeast Region, HFR-NE27

On May 6, 2008 and May 7, 2008, Maine's Department of Marine Resources Water Quality Laboratories in Lamoine and Boothbay Harbor were each evaluated to determine their respective capabilities to continue supporting Maine's Shellfish Sanitation Program. Each Laboratory's operations and performance were reviewed and assessed against requirements established in the *Guide for the Control of Molluscan Shellfish* as part of the National Shellfish Sanitation Program (NSSP). A broad based, comprehensive pre-evaluation records review of each Laboratory coupled to the onsite evaluations of both Laboratories demonstrated that they met and exceeded NSSP requirements. This accomplishment earned them **conforming** status. Conforming status is required for laboratories to continue to support a state's Shellfish Sanitation Program. The Lamoine and Boothbay Harbor Water Quality Laboratories having been found to conform have proven themselves capable to continue to support Maine's Shellfish Sanitation Program.

This is the first evaluation since the Laboratories switched from the A-1 MPN to the mTEC membrane filtration method for fecal coliform analysis. In making this change in methodologies, the Laboratories have been faced with many challenges and each challenge has been successfully met and effectively overcome oftentimes setting a model for other laboratories to emulate.

The evaluation of each Laboratory was conducted in accordance with Chapter III of the NSSP *Guide for the Control of Molluscan Shellfish*, 2005 Revision. No nonconformities were found in either Laboratory. Specific details of the evaluations are given in the attached narrative report. The hospitality and cooperation shown by each Laboratory is acknowledged and appreciated. If you have any questions or concerns about the evaluations or the content of the report, please let me know.


Linda A. Chandler

Enclosures

Evaluation Report

On May 6, 2008 and May 7, 2008, Maine's Department of Marine Resources Water Quality Laboratories in Lamoine and Boothbay Harbor were each evaluated to determine their respective capabilities to continue supporting Maine's Shellfish Sanitation Program. For this purpose, each Laboratory's operations and performance were reviewed and assessed against requirements established in the *Guide for the Control of Molluscan Shellfish* as part of the National Shellfish Sanitation Program (NSSP). An offsite comprehensive records review of each Laboratory was conducted prior to the evaluations. This review coupled with the observations and findings from the onsite evaluations demonstrated each Laboratory's ability to meet and exceed NSSP requirements earning them a laboratory status of **conforming**. Conforming status is required for laboratories to continue to support a state's Shellfish Sanitation Program. These Laboratories having been found in conformity have proven themselves to be quite capable to continue to support Maine's Shellfish Sanitation Program.

The staff of each Laboratory is comprised of a cadre of well trained, capable individuals dedicated to achieving the high quality data necessary to have an maintain an effective Shellfish Sanitation Program. By Laboratory, the staff includes in Lamoine, Mercuria Cumbo, Microbiologist III, Water Quality Laboratory Manager for both facilities, Robert Goodwin, Scientist I and Growing Area Supervisor and John Fendl, Specialist I in Boothbay Harbor, Cathy Vinning, Microbiologist II, Gail Parsons, Microbiologist I and Ed Their, Marine Resources Technician.

This is the first evaluation since the Laboratories switched from the A-1 MPN to the mTEC, membrane filtration procedure for fecal coliform analyses. In making this change in analytical procedures, the Laboratories have had to face a number of challenges from how to test the relative performances of mTEC agar and membrane filters having no previous lots of either material with which to compare performance to which dilutions to filter to maintain a sufficiently broad count range for Systematic Random Sampling to be effectively employed. Each challenge has been successfully met and effectively surmounted oftentimes setting the norm for other laboratories to follow.

The evaluations of both Laboratories were conducted in accordance with Chapter III of the NSSP *Guide for the Control of Molluscan Shellfish*, 2005 Revision. No nonconformities were found in either Laboratory. Details of the evaluations are presented below.

Quality Assurance

Both Laboratories operate under the same quality assurance (QA) plan. However, there are some differences between the Laboratories and these are reflected in the document. The QA plan is comprehensive in scope covering all aspects of laboratory operation. More importantly, it is effectively implemented so as to ensure the high quality data needed for adequate water quality monitoring. A copy of the QA plan was provided for

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the top of the hot air oven being mindful when placing the thermometer of the need to maintain it at proper immersion for accuracy while preserving the ability to read temperatures within the target range of 160-180°C.

Both Laboratories check the ice points on their standards thermometers yearly. Any change in the ice point is incorporated into each calibrated temperature point. Working thermometers used in the incubator and waterbaths are checked against their respective standards thermometers at in-use temperatures yearly. Working thermometers meeting an established tolerance when compared to the standards thermometer ($\pm 0.2^{\circ}\text{C}$ for the incubator and $\pm 0.1^{\circ}\text{C}$ for the waterbath) are placed back in service. Those failing to meet the tolerance are used elsewhere as appropriate or retired.

Labware and Glassware Washing

In both Laboratories, laboratory utensils, sample containers, culture tubes, tube closures, MF culture plates and pipets are consistent with NSSP requirements. In both Laboratories reusable labware is appropriately washed and effectively rinsed. The labware washing/rinsing procedure leaves no toxic or inhibitory residues behind as demonstrated by the results of the Inhibitory Residues Test (IRT) performed by both Laboratories yearly and with each change in detergent lot. Bromthymol blue (BTB) solution is used routinely by each Laboratory as an adjunct to the IRT in monitoring the effectiveness of the labware washing/rinsing procedure in eliminating detergent residue.

Media Preparation, Sterilization, Storage and Decontamination

In each Laboratory, the preparation of culture media, its storage, sterilization and the decontamination of spent cultures is consistent with the requirements of the NSSP. Both Laboratories use the mTEC membrane filtration method for analyzing growing water samples. The mTEC agar is commercially dehydrated and it along with other dehydrated media used in each Laboratory is properly stored and appropriately labeled. Caked or expired dehydrated media is discarded and none was in evidence in either Laboratory.

In both Laboratories all culture media is rehydrated with water of appropriate quality, free of detectable chlorine, designated trace metals and excessive microbial contamination. Rehydrated media is sterilized according to manufacturer's instructions. Exposure of culture media to autoclave temperatures in either Laboratory does not exceed a total cycle time of 45 minutes and is within the temperature range of $121\pm 2^{\circ}\text{C}$ or it is discarded. Autoclave chamber temperatures are monitored routinely in both Laboratories using accuracy checked maximum registering autoclave thermometers. Because of the extremes in temperature to which autoclave thermometers are routinely subjected, both Laboratories are cautioned to frequently check these thermometers under magnification for breaks in the mercury column. If breaks are apparent, the thermometer should not be used as temperature readings will be affected. Both Laboratories should also occasionally check all mercury in glass thermometers in use under magnification for breaks in the mercury column as well. These breaks no matter how small will affect temperature readings.

Spores are used monthly by both Laboratories for monitoring the effectiveness of autoclave sterilization. For monitoring culture media sterilization, it was recommended that both Laboratories use spore strips/suspensions specifically designed for monitoring 15 minute cycles as this cycle length is more representative of the length of most culture media cycles than the 30 minute exposure spore test being used in each Laboratory at the time of these evaluations.

Autoclave operation is completely documented in both Laboratories through the use of an autoclave log. The log also documents the disposition of culture media/materials failing to meet temperature tolerance and/or heat exposure time limits.

In each Laboratory prepared culture media is stored appropriately with storage times not exceeding seven (7) days at room temperature for tubed liquid media, one (1) month under refrigeration for tubed media with loose fitting caps and two (2) weeks for MF agar (mTEC) plates under refrigeration. In both Laboratories media sterility and positive and negative media productivity controls are run as required using media appropriate control cultures. The only exception in both Laboratories is their use of an inappropriate negative control with mTEC media. Because the purpose of the productivity control is to determine the effect of the media's constituents on the pattern of growth, it was recommended that the Laboratories use *Staphylococcus aureus* rather than *Enterobacter aerogenes* as their negative control for mTEC. With *Staphylococcus aureus*, the media constituents control growth rather than the temperature of incubation as would be the case with *Enterobacter aerogenes*. After being used, spent culture plates and liquid tubed media are decontaminated by autoclave sterilization before being discarded.

Collection and Transportation of Seawater Samples

Seawater samples are collected and transported in a manner consistent with NSSP requirements. Samples are collected in pre-sterilized Whirl Pak Bags. The actual sterility of these Whirl Pak Bags is documented through testing of individual lots. The size of the Whirl Pak Bags used in both Laboratories provides a sufficient volume of sample for filtering of the required sample aliquots while maintaining adequate headspace for proper shaking. Samples are labeled with the appropriate identifying information and transported in coolers maintained between 1 and 10°C. Samples are analyzed immediately after reaching the laboratory or placed under refrigeration at 0 to 4°C until analyzed. In no case are samples analyzed by either Laboratory if they have been held for more than 30 hours from the time of collection.

Analysis of Seawater Samples

Both Laboratories use MPN based tube methods where required to support their non-growing area work. Because of time constraints these methods were not observed directly during the evaluation but were evaluated through the detailed SOPs provided by the Laboratories as part of their quality system. No problems with these methods were noted.

Both Laboratories use the mTEC membrane filtration method for their growing water work. Two volumes are filtered consistent with the requirements of Systematic Random Sampling (SRS) for a method capable of generating a broad count range. Using 50 and 5 ml aliquots, the mTEC method as employed in both Laboratories produces a count range of 2 to 1600 cfu/100 ml which is sufficiently broad to detect excessive variability in water quality.

Commercial dehydrated mTEC agar is used for the analysis. When initially placed in service, the performance of the mTEC agar was checked against plate count agar to determine lot acceptability as there was no previous lot of mTEC to test against. Recovery of a known mTEC positive culture by each agar was found to be similar. Accordingly the mTEC agar was placed in service in both Laboratories. Subsequently, the performance of new lots of mTEC agar are checked against the previous lot and a t-test run to determine if a significant difference exists. Lots of mTEC agar that exhibit similar performance characteristics are placed in service.

Pre-sterilized membrane filters of the appropriate composition and pore size are used in the analysis. When initially placed in service, the performance of the membrane filters using a known positive culture was checked against a spread plate to determine lot acceptability using plate count agar to support growth in both the filtered and the unfiltered spread plate. Recovery by both filtering and spread plate was found to be similar. As a result, the membrane filters were placed in service. Subsequently the performance of new lots of filters are tested against previous lots to ensure consistency in performance.

The sterility of the pre-sterilized membrane filters is lot tested before use. Although sample blanks are run at fixed intervals during the analysis as a check on the sterility of the test system including culture plates, it was suggested that the Laboratories consider lot testing of the pre-sterilized culture plates to avoid test system failure due to an unlikely but possible lapse in the sterility of the culture plates.

Membrane filter assemblies are autoclaved sterilized prior to the start of a filtration series. UV sterilization is used to disinfect these assemblies between filtration runs. The efficacy of the UV disinfection is determined by biological testing monthly. Since the efficacy of the UV disinfection is a function of the effective operation of the UV bulbs, it was suggested that the Laboratories develop an SOP for the care, maintenance and replacement of the UV bulbs to ensure maximal operational effectiveness. Results of the testing of the efficacy of UV disinfection are recorded and maintained as required.

Indelible graduation marks are used on the filter funnels to measure 100 and 50 ml volumes. The 5 ml sample volume, however, is pipetted for accuracy. The accuracy of these indelible 100 and 50 ml graduation marks is subsequently verified by weight to within 2.5% of the target volume. Filter funnels are numbered and the results of these volume checks are recorded and maintained as a permanent record in each Laboratory.

Sterile phosphate buffered saline (PBS) is used both as a sample diluent and a filter funnel rinse. PBS is prepared in bulk and bulk sterilized in both Laboratories. The sterility of the PBS so prepared is continually monitored throughout sample analysis as one component of the multi-component blank testing. Blanks are run at the appropriate intervals to check the sterility of the test system. In addition to PBS, blanks test the continued sterility of filter funnels, the sterility of the forceps used to manipulate the membrane filter, the filter itself, the culture medium and the culture plate.

Samples are filtered under a vacuum using a vacuum pump operating within the prescribed pressure range. Filtered plates are placed in watertight plastic bags, double-bagged, sealed and incubated at 35°C in air for two (2) hours of resuscitation. The remaining 22 hours of incubation is at 44.5°C completely submerged in a circulating waterbath. Positive and negative process controls accompany each set of samples analyzed through resuscitation and waterbath incubation. The positive process control used by both Laboratories is *E. coli* and the negative process control is *Enterobacter aerogenes*. The positive process control, *E. coli* was developed to produce 10 to 30 colonies per plate to mimic the anticipated low density of fecal coliforms in the samples. Conversely, a high count of the negative process control is used to provide a stringent test of the efficacy of the incubation regime.

All yellow, yellow-green and yellow-brown colonies are counted and results reported as cfu/100 ml of sample. Because of the need to filter multiple dilutions under the Systematic Random Sampling water quality monitoring regime, the Laboratories have developed an effective approach to calculating fecal coliform density from the various combinations of countable (plates having 20 to 80 colonies per plate), non-countable (plates having <20 or >80 colonies per plate) and indeterminate plates (plates showing no growth) obtained.

Collection, Transportation and Analysis of Shellfish Samples

Shellfish Samples are labeled with the appropriate identifying information, transported in coolers maintained at 0 – 10°C and analyzed as soon as possible after reaching the Laboratories. In no case are samples analyzed if they have been held for more than 24 hours from the time of collection.

In both Laboratories proper shucking technique is observed and a representative number of shellfish used in the analysis. Process controls, *E. coli* as the positive control and *Enterobacter aerogenes* as the negative control accompany each set of shellfish samples analyzed. The positive process control is appropriately diluted to mimic anticipated fecal coliform densities in the samples. The negative control provides a heavy inoculum to test the robustness of the incubation regimen in preventing the growth of non-fecal coliform organisms. Although process controls are routinely used by both Laboratories, their use is not expressly covered in the SOP governing the analysis. Consequently it is recommended that the use of process controls be incorporated into the SOP covering the analysis.

The standard plate count is performed in association with the analysis of shellfish meat samples. Because of time constraints, this analysis was not directly observed as part of the evaluation in either Laboratory. The review of the SOP indicates that this analysis as described is consistent with NSSP requirements. Moreover, Laboratory proficiency is consistently demonstrated by the successful participation of both facilities in the aerobic plate count portion of the annual FDA Shellfish Split Sample Program.