Proposal for Task Force Consideration at the 2009 Biennial Meeting Interstate Shellfish Sanitation Conference	☒ **Growing Area** ☐ **Harvesting/Handling/Distribution** ☐ **Administrative**
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Proposal Subject:	Re-Opening Criteria Based on New Indicator of Sewage-Borne Viral Pathogens
Specific NSSP Guide Reference:	NSSP Guide Model Ordinance Section II, Chapter IV. Shellstock Growing Areas @ .03 Growing Area Classification A. General (5) Status of Growing Areas (c) Reopened Status
Text of Proposal/ Requested Action	(c) Reopened Status. A growing area temporarily placed in the closed status as provided in (b) above, shall be returned to the open status only when: (i) The emergency situation or condition has returned to normal and sufficient time has elapsed to allow the shellstock to reduce pathogens or poisonous or deleterious substances that may be present in the shellstock to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of contaminant levels in the shellstock to pre-closure levels. In addressing pathogen concerns, the study may establish criteria for reopening based on coliform levels in the water~~; or~~ **and;** **(ii) For emergency closures (not applicable for conditional closures) of harvest areas caused by the occurrence of sewage contamination events, such as sewage collection system failures, the analytical sample results shall not exceed background levels or a level of 50 male-specific coliphage per 100 grams from shellfish samples collected no sooner than 7 days after contamination has ceased and from representative locations in each growing area potentially impacted; or** ~~(ii)~~ **(iii)** The requirements for biotoxins or conditional area management plans as established in §.04 and §.03, respectively, are met; and ~~(iii)~~ **(iv)** Supporting information is documented by a written record in the central file. NOTE: An analytical method for shellfish meats and a draft laboratory checklist are separately proposed to enable the use of the new, optional re-opening criteria.
Public Health Significance:	The absence of bacterial pathogens such as *Salmonella* species can be reliably determined using the coliform bacterial indicators of the National Shellfish Sanitation Program (NSSP). However, when growing areas are implicated as the source of shellfish causing

illness consistent with viral etiology, the NSSP requires closure for a minimum of 21 days.[1] That is because viruses and bacteria persist differently in growing waters and in shellfish[2,3,4,5], it takes considerably longer for shellfish to eliminate viruses[2,3,4,5], and the coliform bacterial indicators of contamination currently stipulated in the NSSP do not index risks from enteric viral pathogens very well[3,6,7]. This means that if open harvest areas become unexpectedly contaminated, the likelihood exists that viral pathogens may remain viable in shellfish long after growing waters appear safe according to the NSSP bacteriological criteria. Recognizing these facts, and lacking an alternative viral indicator or any other reasonable way to judge, the NSSP has stipulated 3 weeks as the criterion for achieving safe shellfish when viral pathogens are known or suspected to be involved.[1]

The NSSP needs an alternative viral indicator. Coliform bacteria do not reliably reflect the presence or absence of viral pathogens such as Noroviruses and hepatitis A[2,3,6]. Events such as sewage spills and bypasses, sewer pipe breaks, sewage pumping and lift station leaks, and wastewater treatment plant failures can heavily contaminate shellfish areas, at least temporarily. Every harvest area even remotely impacted by sewage effluent is at risk for a lengthy closure if and when a mishap in the sewage collection system or at the sewage treatment plant occurs. Already some State Shellfish Control Authorities (SSCA) have issued 3 week emergency closures to harvest areas after power failures disrupted sewage treatment and following pipe ruptures and sewage spills. When such contamination events occur, there currently is no choice. In the absence of an alternative viral indicator, there exists no way under the NSSP to judge whether a lengthy closure can be avoided. Logically, if the NSSP had an indicator that better resembled the enteric viral pathogens of concern, then lengthy closures might be averted.

Male-specific coliphage:
Studies[4,7,8,9,10] demonstrate that a group of bacterial viruses called male-specific coliphage (MSC) appear to be good candidates as an alternative viral indicator in the NSSP. That is, MSC appear to be conservative indicators of sewage-borne enteric viral pathogens in shellfish and can be relied upon to indicate the virtual absence of these viral pathogens when derived from sewage. MSC occur universally in sewage in large numbers[6]. They only replicate in F+ (palliated) *E. coli* cells but do not reproduce below 30°C[5,,11,12]. Quantitative analysis for MSC is easy, inexpensive, and takes only 18-24 hours[13,14]. MSC persist in waters and in shellfish much like the infectious hepatitis and Norwalk-like viruses of concern to the NSSP[2,3,4,8]. The physical size and shape of most MSC closely resemble those of these pathogens as well, and they all contain RNA as their genetic material[5]. More importantly, studies further show that when shellfish are contaminated with sewage, male-specific coliphage provide a better measure of the potential presence of enteric viruses than do coliform bacteria[4,8].

To establish an alternative safety standard for shellfish based on MSC, a quantitative relationship between measurable levels of the indicator and the absence of viral pathogens is needed. Researchers in the United Kingdom studied shellfish harvested over a 2 year period, and their findings[7] show that when mean levels of F+ RNA bacteriophage remained below 50 per 100 grams; Norwalk-like viruses were not detected in any samples. Conversely, when the mean level of MSC exceeded 125 per 100 gram, 37% of the same shellfish samples were positive for enteric viruses. These data provide a scientific basis for establishing a level of MSC, one that is readily detectable and that provides a measurable indication that the sewage contamination levels in shellfish are below the threshold for containing enteric viral pathogens. Applied as an indicator following sewage contamination events in the

U.S., if the levels of MSC are found below 50 per 100 grams throughout the harvest area, then those shellfish should be as safe from sewage-borne enteric viral pathogens as they are under normal conditions.

Limitations of MSC:
Though abundant in sewage, sewerage collections systems, and most wastewater effluents, the MSC group is not reliably detected in fresh human waste, small point sources, vessel discharges, and vomit, all of which can transmit viral pathogens.[5,10] Therefore, it is not proposed as an index of enteric viral pathogens from these sources. Nonetheless, MSC are a useful alternative indicator for signaling the presence of sewage contamination in shellfish, and provide a science-based means for determining whether shellfish areas are safe from viral pathogens, such as Noroviruses and hepatitis A viruses, following sewage contamination events.

Proposal of Male-specific Coliphage as an Indicator:
It is proposed that MSC can, at the discretion of the SSCA, be used as an alternative to the 3 week period for re-opening shellfish areas to harvest activities after a sewage contamination event causes emergency closure. This re-opening option is based upon analytical results from shellfish samples collected at least 7 days after contamination has ceased and from representative locations in the growing area, whereby no samples exceed background levels or a level of 50 MSC per 100 grams.

Benefits:
The MSC option for re-opening could decrease currently practiced closure periods by as many as 13 days.
References:
[1] NSSP Model Ordinance, Chapter II @.01 H (2).
[2] Sobsey, M.D., A.L. Davis, and V.A. Rullman. 1987. Persistence of hepatitis A virus and other viruses in depurated eastern oysters. Proc. Oceans '87, Halifax, Nova Scotia. 5:1740-1745.
[3] Richards, G.P. 1988. Microbial purification of shellfish: a review of depuration and relaying. J. Food Prot. 51:218-251.
[4] Burkhardt, W., III, S.R. Rippey, and W.D. Watkins. 1992. Depuration rates of northern quahogs, *Mercenaria mercenaria* (Linnaeus, 1758), and eastern oysters, *Crassostrea virginica* (Gmelin, 1791), in ozone- and ultraviolet light-disinfected seawater systems. J. Shellfish Res. 11:105-109.
[5] Dore, W.J. and D.N. Lees. 1995. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration**.** Appl. Environ. Microbiol. 61: 2830-2834.
[6] Goyal, S.M., C.P. Gerba, and G. Britton (Eds). 1987. Phage Ecology. John Wiley & Sons, New York.
[7] Dore, W.J., K. Henshilwood, and D.N. Lees. 2000. Evaluation of F-Specific RNA Bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Appl. Environ. Microbiol. 66(4):1280-1285.
[8] Cabelli, V.J. 1988. Microbial indicator levels in shellfish, water, and sediments from the upper Narragansett Bay conditional shellfish-growing area. Report to the Narragansett Bay Project, Providence, RI.
[9] Burkhardt, W., III, W.D. Watkins, and S.R. Rippey. 1992. Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. Appl. Environ. Microbiol. 58:826-831.
[10] Calci, K.R., W. Burkhardt III, W.D. Watkins, and S.R. Rippey. 1998. Occurrence of male-specific bacteriophage in feral and domestic animal waste, human feces, and

	human-associated wastes. Appl. Environ. Microbiol. 64:5027-5029. [11] Novotny, C. P. and K. Lavin. 1971. Some effects of temperature on the growth of F pili. J. Bacteriology. 107: 671- 682. [12] Woody, M.A. and D.O. Cliver. 1995. Effects of temperature and host cell growth phase on replication of F-specific RNA coliphage Qβ. Appl. Environ. Microbiol. 61:1520-1526. [13] DeBartolomeis, J. and Cabelli, V.J. 1991. Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. Appl. Environ. Microbiol. 57:1301-1305. [14] Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure, USEPA, EPA 821-R-01-030, April 2001.
Cost Information (if available):	The proposal provides optional re-opening criteria that are not required. Therefore, there are no added costs to State programs or industry. However, State laboratories that do not have requisite equipment already would incur such costs if the State chooses to implement the optional re-opening criteria described.
Action by 2005 Task Force I	Recommended referral of Proposal 05-105 to the appropriate committee as determined by the Conference Chairperson.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 Growing Area Classification Committee	Recommended referral of Proposal 05-105 to the appropriate Committee as determined by the Conference Chairman. Additionally, the Committee recommended the Executive Board establish a work group to evaluate the appropriateness of MSC as a viral indicator, and to identify appropriate applications. The workgroup should report its findings at the March 2008 Board meeting.
Action by 2007 Task Force I	Recommended adoption of the Growing Area Classification Committee recommendation on Proposal 05-105.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action.