Interstate Shellfish Sanitation Conference (ISSC)
Male Specific Coliphage Meeting Report
August 18-19, 2014 – Charlotte, NC

I. Purpose

The ISSC held a Male Specific Coliphage (MSC) Informational Meeting in Charlotte, NC. The purpose of this meeting was to discuss the appropriate use of Male Specific Coliphage (MSC) as an enteric virus risk indicator in the National Shellfish Sanitation Program (NSSP). Currently, MSC is used in the NSSP to assess the impact of raw sewage spills. Since 2005, the ISSC has continued to debate the appropriateness of expanding the use of MSC to other types of classification. At the 2013 Biennial Meeting, the ISSC took the following action on Proposals 11-101 and 11-102:

> To organize a meeting of MSC experts, academia, and scientists to present current information and science on MSC and develop recommendations for the Growing Area Classification Committee.

This meeting brought together expert panelists on MSC to present current data regarding the utility of MSC as an indice of human enteric viruses (norovirus; NoV). The ISSC Growing Area Classification Committee participated in the meeting and developed recommendations for Conference action. This format allowed the committee to hear supporting science prior to discussing the most appropriate expanded uses of MSC in the NSSP.

II. Introduction

Male specific coliphage is a very specific group of viruses. It is one of many viruses labeled as bacteriophage because it infects bacterial cells. Coliphage viruses infect a certain type of bacteria called *Escherichia coli*. *Escherichia coli* is a bacteria commonly found in the lower intestine of humans and is expelled from the body through fecal materials. When *Escherichia coli* is grown at temperatures of 98.6 ° or above, they express small appendages called pili. Bacterial cells that have pili are referred to as "male" bacteria. Male-specific coliphage are viruses that infect male *Escherichia coli* cells by attaching to the pili of the bacteria. MSC belong to two different morphological families: *Leiviridae*, which consists of icosahedral viruses containing single-strand RNA, and *Inoviridae*, which consists of filamentous viruses containing single-strand DNA. The icosahedral shaped coliphages (i.e.: bacteriophage MS-2) are similar to many enteric viruses in shape, transport and survival characteristics. Due to their similarity, abundance and ease of detection, coliphage can be useful in monitoring what happens to enteric virus populations in water. Male Specific Coliphage is found in feces and sewage that contains *Escherichia coli* and is also be found in low levels in water that has not been contaminated. Since MSC is an easily detected fecal coliphage, the detection of Male

Specific Coliphage allows for fecal contamination detection in water which can provide an insight into the possible levels of harmful human enteric viruses such as Norovirus.

III. Relevance to Molluscan Shellfish

The National Shellfish Sanitation Program (NSSP) Guide Model Ordinance (MO) Section II, Chapter IV.@.03 A.(5)(c)(i) requires that before a growing area can be reopened after an emergency situation, sufficient time must have elapsed for the shellfish to cleanse themselves from pathogens or poisonous and deleterious substances. It further requires that studies be conducted to determine the time sufficient for the reduction of contaminants to pre-closure levels. 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, viruses and bacteria persist differently in growing waters and in shellfish it takes considerably longer for shellfish to eliminate viruses. The coliform bacterial indicators of contamination currently stipulated in the NSSP do not index risks from enteric viral pathogens. This means that if open harvest areas become unexpectedly contaminated, the likelihood exists that viral pathogens may remain viable and infectious in shellfish long after growing waters appear safe according to the NSSP bacteriological criteria.

IV. Role and Application of Indicators

Historically the NSSP primarily has relied on indicator microorganisms (rather than pathogens) to assess the sanitary quality of shellfish growing areas. The indicators are used to indicate the presence or absence of fecal pollution. Utility of the indicator based system is inherently more proactive and cost effective than the detection of the multiple pathogens associated with municipal wastes. Additionally, the presence of pathogens at any one time is generally unpredictable depending on the health of the contributing population. To reduce the risk of illness it is the basic premise of the NSSP to make decisions based on the potential for pathogen contamination. The potential risk of pathogen contamination is estimated by detecting the presence of fecal pollution.

For an indicator to be effective it should have the following characteristics:

- The ideal contamination indicator:

1. Should be a derived intestinal microflora of warm-blooded animals
2. Should be present whenever pathogens are present
3. Should occur in greater numbers than the pathogen of concern
4. Should be absent or at least very few numbers in clean waters
5. Should be detectable and quantifiable by easy, rapid, inexpensive methods
6. Should be non-pathogenic
7. Should not multiply in the environment
8. Should respond to natural environmental stress and wastewater treatment processes and disinfectant in a manner similar to the pathogen of interest

The largest application of indicators in the growing area program classification is fecal coliforms for the purpose of classifying growing area harvest waters. Fecal coliforms are used to index presence and relative amounts of fecal contamination in seawater. As an indicator organism group, fecal coliforms satisfy many of criteria listed above (#1-#7). However, in terms of persistence in the environment and wastewater treatment, fecal coliforms are inadequate for certain enteric viral pathogens (i.e. norovirus and hepatitis A virus). In addition, viruses can be bioaccumulated and eliminated or inactivated by molluscan shellfish differently than vegetative bacteria (fecal coliforms/E. coli). When ingested by molluscan shellfish viruses require a longer period of time for the shellfish to eliminate the viruses. Where viral contamination is an issue for shellfish an indicator microorganism is needed that shows similar persistence in the environment and in shellfish. Recently MSC was added as an alternative indicator for assessing the impact of raw untreated sewage spills.

V. Previous ISSC Actions Related to MSC

The ISSC first debated the use of MSC in the NSSP in 2005 (Proposal 05-105). The ISSC also discussed the adoption of a laboratory method for enumeration of MSC. To facilitate discussions of 05-105, the ISSC Growing Area Classification Committee in 2009 developed a white paper which provided background information on MSC (attached). After much debate, the ISSC in 2009 incorporated language into the NSSP which allows states to use a MSC level of 50 per 100 grams as reopening criteria for spills of raw untreated sewage. The ISSC also approved laboratory method for enumeration of MSC for soft shell clams and oysters (Proposal 05-114) and a microbiological checklist for the method was also adopted in 2009 (Proposal 05-113). Additional proposals were submitted for ISSC discussion in 2011 (Proposal 11-101 and Proposal 11-102). These proposals recommended that the use of MSC be expanded to allow states in certain situations to use MSC sampling data to classify harvest waters adjacent to wastewater treatment plants.

VI. Historical Application of MSC (to be included)

Background

USFDA use of male-specific coliphage as an adjunct indicator to coliforms began in 1986. These initial investigations assessed the seasonal bioaccumulation and depuration of rates of viral indicators to those by the conventional indicators of sanitation. The findings of these investigations have served as the basis for MSC application in the NSSP.

Since 1986 MSC have been successfully used in multiple instances to provide additional information for assessing viral risk;

A. Reopening of Shellfish Harvest Areas following Catastrophic Wastewater Treatment Failures (Emergency Closure);

The initial application of MSC by ISSC member States to reopen harvest areas impacted by raw municipal wastewater occurred followed the failure (rupture) of a large pipe delivering raw wastewater to a wastewater treatment plant. In this failure 100's of millions of gallons of wastewater was discharged into the New York Bight area (NY/NJ waterway). Advice was sought and the state was advised by FDA (Watkins/Burkhardt) that based upon evidence of viral elimination rates of MSC by shellfish and the association of MSC and incidence of illness by NoV in Europe that shellfish areas should be allowed to reopen after shellfish in the impacted areas after MSC levels were <50 pfu/100g or achieved background levels. This application of MSC allowed areas to reopen earlier than the 21 days described in the model ordinance.

In 2013, the New Jersey shellfish industry was impacted by Superstorm Sandy which caused catastrophic failures of the wastewater treatment infrastructure. MSC were used as means to reopen harvest areas that had been impacted prior to a mandatory 21 day closure. While most harvest areas reopened soon after using this criterion, a small area remained closed for several months due to public health concerns that the shellfish were not purging acquired contaminants due to low water temperatures. This hypothesis was confirmed by laboratory based depuration trials conducted by SCA.

B. Allowing the Harvest of Shellfish from Areas Previously Prohibited
In a joint effort by Spinney Creek Shellfish, Maine's Department of Marine Resources and FDA investigations were undertaken to:

1. Characterize the seasonal influence and virological impact of a treated wastewater on soft shelled clams in the Royal and Cousins River in ME; assessment was based upon levels of MSC and NoV detection.

2. Determine the rates of MSC and NoV elimination from naturally contaminated soft shelled clams undergoing depuration

Based upon the findings of this effort Spinney Creek were allowed to implement a pilot project to seasonally harvest soft shelled clams in the Royal River for depuration purposes only in area previously prohibited to harvest; end product testing of shellfish for MSC was used to verify depuration effectiveness. This project ran successfully for 3 years.

C. Pollution Source Identification/Strength

In 1987, FDA in collaboration with EPA and State entities performed the Narragansett Bay Wet Weather project. The objective of this investigation was to determine the relative strengths of pollution sources following appreciable rainfall events that impact the sanitary quality of the estuary. Fecal coliforms in conjunction with MSC were used to assess the impact of these pollution sources. In several instances, the potential virological impact of pollution sources would not have been evident by monitoring coliforms alone. Since this initial study, FDA in collaboration with multiple shellfish control authorities has used MSC to identify pollution sources that potentially impact shellfish growing areas. To date, over 10 ISSC member States and International Partners (Canada, Korea) have successfully used MSC to identify pollution sources; in several instances MSC was successfully used to identify infrastructure failures that posed a significant health concern.

D. Assessing the Efficacy of Wastewater Treatment Plants to Reduce Virus Loads into Estuaries and Impact on Impact on Shellfish in Adjacent areas.

VII. Format and Meeting Objectives

As part of the planning for the MSC Informational meeting, a steering committee was established and tasked to develop a meeting strategy to encourage and facilitate the exchange of MSC information. The steering committee selected a panel of nine (9) MSC experts to answer questions, share field study results and offer consensus opinion regarding the use of MSC in the NSSP. The MSC meeting was formatted to allow a MSC Expert panel to share current scientific information, current research and results from relative field studies. MSC is a relatively new tool for assessing the persistence of human viruses in shellfish growing areas. The format was used to allow members of the ISSC Growing Area Classification Committee the opportunity to learn and understand more about the viral indicator and its potential benefits for use in both reopening of shellfish waters following emergency closures and classification of harvest waters adjacent to waste treatment plants.

Prior to the MSC Informational Meeting, the ISSC solicited MSC related questions from the membership. There were 64 questions submitted. The steering committee reviewed and selected 37 questions for the expert panelists to address. Each question was assigned to a panelist to answer. Other panelists commented on the response of the assigned panelist.

Members of the Growing Area Classification Committee and attendees were given an opportunity to ask questions following the presentations of field study results. The

panelists were then asked for their opinion regarding the use of MSC in the NSSP. The assigned questions, summaries of the field studies and panelist opinions regarding uses of MSC are included as a part of this report.

VIII. Expert Panelists

The ISSC invited several panelists with expertise in the use and applicability of MSC as an indicator of the risk of enteric viruses in shellfish. The panelists are listed below. For biographical information, see attachment.

A. Bill Burkhardt
Director of FDA's Division of Seafood Science and Technology at the Gulf Coast Seafood Laboratory located on Dauphin Island, AL

B. Kevin Calci
FDA microbiologist at the Gulf Coast Seafood Laboratory located on Dauphin Island, AL

C. Thomas L. Howell
Spinney Creek Shellfish

D. Lee-Ann Jaykus
Professor in the Department of Food, Bioprocessing, and Nutrition Sciences Department at North Carolina State University (NCSU)

E. David Lees
Director of the European Union Reference Laboratory (EURL) for bacterial and viral contamination of bivalve molluscs

F. David Love
Assistant Scientist in the Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health (JHSPH)

G. Kim Reese
Professor of Marine Science at the Virginia Institute of Marine Science and chair of the Aquatic Health Sciences Department

H. Chris Roberts
Regional Manager in Environment Canada's Marine Water Quality Monitoring Program

I. Chip Simmons
Departments of Biological and Agricultural Engineering and Food, Bioprocessing, and Nutrition Sciences at North Carolina State University

IX. Field Study Overview

The expert panelists were asked to respond to a number of questions (See Attachment A). Several of the expert panelists who had field experience utilizing MSC presented results from field studies. Below is a synopsis of the studies that were presented.

A. Tom Howell, Spinney Creek Shellfish, Inc.:

Tom presented three field studies. The first was conducted in New England using samples from the Royal River, Fore River, and Presumpscot River in a multi-year collaboration with Spinney Creek Shellfish, FDA Gulf Coast Shellfish Lab, Maine Department of Marine Resources, and Massachusetts Division of Marine Fisheries. Seasonal persistence and temperature-dependent depuration rates were investigated for fecal coliforms (FC), MSC, NoV genogroups GI&II, and human Adenovirus (AdV) in soft-shelled clams. Viral persistence was shown to be low in the summer months and 2 to 3 log higher in the winter. Viral depuration rate was shown to be highest in the summer months (log reduction in 2 days) and lower in the winter months (log reduction in 23 days).

The second study was performed in the Royal River and looked at spatial variation of MSC in soft-shelled clams as a function of distance from the outfall in comparison with the dilution model in collaboration with Spinney Creek Shellfish, FDA Gulf Coast Shellfish Lab, FDA Division of Shellfish Safety Dye Study Group, and the Maine Department of Marine Resources. The results of this study show a high degree of consistency between MSC levels in shellfish and dilution levels as a function of distance from the outfall.

The third study was conducted at multiple sites in Marblehead Harbor, MA and the Piscataqua River in Maine in collaboration with Spinney Creek Shellfish, MA Department of Marine Fisheries, UNH Sea Grant, FDA Gulf Coast Shellfish Lab, and Maine Department of Marine Resources. Species-specific bio-accumulation studies were performed to investigate species-specific anomalies in bio-accumulation of FC, MSC, NoV, AND AdV. Quahogs and American oyster demonstrated different viral bio-accumulation patterns than Pacific oysters, European oysters, soft-shelled clams due to their tendency to stop pumping as water temperatures approach 10°C.

B. David Lees, Centre of Environmental Fisheries and Aquatic Science:

David presented multiple studies conducted throughout the EU, including the UK Harvest Area Study (circa 2001) and data from the Centre for Environment, Fisheries & Aquaculture Science (2000-2003). These studies addressed the applicable use of MSC as an indicator in classifying shellfish growing areas.

C. Kevin Calci, US FDA Dauphin Island:

Kevin presented two potential areas in which the expanded use of MSC could be applied: wastewater treatment plant efficiency and as a shoreline survey tool. Data from treatment plants and shoreline surveys came from numerous locations across the US. He also gave a brief overview of the US/Canadian Molluscan Shellfish Risk Assessment which, in part, used a meta-analysis of influent and effluent values of NoV and MSC from peer reviewed journals and FDA and Canadian surveillance to model viral reductions.

D. Chris Roberts, Environment Canada Marine Water Quality Monitoring:

Chris presented how Canada is applying MSC concentrations in influent and effluent as an indicator of the efficacy of different wastewater treatment levels and plant types and how log reduction values for MSC compared to fecal coliform data in Canadian wastewater treatment plants. Also, he described the effect seasonality has on MSC in Atlantic Canada.

E. Kim Reece, Virginia Institute of Marine Science:

Kim presented an *in vitro* and *in situ* study on whether MSC's are suitable to assess stability (temperature/sunlight) of enteric viruses in the marine environment. Also mentioned a protocol developed by David Kinsley of USDA/ARS for distinguishing infectious from non-infectious NoV by utilizing binding to pig mucine. The binding would infer the capside region was intact and the binding sites were available.

X. <u>Questions Answered by Panelists</u>

A. Tom Howell

7. What is the estimated rate of false positives or false negatives utilizing existing MSC analysis?

   The chances of false positives are low due to the specificity of bacteriophages. These are for meat testing.
   See slide 14 of David Lees "Answers to questions" PowerPoint.
   Dave Love agreed citing methods comparisons EPA 1601 and EPA 1602 from a study in 2003-2005. EPA detected 60% of samples positive and 1602 was much lower at 24%.

10. What are the estimated costs to the industry, nationwide as a result of adopting more stringent growing area standards?

    MSC is not being recommended to replace the existing indicator.
    The impact of using MSC adjacent to WTPs may not reduce harvestable acreages and would not result in a cost to the industry.

14. What do we know about the dynamics of viral depuration rates and what factors/processes influence the rates of inactivation or elimination of enteric viruses or MSC?

    Temperature and season are influencing factors. As levels increase in winter and temperatures decrease, depuration becomes more difficult as well. For summer the opposite occurs with lower levels, higher temperatures and more efficient depuration.
    Also see question 6.

15. Since warm temperatures are required for shellstock to "purge" during relay or depuration (must be actively pumping) and MSC levels are low when temperatures are warm, how can it be an effective measure to reduce relay and depuration times?

    Winter months would probably require the heating of water and would not be economically feasible in tanks. It is *possible* to make it work year-round, but most economically in warmer months.

29. Did any studies determine the background levels of MSC in shellfish in prohibited areas/closed safety zones that are continuously exposed to adequately treated effluent from a wastewater treatment plant?

    Yes
    Tom Howell's PowerPoint Presentation: See graph on slide 4. The graph illustrates seasonal variation in multiple locations.

30. Any studies/data on the background levels of MSC in shellfish in the conditionally approved and/or approved areas, lying down stream/down tide from the adjacent or nearby prohibited area/closed safety zone around the sewage outfall that are continuously exposed to some amount of adequately treated effluent from a wastewater treatment plant?

    Kevin Calci indicated that the wording of this question, particularly "adequately treated", does not allow for a definitive answer.

35. Did any studies involve determining the levels of MSC in shellfish in an approved area, which is not near a WWTP outfall and thus not downstream/down tide from an outfall and not regularly exposed to dilute, adequately treated effluent from a WWTP, but which had been temporarily affected by raw, untreated sewage discharged from a break in a sewage collection line or pump station overflow, that is adjacent to that approved area but which normally sends raw sewage to a WWTP that discharges to another area?

*See answer to question 30.

B. Kevin Calci

2. There is some evidence that MSC replicate in the environment in the absence of a pollution source. How does this impact its use as an indicator of viruses?

   There is no evidence that MSC grows in the environment. He added that background in the absence of fecal pollution should be less than the lower limit of detection for the analysis currently employed (<10/100g of shellfish)
   Dave Love: Environmental conditions make it unlikely for this to happen.

11. What is the estimated reduction in the number of days opened to harvest of conditionally approved shellfish growing areas, anticipated as a result of adopting more stringent growing area standards?

    Instead of this being “more stringent” it could perhaps be a benefit by being “less stringent”.

12. What are the estimated reductions of approved or conditionally approved shellfish growing areas acreage, nationally, anticipated as a result of adopting more stringent growing area standards?

    See question 10.
    See slides 9 - 11 of David Lees “Answers to questions” PowerPoint.

20. Researchers have been looking for suitable Norovirus surrogates for decades, however each of the candidates (culturable viruses such as Feline calicivirus, Murine norovirus, Tulane virus) has drawbacks because apparently they don't respond to treatments (HPP, antiseptics, UV, chlorine etc.) in the same way as NoV. Why is MSC a superior surrogate to the other viral candidates?

    MSC is a cheap and quick assay for indicating municipal pollution.
    Lee-Ann Jaykus clarified that MSC is an indicator, not a surrogate. The other viruses mentioned here are surrogates and would not be appropriate for the purposes discussed in this meeting.

    Also, see questions 9 and 13.

33. What applicability is there when the discharge is other than "raw, untreated sewage" but involves, for example, partially-treated sewage that was chlorinated?

MSC is applicable when discharge is other than “raw untreated sewage”. The levels of chlorination presently used do not efficiently eliminate NoV or MSC. MSSC can actually be used to determine the level of treatment with respect to enteric virus.

34. Did any studies determine the change in the levels of MSC in shellfish (any species) over various intervals (days) after a discharge of raw, untreated, non-disinfected sewage? Of partially treated sewage, with disinfection by chlorination? Disinfection by UV radiation?

Information was not available to differentiate all of the treatment options listed, however Bruce Friedman provided data from the MSC samples following Hurricane Sandy that shows a relationship between water temperature and depuration of MSC. The data can be found at: www.nj.gov/dep/bmw/sandy.html.

C. Bill Burkhardt

1. Little is known on the distribution of phages in growing areas. What is the significance of background levels in the absence of sewage?

In general, municipal wastewater treatment effluents are the largest contributor of MSC into the estuarine environment and shellfish harvest areas. However under certain specific circumstances animal waste can be contribute MSC. Their presence in animal waste however does not diminish their utility since they remain an indicator of fecal waste impact.

3. What differences in winter vs. summer, if any, were found in the levels of MSC in water in areas of those different classification types around WWTP outfalls?

The vast majority of shellfish related norovirus illnesses occur in the colder months (late fall and early winter). Studies in Europe have been performed on shellfish show a strong relationship between norovirus occurrence/ levels and MSC levels. Independent studies have demonstrated bioaccumulation of MSC by shellfish to be seasonal, occurring at generally the same time when norovirus illnesses are reported.

David Lee’s PowerPoint “Answers to questions” slide 2 supports this statement.

9. How hard is it to learn the MSC assay, what is the cost per sample and are labs being validated independently to ensure that methods are repeatable between operators and labs?

The MSC assay for shellfish is relatively easy to perform and staffs from multiple state laboratories have already been trained on it. The cost of MSC for shellfish is roughly equivalent to that of performing fecal coliform testing. Initial cost to prepare laboratory to perform analysis is approximately $8,000- 10,000. (Refrigerated centrifuge)

16. Does the presence of food particles in the water influence depuration rates?

Yes. Shellfish in artificial seawater versus natural seawater had different effects on coliphage but not on fecal coliform. If food is present, shellfish feed and depurate more readily.

32. MSC are rarely detected in human feces, suggesting that their presence in water or shellfish meats do not necessarily indicate human fecal pollution. This needs further study. What size waste water treatment plant or size of human population served is too small to apply MSC?

MSC are indeed found at a low prevalence in fresh human fecal waste but the strength of their utility is based upon their ubiquitous nature in human wastewater. Is unaware of a situation where MSC was not found in the wastewater from a sizable population. They should be considered wastewater indicators not an indicator of fresh human fecal waste.

Dave Love indicated that approximately 5% of population shed MSC in their fecal waste.

D. Chris Roberts

4. What differences in winter vs. summer, if any, were found in the background levels of MSC in shellfish (any species) in areas of the different classification types around WWTP outfalls which are continuously exposed to some amount of adequately treated effluent?

See answer to question 3.

5. Do the accepted levels for regulatory decision making in the US and internationally vary by season or temperature?

The only established level that exists is for assessing the impact of waste treatment plant failure or collection system failures. There is not a seasonal or temperature variable.

21. Is the correlation between NLVs and other enteric viruses and MSC known? If known, how is the correlation impacted by type of treatment,

size of plant, and environmental and seasonal conditions at the discharge point. Is MSC a good indicator of NLVs or norovirus under all conditions?

See answer to question 20 and 13.

23. What are the limits in using MSC as an indicator of enteric virus concentrations in growing area overlay waters and shellstock?

See answer to question 20

28. How shall we consider the seasonal effects on efficacy of MSC as an indicator of the presence of pathogenic viruses: i. Variations in human population contributing to the WWTP? Perhaps very low in winter, ~15% of summer levels; ii. Persistence of MSC in the environment (water and/or shellfish)?; iii. Feeding activity of different shellfish species? Very low to inactive in winter, when water temps drop below 50^0F down to 30^0F.

See answer to question 20 and 3.

31. Do MSC levels in water and molluscan shellfish reflect the magnitude (dilution) of wastewater contamination? Do they overestimate or underestimate the level of contamination?

See answer to question 20 and 6.

E. Kim Reece

6. Are MSC's suitable to assess stability (temperature/sunlight) of enteric viruses in the marine environment?

See slides 2 – 5 in Kim Reece's "Reece Questions ISSC" PowerPoint.
At 20°C-30°C, there is a difference in inactivation rates. For the seasonal data, inactivation rates were more comparable in winter between FRNA coliphage and treated norovirus.
Dave Love mentioned the difference between DNA-MSC and RNA-MSC and whether these should be considered differently.

Kevin Calci: It is hard to compare the viability of the MSC with a molecular target NoV.

19. What is the relationship or correlation between live infectious norovirus and MSC?

Also, see David Lees PowerPoint presentation in response to his questions (slide 13). This data compares MSC levels, *E. coli* levels, and norovirus outbreaks. There was a relationship to MSC and NoV outbreaks.

22. MSC testing detects infectious agents while current RT-qPCR assays likely detect infectious and non-infectious NoV. Does this level of potential overestimation by RTqPCR err on the side of public health safety; is this overestimation acceptable? If not, why?

    Yes, but more work could be done on the protocols.

25. Is there a general association between MSC and NoV levels in naturally occurring shellfish? Is there an association between these levels and rates of illness? Is this association season/temperature association?

    Kim Reece's data did not provide correlation between MSC and NoV. See her slides in "Reecequestion2_ISSC". There was no association between MSC and rates of illness.

    Provided data that showed correlation between MSC levels and prelance of illness; published literature. See slides 2 - 5 of David Lees "Answers to questions" PowerPoint.

F. David Lees

3. What differences in winter vs. summer, if any, were found in the levels of MSC in water in areas of those different classification types around WWTP outfalls?

    Although the UK is using *E. coli* in shellfish meats for classification, differences were found.
    See slides 2 - 5 of David Lees "Answers to questions" PowerPoint.
    Kevin Calci's slides did not have winter versus summer data.

8. What are the options for reducing the lower limits of quantification in existing analysis methods for MSC in water?

    Adjust sensitivity.
    See slide 7 of David Lees "Answers to questions" PowerPoint. Dore et al., 2003.

12. What are the estimated reductions of approved or conditionally approved shellfish growing areas acreage, nationally, anticipated as a result of adopting more stringent growing area standards?

See answer to question 10.
See slides 9 - 11 of David Lees “Answers to questions” PowerPoint.

27. What is the relationship or correlation between illness and MSC?

See slides 13 - 16 of David Lees “Answers to questions” PowerPoint

G. Dave Love

7. What is the estimated rate of false positives or false negatives utilizing existing MSC analysis?

See answer to question 7 for Tom Howell.

13. What are the limitations of the MSC assay and when should it not be used? (aside from non-point source pollutants)?

Compared to fecal coliform, MSC is much more similar to NoV. MSC is also an inexpensive assay.

17. What differences between shellfish species (oysters, hard clams, mussels and soft clams), if any, were found in the levels of MSC) over various intervals (days) after the discharge of raw, untreated, non-disinfected sewage ended? Seasonal differences in uptake and purging of MSC in different species?

Kevin Calci: Generally, what we have found in studies is oysters have the highest bio-accumulation levels, then clams, then mussels.

18. How do different species eliminate NoV or MSC? What is the impact of temperature? or more specifically, what do we know about the elimination of NoV and MSC at temps below 50F for hard clams and oysters?

See answer to question 14 and 15.

24. Do MSC accurately reflect the bioaccumulation and elimination rates observed for NoV from molluscan shellfish? How do these rates of accumulation and elimination compare to those of fecal coliforms and E. coli? Is there a season/ temperature association?

Depuration studies were repeated month to month for E.coli and MSC to see if there was any difference in depuration rates due to environment temperature and no difference was found.
See slides 2 – 5 of David Lees “Answers to questions” PowerPoint.

26. NoV appears to be rapidly inactivated in summer by UV light. Is the same true for MSC?

Yes, viruses are sensitive to UV based on the size of their genome. Viruses with bigger genomes die sooner. DNA is more sensitive due to thymine nucleotide.

H. Unassigned

36. 11-102 – Use of shellstock meat samples to define and determine prohibited areas around treatment plants without conducting dye dispersion studies or models may not provide equivalent protection. Without knowledge of the hydrographics impacting the discharge dispersion and dilution, how can the Authority determine where shellstock samples should be collected? How many, how often, what time of year should shellstock be sampled? How often would meat sampling need to occur to be able to account for poor performance or temporary loss of disinfection?

See Committee Recommendations

37. Questions re 11-101 Does size matter? Do we have the right kind of information to determine what a "large" spill is? How does "partial treatment" impact MSC levels in effluent? Differences at sewage treatment plants may produce a vast number of different quality of effluent labeled as "partially treated effluent", how would that be defined?

See Committee Recommendations

XI. <u>Expert Panelists Consensus</u>

A. MSC should not be used to replace Fecal Coliform as an indicator for shellfish growing area classification.

B. MSC should continue to be used in conjunction with sanitary surveys to assess impacts of raw untreated sewage discharged from waste treatment plant failures.

C. MSC testing could be used in re-opening conditional growing areas adjacent to waste treatment plant outfall after waste treatment plant bypass or malfunction following the required 7 days closure.

D. MSC could be used to evaluate the impact of rainfall events for combined sewer systems and hydraulically-overburdened sanitary systems. Based on the efficiency of the plant, MSC could be used to evaluate the changes in MSC levels in water and shellfish meat.

E. MSC sampling data comparing influent and effluent quality could be used under various flow conditions to evaluate waste treatment plants for determining the size of prohibited, restricted, and conditionally approved area adjacent to outfalls. This could include determining the size of harvest areas for relaying and depuration.

F. MSC sampling data comparing waste treatment plant influent and effluent quality could be used for evaluating viral reduction. This information could be valuable as critical input for dilution models and hydraulic modeling.

G. MSC sampling data could be used as a classification and assessment tool to determine viral persistence in shellfish meats harvested from growing areas adjacent to waste treatment plant outfall for determining seasonal, spatial, and meteorological variations.

H. MSC sampling data could be used as a classification and assessment tool for verifying viral persistence in shellfish meats harvested from growing areas adjacent to waste treatment plant outfalls. This information could be used in ground truthing studies and dilution models

I. MSC could be used in source water tracking for shoreline survey problems associated with waste treatment plant collection systems and pump stations.

J. MSC, in conjunction with fecal coliform, could be used as an optional indicator for sampling to determine effectiveness studies and process controls for relaying and container relaying.

K.. MSC, in conjunction with fecal coliform, could be used as an optional Indicator for sampling to determine effectiveness studies and process controls for depuration plants.

XII. Conclusions From Expert Panel Consensus

Section IV of this report outlines eight (8) characteristics of an effective indicator.

The primary indicator used in the NSSP is fecal coliform. Total coliform is allowable under the NSSP along with MSC which presently has a very limited application. Fecal coliform is used by most state control programs and is superior to total coliforms in addressing the eight (8) characteristics listed below

Although fecal coliform satisfies many of the criteria, it has weaknesses in addressing others. The information provided by the expert panels suggest that the use of MSC in conjunction with fecal coliform can provide more insight into sources and risk of enteric viruses. The criteria as listed below outlines the benefits of

combining the use of MSC with fecal coliforms in situations involving human fecal waste from large populations.

1. Should be a derived intestinal microflora of warm-blooded animals

Fecal and total coliform are found in the intestinal microflora of warm blooded animals. Although MSC has been found in the intestinal microflora of warm blooded animals, it is most often associated with humans. This unique characteristic allows MSC to compliment fecal and total coliform

2. Should be present whenever pathogens are present

Chlorination can eliminate fecal and total coliform without eliminating certain enteric virus like norovirus . MSC are more akin to enteric viruses and with coliform would better define the risk of enteric viruses.

3. Should occur in greater numbers than the pathogen of concern

When present, fecal coliform, total coliform and MSC are all found in numbers greater than the pathogen of concern, notable wastewater

4. Should be absent or at least very few numbers in clean waters

Total and fecal coliforms and MSC are usually absent or found in very low numbers.

5. Should be detectable and quantifiable by easy, rapid, inexpensive methods

Fecal Coliform, total coliform and MSC are all detectable utilizing an easy, rapid and inexpensive method.

6. Should be non-pathogenic

Fecal coliform, total coliform and MSC are not pathogenic

7. Should not multiply in the environment

In most instances total coliforms and fecal coliform do not multiply in the environment. MSC cannot multiply in the environment.

8. Should respond to natural environmental stress and wastewater treatment processes and disinfectant in a manner similar to the pathogen of interest

Fecal and total coliform do not respond the same as enteric viruses. MSC does respond similarly to enteric viruses (ex. Norovirus)

XIII. Growing Area Committee Action

Following the Expert Panel activities, the Growing Area Classification Committee discussed possible expansion of the use of MSC in the NSSP. The discussion and actions taken by the Committee are addressed in the Committee report(which is attached).