

Submitter	Executive Board Interstate Shellfish Sanitation Conference (ISSC) issc@issc.org
Proposal Subject	Pre-Proposal for Male-Specific Coliphage Enumeration in Wastewater by Direct Double-Agar Overlay Method
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	The submitter of the pre-proposal requests approval to submit a full proposal to the ISSC for approval of the analytical method for use in the NSSP. Submitted by the developer Kevin Calci (FDA Gulf Coast Seafood Laboratory) Proposed Use of the Method: This method is applicable for the enumeration of MSC wastewater influent, effluent and sewage contaminated surface waters. The method will directly determine the quantity of MSC in wastewater to provide information of the viral reduction efficiencies of wastewater treatment plants. Method is also applicable for the analysis of surface source waters as part of a shoreline survey. Description of Method: This method employs E. coli HS (pFamp) RR as a male-specific coliphage host in a direct double agar overlay for the quantification of plaque forming units. All sample volumes are plated in triplicate. Briefly, 2.5ml of sample is mixed with 2.5ml of soft agar and 0.2ml of Famp host and then poured onto bottom agar petri plate. One ml of the sample is serially diluted down to 1:10 and 1:100. Those two dilutions are then plated by placing 2.5ml of sample is mixed with 2.5ml of soft agar and 0.2ml of Famp host and then poured onto bottom agar petri plate. The plates are incubated at 35-37°C for 16-20 h. Under indirect light the plaque forming units are counted. The working range of the 9 plate method would be 14pfu/100ml to 1.0 x 10 ⁶ pfu/1 00ml.
Public Health Significance	Scientific consensus at the MSC informational meeting supported the use of MSC to evaluated wastewater treatment plant viral reduction efficiency to better inform the SSCA's conditional management plans impacted by wastewater treatment plant operations. This method would identify a consistent and accurate measure of MSC load in wastewater influent, effluent and surface waters.
Cost Information	
Action by 2015 Laboratory Methods Review Committee	Recommended referral of Proposal 15-114 to an appropriate committee as determined by the Conference Chair to await SLV data.
Action by 2015 Task Force I	Recommended adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-114.
Action by 2015 General Assembly	Adopted recommendation of Task Force I on Proposal 15-114.
Action by FDA January 11, 2016	Concurred with Conference action on Proposal 15-114.
Action by 2017 Laboratory Committee	Recommended referral of Proposal 15-114 to an appropriate committee as determined by the Conference Chair.
Action by 2017 Task Force I	Recommended adoption of Laboratory Committee recommendation on Proposal 15-114.

Action by 2017 General Assembly	Adopted the recommendation of Task Force I on Proposal 15-114.
Action by FDA February 7, 2018	Concurred with Conference action on Proposal 15-114.
Action by 2019 Laboratory Committee	Recommended referral of Proposal 15-114 to an appropriate committee as determined by the Conference Chair.
Action by 2019 Task Force I	Recommended adoption of Laboratory Committee recommendation on Proposal 15-114.
Action by 2019 General Assembly	Adopted recommendation of Task Force I on Proposal 15-114.
Action by FDA February 21, 2020	Concurred with Conference action on Proposal 15-114.
Action by 2023 Laboratory Committee	Recommended adoption of Proposal 15-114 as amended.
Action by 2023 Task Force I	Recommended adoption of the Laboratory Committee recommendation for Proposal 15-114.
Action by 2023 General Assembly	Adopted recommendation of Task Force I on Proposal 15-114.
Action by FDA July 7, 2023	Concurred with Conference action on Proposal 15-114.