Proposal Subject: Domoic Acid Test Kit

Specific NSSP Guide Reference: Section IV. Guidance Documents, Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.

Text of Proposal/ Requested Action

Mercury Science Inc., in collaboration with the NOAA Center for Coastal Fisheries and Habitat Research has developed a new quantitative immunoassay for the detection of Domoic acid. The assay has been commercialized and is currently sold for research use as the Domoic Acid Test Kit (product # DAK-36) (Information online at http://mercuryscience.com/DA).

This product underwent thorough testing by Mercury Science to define the performance characteristics of the assay prior to commercialization. In addition, the product has been independently validated in several labs in a variety of matrices. The results of these internal and external validation studies strongly suggest that the Domoic Acid Test Kit is a rapid, low-cost, and accurate method for analysis of food, water and phytoplankton samples.

At this time, Mercury Science would like to submit a partially complete Method Application to the ISSC Laboratory Methods Review Committee. Please note that the Method Application at this time does not include the completed Single Lab Validation report. The DA analyses to complete Section C. Validation Criteria are currently in progress and will continue throughout the summer. My laboratory has just received funding from the North Pacific Research Board and will be running ISSC Single Laboratory Validation Testing on butter clams (*Saxidomus giganteus*), blue mussels (*Mytilus edulis*), geoducks (*Panopea abrupta*), manila clams (*Venerupis japonica*), oysters (*Crassostrea virginica*) and razor clams (*Siliqua patula*) from Alaska later this summer. The NOAA CCFHR laboratory has similarly received their MERHAB funds last week and will be conducting a parallel Single Laboratory Validation study on butter clams, blue mussels, geoducks, manila clams, oysters, and razor clams from California, Oregon and Washington, oysters from North Carolina and quahogs (*Mercenaria mercenaria*) from Georges Bank, Massachusetts. The goal is to test a broad array of commercial species to ensure that matrix affects do not affect the assay. The results will be made available to the ISSC as they become available.

The work to date includes 1) publishing the complete ELISA methodology and initial validation studies in the December 2008 issue of the Journal of Shellfish Research and 2) completing the first validation series using oysters from North Carolina. The technique was also independently validated by the Quinault tribe in Washington State. They ran the ELISA on razor clam samples gathered by the tribe for a year and sent duplicate samples to the Washington Department of Health HPLC for analyses and have made their results available for inclusion in this preliminary application.

The purpose of this submission is to bring the new method to the attention of the committee in a manner that enables the method to be evaluated in a timely way. I am also seeking the committee's advice and guidance on the validation studies that will be conducted this coming summer by my laboratory and that of Wayne Litaker at NOAA. In the initial study using the oyster tissues I have closely followed the ISSC guidelines, but wanted to ensure that my interpretation was correct. I would therefore request the committee to review the methodology used in the initial oyster validation study to ensure the procedures used meet current requirements and that no additional data need to be gathered. If necessary, the protocol can be altered to meet the committee requirements.

Please find in association with this cover letter a series of materials relevant to the evaluation of the Domoic Acid Test Kit by the ISSC Laboratory Methods Review Committee.

These items included:

- ISSC Method Application with Section A, Section B**,** and Section D completed (see below).
- A pdf file containing the User Guide for the Domoic Acid Test Kit (DAK-36) that is included in the commercial product. (Also available online at: http://www.mercuryscience.com/DA User Guide 2007A.pdf)
- A pdf file containing a reprint of the research paper entitled " RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID," published in the December, 2008 issue of Journal for Shellfish Research. This paper describes correlation data comparing the Domoic Acid Test Kit versus HPLC analysis using several sample matrices. (Also available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf)
- An Excel file showing the results of a study done by the Quinault Indian Nation and the Washington Department of Health comparing razor clam analysis performed by the Domoic Acid Test Kit versus HPLC analysis. This independent study used samples collected over a nineteen month period and was planned and performed without any input from Mercury Science or NOAA. (also available online at: http://mercuryscience.com/QINWDOHdata.xls)
- Preliminary tests using oyster spiked materials (see below)

The ELISA method has been used independently in six laboratories and provided results equivalent to those obtained using HPLC, FMOC-HPLC and LC-MS. This is detailed in the Litaker et al. 2008 publication listed above. Based on the correlation studies conducted so far, I request that this method be considered for interim approval by the LMR committee until the remaining validation data can be provided over the next six months. Upon completion of the SLV, consideration for approval of the assay as a Level 4 method will be requested.

Public Health Significance: The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples.

Cost Information (if available): Anticipated cost is $7.00 per duplicate reaction

Proposed Specific Research Need/Problem to be Addressed:

This research focuses on the development is an accurate, rapid, cost-effective ELISA for use by environmental managers and public health officials to monitor Domoic Acid concentrations in environment samples. The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected.

In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. The high throughput capacity of the assay also allows for much faster response times when Domoic acid events occur. The relatively low cost of the assay means that significantly more sampling is also possible on the same or smaller budget.

How will addressing this research support/improve the mission/role of the ISSC/NSSP/Industry? Support need with literature citations as appropriate.

This Assay will allow better protect public health and provide a rapid response capability when DA outbreaks occurs. It can also be adapted to monitoring phytoplankton samples so that toxic blooms can be identify and tracked. Toxic phytoplankton cells generally appear several weeks before the shellfish become toxic and can be used as an early warning system for when shellfish are likely to become toxic/

More detailed information on the assay and its potential uses is provided in a recently published article:
RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID, Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008.
Available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf

Relative Priority Rank in Terms of Resolving Research Need:

Immediate	☐	**Important**	☐
Required	☐	**Other**	☐
Valuable	☐		

Estimated Cost: $7.00 per duplicate sample (~$200.00 for ELISA kit capable of analyzing 36 duplicate samples in 1.5 h)

Proposed Sources of Funding/Support: Grants have been awarded by NPRB and NOAA MERHAB program for the completion of the validation studies.

Time Frame Anticipated: Validation should be completed by January or February 2010.

Action by 2009 LMRC	Recommended referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-105.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 09-105.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-105.
Action by 2011 LMRC	Recommends referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman to await further data to be provided by Mercury Science the developer of the method to determine if the method is fit for purpose within the NSSP as a screening tool.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 09-105.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-105.

Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-105.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 09-105. Rationale - There is insufficient data to determine if the method is fit for purpose within the NSSP
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 09-105.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 09-105.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 09-105.
NOTE:	Click here for Proposal 09-105 Supporting Documentation