Proposal Subject: Rapid Extraction Method for PSP and ASP

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter III Laboratory @.02 Methods
ISSC Constitution, Bylaws, and Procedures
Procedure XVI.

Text of Proposal/ Requested Action

Procedure for Acceptance and Approval of Analytical Methods for the NSSP

Marine Biotoxins affect farmed and wild fish and shellfish, as well as having a deleterious effect on humans. Jellett Rapid Testing has designed and developed rugged tests for the presence of Paralytic Shellfish Poison, Amnesic Shellfish Poison and Diarrhetic Shellfish Poison (under development at the time of this submittal). To facilitate the use of these tests in the field (for aquaculturists, campers, regulatory officials, etc.), Jellett Rapid Testing has developed a "low-tech" rugged alternative to the standard AOAC method designed to extract the toxins in the field as well as the laboratory. The AOAC method requires the sample to be boiled in acid at low pH and the pH adjusted with strong acids. This requires a fully equipped laboratory and significant safety precautions. The JRT Rapid Extraction Method was designed for use in remote areas, with little sophisticated backup support, by average individuals with little training and education. It is faster, less labor-intensive and less expensive than the other available method.

The rapid extraction method requires vinegar and rubbing alcohol to extract the toxins. A simple, rapid, safe method such as this would make rapid tests for marine Biotoxins available in remote areas, to fishermen, aquaculturists, and regulatory officials on an instant basis.

The method developed by Jellett Rapid Testing Ltd has been presented to regulatory bodies over the past several years. In cooperation with individuals, governments and those organizations, the analytical method has been refined and improved. The Rapid Extraction Method is being tested in several states and foreign countries. Publications will be forthcoming.

The CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE allows the ISSC, through the Laboratory Methods Review Committee, to accept analytical methods that are sufficiently validated but are not AOAC or APHA methods. This is defined in the Constitution, PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP. Two possible reasons for considering a method are found in Subdivisions i and ii.

Subdivision i. Meets immediate or continuing need;

Subdivision ii. Improves analytical capability under the NSSP as an alternative to other approved or accepted method(s)

Currently, only the AOAC extraction for PSP and ASP are accepted. The need for a simple safe extraction method has been expressed by regulatory agencies, governmental organizations and industry for many years. The Jellett Rapid Extraction Method is being validated over a wide geographic area to demonstrate its simplicity, reliability, precision and accuracy. As a result of demonstrations of efficacy and the need that has been expressed by industry and state agencies, the Jellett Rapid Extraction Method is presented as an alternative extraction method for PSP and ASP for the NSSP as a Type III or Type IV method.

Please see attached additional information.

Suggested wording:

Section II, Chapter III Laboratory @.02 Methods

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and

(2) The current APHA method used in bioassay for *Karemia breve* toxins.

(3) The Jellett Rapid Extraction Method may be used for extracting PSP and ASP toxins from Shellfish by regulatory and industry laboratories.

Public Health Significance:

Currently, only the AOAC extraction for PSP and ASP analyses are accepted. Because of many significant constraints, in practical terms, this means that analyses can be conducted only in laboratories, and then under dangerous conditions. Acceptance of the Jellett Rapid Extraction Method for PSP and ASP would allow harvesters, processors, and regulatory agencies to screen for PSP and ASP with an accepted standardized method that provides valid useable data.

The Jellett Rapid Extraction Method for PSP and ASP was developed over several years in answer to the oft-stated need for a rapid, reliable, rugged, simple and safe sample preparation method. The Jellett Rapid Extraction Method for PSP and ASP is not meant to be a definitive "Standard Method", but rather to provide a supplementary extraction method that can be used in the field as well as in the lab.

Possible applications for The Jellett Rapid Extraction Method for PSP and ASP include:

- as a supplement to analytical methods of screening out negative samples in shellfish regulatory labs;
- as a harvest management tool at aquaculture facilities or in wild shellfish harvest areas (especially near shore areas) to supplement available methods to determine if shellfish are free of PSP or ASP and safe to harvest;
- as a supplement to quality control methods for shellfish processing plants, distributors and wholesalers to ensure incoming shellfish are free of PSP and ASP toxins before processing or further distribution (this test could become part of the plant's HACCP program);
- as a supplement to analytical methods for water classification for Biotoxins; and
- as a supplement to analytical methods for broad scale ecological monitoring.

The rationale for using the Jellett Rapid Extraction Method for PSP and ASP is that the method provides a rapid, reliable, rugged, simple, safe and cost-effective extraction method (especially in low-volume laboratories) for PSP and ASP that can supplement accepted tests and substantially reduce the cost of analyses. Used in conjunction with other rapid methods, the Jellett Rapid Extraction Method for PSP and ASP will supplement regulatory agency efforts and help prevent the harvest of contaminated product. Having the ability to conduct tests using an accepted rapid extraction method will allow those processors who choose to use this test to demonstrate that they are truly controlling for PSP and ASP hazards in the harvested shellfish.

The Jellett Rapid Extraction Method for PSP and ASP could contribute to building long-term databases on broader scales than a regulatory lab can afford and, by using an accepted standardized method, will provide consistent results. These databases could be supplemented with industry testing in areas where there is no testing currently. This would extend, augment and strengthen the current food safety system broadening and refining the food safety net by increasing the number of testing sites and generating long term data in more areas.

A simple, rapid, rugged, effective, reliable, safe and cost-effective extraction method, available to all harvesters, regulators, and processors, would increase the monitoring and reduce the chance that shellfish containing ASP toxins above the regulatory limit would be harvested or marketed.

Cost Information (if available): It is difficult to determine exact costs because many government cost models do not consider capital costs. Both extraction methods are the same through puree step, the chemicals used in both cases are minimal, as is the cost of incidental equipment (blender, pipettes, etc.). However, a comparison of time required using the Rapid Extraction Method (Add rapid liquid; Filter) with the time required using the AOAC Extraction (Add HCL; Boil; Wait; Filter; Pour in tube; Check PH) shows a significant difference. Our experience shows that it takes about 22 minutes for this portion of the AOAC extraction while it takes less than 2 minutes to complete the Jellett Rapid Extraction Method. At a salary of $33 / hour, that is a savings of $11.00 per sample extract.

Action by 2005 LMRC Recommended referral of Proposal 05-111 to the appropriate committee as determined by the Conference Chairman.

Action by 2005 Task Force I Recommended adoption of the Laboratory Methods Review Committee recommendation of Proposal 05-111.

Action by 2005 General Assembly Adopted recommendation of 2005 Task Force I.

Action by USFDA Concurred with Conference action.

Action by 2007 LMRC Recommended no action on Proposal 05-111. Rationale – Alternative extraction method for JRT PSP should be adopted to expand utility of the test; however there are insufficient data for acceptance at this time. The submitter will send data to the Executive Office for Conference approval.

Action by 2007 Task Force I Recommended referral of Proposal 05-111 to an appropriate committee as determined by the Conference Chairman.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force I.

Action by USFDA December 20, 2007
Concurred with Conference action with the following comments and recommendations for ISSC consideration.

The Conference has made considerable progress in its efforts to recognize new and developing analytical methods for the detection of indicators, pathogens, and marine toxins. Much credit goes to the Laboratory Methods Review Committee and its leadership for ensuring a scientifically defensible process for adopting analytical methods under the NSSP.

At the 2007 meeting numerous analytical methods were proposed for ISSC adoption. However, many of these methods were lacking the validation and associated data needed by the Laboratory Methods Review Committee to make a final determination regarding their efficacy for use in the NSSP. As a result the General Assembly voted "No Action" on analytical method Proposals 05-107, 05-108, 05-109, 05-111, 05-113, and 05-114. It is FDA's understanding that the intent of the "No Action" vote was not to remove these Proposals from ISSC deliberation as "No Action" normally suggests, but rather to maintain them before the Conference pending submission of additional

data for further consideration. The Voting Delegates, by requesting the Proposal submitters provide additional data to the Executive Office for methods approval consistent with Procedure XVI, clearly recognized the importance and utility of these methods and intended to maintain them before the Conference for possible adoption following additional data submission. FDA requests that the ISSC Executive Board confirm FDA's understanding of this outcome. FDA fully supports such a Conference action and encourages the Executive Office to pursue submission of additional data as necessary to move forward with acceptance of these methods.

Action by 2009 LMRC Recommended no action on Proposal 05-111. Rationale: Requested additional information has not been submitted.

Action by 2009 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation of Proposal 05-111.

Action by 2009 General Assembly Referred Proposal 05-111 to the Laboratory Methods Review Committee.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 05-111.

Action by 2011 LMRC Recommended acceptance of the rapid extraction method in Proposal 05-111, specifically 70% isopropanol: 5% acetic acid 2.5:1, only for use with the Abraxis shipboard ELISA for PSP as an Emerging Method solely for use in the onboard screening dockside testing protocol in the Northeast region, including George's Bank.

The Laboratory Methods Review Committee further recommends:

1. The data collected during the dockside testing study be submitted to the LMRC in the SLV Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions.

2. The validation study conducted by the State of Maine of the Abraxis laboratory ELISA with the extraction method in Proposal 05-111 be submitted to the LMRC in the SLV Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions.

3. No action on the requested language change in Proposal 05-111 for the Model Ordinance Section II, Chapter III Laboratory @.02 Methods.

   Section II, Chapter III Laboratory @.02 Methods
   C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:
   (1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and
   (2) The current APHA method used in bioassay for *Karenia breve* toxins.
   (3) The ~~Jellett Rapid Extraction Method may be used for extracting PSP~~ ~~and ASP toxins from Shellfish by regulatory and industry~~ ~~laboratories.~~

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 05-111.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 05-111.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 05-111.

Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 05-111 Rationale - Proposal 05-111 is resolved by action on Proposal 13-109.
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 05-111.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 05-111.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 05-111.

Lab #	CFIA Sample #	CFIA Result HPLC (µg/g)	Jellett Result Approx. (µg/g)
04-01847	1	24.1	16-24
04-02156	2	1.4	0-4
04-01784	3	70.0	72-80
04-01968	4	71.9	72-92
04-01647	5	8.9	12-16
04-02328	6	9.3	6.4-11.2
04-02467	7	4.2	6.0-7.2
04-01646	8	31.2	40-64
04-02351	9	9.4	9.6-12
04-02238	10	4.7	4-5.6
04-01862	11	96.7	60-80
04-02240	12	10.3	12-20
04-01750	13	30.7	24-32
04-02231	14	2.5	0-4
04-01969	15	40.1	64-72

Jellett Rapid Testing Ltd.: NOAA Study - JREM Trial
Sample Record Sheet – Homogenate
State of Alaska - Department of Environmental Conservation

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	Date	Species	Field / Site / Lab Name	Date	Size of Sample (mL)	Field / Site / Lab Name	Date	Batch # - Test	Batch # - Buffer	Result (1=Pos, 0=Neg)	Intensity of C Line as % of T	Lab Name	Date	Toxin Standard Used	# of Mice Dead	Result (µg/100g)	# of Mice Sick
20053168-C	3/06/05	Geoduck Viscera	ADEC-EHL	3/14/05	66[2]	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	71	0
20053169-C	3/06/05	Geoduck Viscera	ADEC-EHL	3/14/05	495	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	<10%	ADEC-EHL	03/15/05	FDA	3	39	0
20053170-C	3/06/05		ADEC-EHL	3/14/05	650	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	71	0
20053183-C	3/13/05	Geoduck	ADEC-EHL	3/15/05	416	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	>0%, <25%	ADEC-EHL	03/15/05	FDA	3	70	0
20053184-C	3/13/05	Geoduck	ADEC-EHL	3/15/05	632	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	54	0
20053185-C	3/14/05	Geoduck	ADEC-EHL	3/15/05	561	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	72	0
20053186-C	3/15/05	Geoduck	ADEC-EHL	3/15/05	301	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	90	0
20053137	03/06/05	Oyster	ADEC-EHL	03/08/05	150	ADEC-EHL	03/08/05	40000-13Aug04	40005-05Nov04	INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053136	03/06/05	Oyster	ADEC-EHL	03/08/05	500	ADEC-EHL	03/08/05	40000-13Aug04	40005-05Nov04	N/A INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053138	03/05/05	Oyster	ADEC-EHL	03/08/05	500	ADEC-EHL	03/09/05	40000-13Aug04	40005-05Nov04	INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053142	03/06/05	Oyster	ADEC-EHL	03/09/05	50	ADEC-EHL	03/09/05	40000-13Aug04	40005-05Nov04	INV	C <50% T	ADEC-EHL	03/09/05	FDA	0	NDT	0
20053124-C	3/5/05	Geoduck	ADEC-EHL	3/7/05	495	ADEC-EHL	3/7/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/07/05	FDA	3	117	0
20053125-C	3/5/05	Geoduck	ADEC-EHL	3/7/05	404	ADEC-EHL	3/7/05	40000-13Aug04	40005-05Nov04	1	75%	ADEC-EHL	03/07/05	FDA	3	58	0
20053006	2/29/05	Oyster	ADEC-EHL	3/3/05	125	ADEC-EHL	3/3/05	40000-13Aug04	40005-05Nov04			ADEC-EHL	3/3/05	FDA	0	NDT	0
20053040-C	03/01/05	Geoduck Viscera	ADEC-EHL	03/02/05	545	ADEC-EHL	03/02/05	40000-13Aug04	40009-06Oct04	1	50%	ADEC-EHL	03/02/05	FDA	3	86	0
20053039-C	03/01/05	Geoduck Viscera	ADEC-EHL	03/02/05	340	ADEC-EHL	03/02/05	40000-13Aug04	40009-06Oct04	1	10%	ADEC-EHL	03/02/05	FDA	3	175	0
20053007-C	02/26/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	25%	ADEC-EHL	02/28/05	FDA	3	59	0
20053010-C	02/26/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	<25%	ADEC-EHL	02/28/05	FDA	3	65	0
2005301-C	02/27/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	0%	ADEC-EHL	02/28/05	FDA	3	151	0

Jellett Rapid Testing Ltd.: NOAA Study
JREM Trial Sample Record Sheet - Homogenate
California - Microbial Disease Lab

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	Collection Date	Species	Field / Site / Lab Name	Date	Size of Sample (mL)	Field / Site / Lab Name	Date	Batch # - Test	Batch # - Buffer	Result (1=Pos, 0=Neg)	Intensity of C Line as % of T	Lab Name	Date	Toxin Standard Used	# of Mice Dead	Result µg/100g	# of Mice Sick
05E-00110	02/05/05	LBMU	CA-DHS-EMDS	02/09/05	>130	CA-DHS-EMDS	02/09/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	2/09/05	FDA	0	<36	0
05W-00099	02/01/05	SSMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<34	0
05E-00096	02/28/05	CBMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<36	0
05W-00093	02/01/05	SBMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<36	0
05W-00079	01/25/05	SSMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/26/05	FDA	0	<35	0
05W-00076	01/22/05	CBMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	1	50%	CA-DHS-EMDS	01/26/05	FDA	3	39	0
05W-00069	01/24/05	SBMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	01/26/05	FDA	0	<36	3
05W-00059	01/18/05	SSMU	CA-DHS-EMDS	01/19/05	>130	CA-DHS-EMDS	01/19/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/19/05	FDA	0	<35	3
05W-00055	01/14/05	CBMU	CA-DHS-EMDS	01/18/005	>130	CA-DHS-EMDS	01/18/05	40000-8/13/04	40005-9/7/04	1	25%	CA-DHS-EMDS	01/18/05	FDA	3	37	
05W-00052	01/17/05	SBMU	CA-DHS-EMDS	01/18/05	>130	CA-DHS-EMDS	01/18/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	01/18/05	FDA	0	<36	0
05W-00025	1/10/05	SBMU	CA-DHS-EMDS	1/12/05	>130	CA-DHS-EMDS	1/12/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/12/05	FDA	0	<35	0
05W-00023	1/11/05	SSMU	CA-DHS-EMDS	1/12/05	>130	CA-DHS-EMDS	1/12/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/12/05	FDA	0	<36	0
05W-00020	1/7/05	CBMU	CA-DHS-EMDS	01/11/05	>130	CA-DHS-EMDS	01/11/05	40000-8/13/04	40005-9/7/04	1	25%	CA-DHS-EMDS	1/11/05	FDA	3	44	0

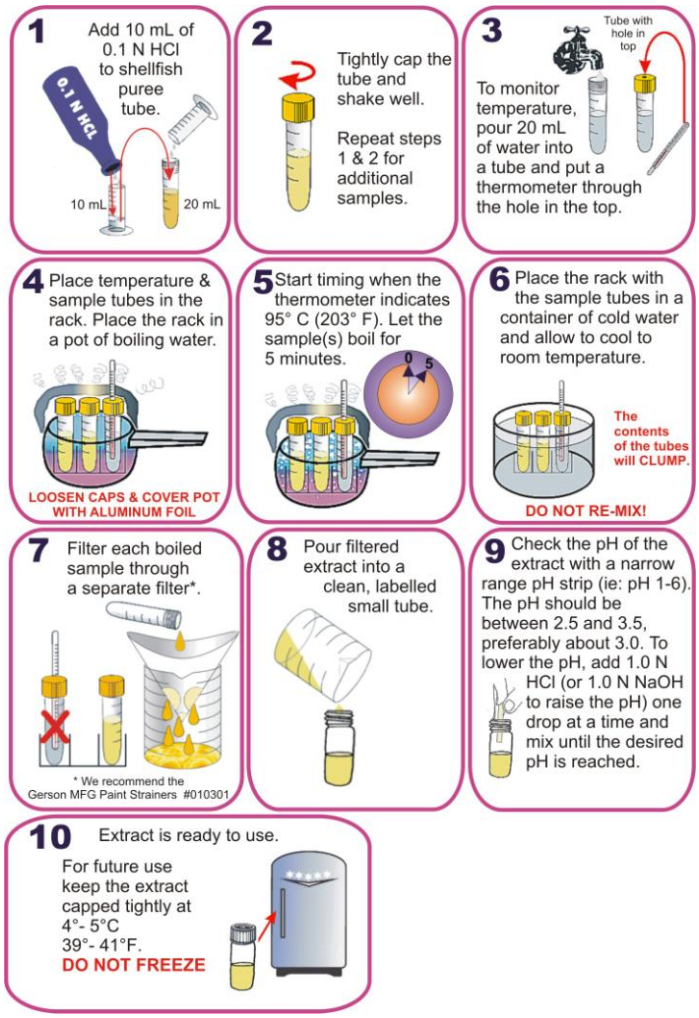
Jellett Rapid Testing Ltd.: NOAA Study
JREM Trial Sample Record Sheet - Homogenate
California - Microbial Disease Lab (CONTINUED)

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	**Collection Date**	**Species**	**Field / Site / Lab Name**	**Date**	**Size of Sample (mL)**	**Field / Site / Lab Name**	**Date**	**Batch # - Test**	**Batch # - Buffer**	**Result (1=Pos, 0=Neg)**	**Intensity of C Line as % of T**	**Lab Name**	**Date**	**Toxin Standard Used**	**# of Mice Dead**	**Result µg/100g**	**# of Mice Sick**
05W-00011	1/3/05	SBMU	CA-DHS-EMDS	1/5/05	>130	CA-DHS-EMDS	1/5/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/5/05	FDA	0	<34	0
05W-00007	1/4/05	SSMU	CA-DHS-EMDS	1/5/05	>130	CA-DHS-EMDS	1/5/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/5/05	FDA	0	<34	0
05W-00002	12/30/04	CBMU	CA-DHS-EMDS	1/04/05	>130	CA-DHS-EMDS	1/04/05	40000-8/13/04	40005-9/7/04	0	75%	CA-DHS-EMDS	1/04/05	FDA	2	36	1
04W-01458	12/28/04	SSMU	CA-DHS-EMDS	12/29/04	>130	CA-DHS-EMDS	12/29/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/29/04	FDA	0	<36	0
04W-01454	12/27/04	SBMU	CA-DHS-EMDS	12/29/04	>130	CA-DHS-EMDS	12/29/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/29/04	FDA	0	<36	0
04W-01457	12/24/04	CBMU	CA-DHS-EMDS	12/28/04	>130	CA-DHS-EMDS	12/28/04	40000-8/13/04	40005-9/7/04	1	<25%	CA-DHS-EMDS	12/28/04	FDA	3	42	0
04W-1446	12/21/04	SSMU	CA-DHS-EMDS	12/22/04	>130	CA-DHS-EMDS	12/22/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/22/04	FDA	0	<34	0
04W-01436	12/20/04	SBMU	CA-DHS-EMDS	12/21/04	>130	CA-DHS-EMDS	12/21/04	40000-8/13/04	40005-9/7/04	0	75%	CA-DHS-EMDS	12/21/04	FDA	0	<34	3
04W-01399	12/13/04	SBMU	CA-DHS-EMDS	12/14/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	1	50%	CA-DHS-EMDS	12/15/04	FDA	2	35	0
04W-01421	12/11/04	CBMU	CA-DHS-EMDS	12/15/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	1	0%	CA-DHS-EMDS	12/15/04	FDA	3	48	0
04W-01424	12/14/04	SSMU	CA-DHS-EMDS	12/15/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/15/04	FDA	0	<35	0

Extraction is a process whereby the prepared shellfish tissue is converted into liquid containing released toxins.

The Association of Official Analytical Chemists (AOAC) is the approved method of extraction used by regulatory agencies. The Mini AOAC Extraction is a scaled-down version of the official method, based on a 10g sub sample from 100g of shellfish puree and was validated by Jellett Biotek Ltd. (Toxicon 40 [2002] 1407-1425). It was shown to be equally as effective as the full 100g official extraction method.

Mini AOAC Extraction Method:



Equipment required to perform the mini AOAC extraction:

- Pot
- Hot plate
- Heat resistant test tube rack*
- Centrifuge tube with hole in cap*
- Thermometer*
- Tin foil

- Timer
- Graduated cylinder 10ml*
- Filters*
- 600 ml plastic beakers*
- Transport tubes 5ml*
- Plastic droppers
- pH strips (2-6 range)*

Solutions required to perform mini AOAC Extraction:

- 0.1 N HCl
- 1.0 N HCl
- 1.0 N NaOH
- Tap water

*Available to purchase from Scotia Rapid Testing as individual components or as an extraction kit.

The Mini AOAC extraction method can be used with PSP and ASP Rapid tests.

Cleaning Up

Clean all used equipment thoroughly with 5% household bleach and rinse well with tap water before re-using with another sample to avoid cross contamination.

Proposal Subject: Saxitoxin (PSP) ELISA Kit

Specific NSSP Guide Reference: Section IV. Guidance Documents, Chapter II Growing Areas, .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods

Section II. Model Ordinance Chapter III. Laboratory @.02 Methods C. Biotoxin

Text of Proposal/ Requested Action See attached ISSC Method Application

Faster, easier, and/or more reliable methods are needed to satisfy the needs of the regulatory community and shellfish industry. The proposed ELISA method is a fast and easy to perform method with ready to use reagents i.e. analyst only needs to extract shellfish sample or dilute water sample before analysis. The proposed ELISA also provides a quantitative and/or semi-quantitative screening for shellfish extracts and/or water samples. This assay is part of Abraxis platform for marine toxin testing and complements the company's other offering for NSP, DSP, and ASP testing. The proposed ELISA can be used on-site (boat, dock) or established analytical laboratories.

Public Health Significance:

Cost Information (if available): As low as $15 per sample.

Action by 2009 Laboratory Methods Review Committee Recommended no action on Proposal 09-107. Rationale: Insufficient data.

Action by 2009 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-107.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-107 with the following comments and recommendations for ISSC consideration.

The Laboratory Methods Review Committee determined that Proposal 09-107 was accompanied by insufficient data necessary for the Committee to make a determination regarding the efficacy of the proposed saxitoxin test method for use under the NSSP. As a result the General Assembly voted "No Action" on the proposed analytical method. It has been FDA's observation and experience that the proposed ELISA method for saxitoxins presents itself as a reliable screening method to supplement existing NSSP tools for managing Paralytic Shellfish Poisoning (PSP). Therefore, FDA Recommended the Conference pursue submission of additional data from Abraxis, LLC via the Proposal submission process to advance a thorough examination of this method for saxitoxin screening.

Action by ISSC Executive Board March 2010 The Executive Office will send a letter to the submitter of Proposal 09-107 to resubmit Proposal 09-107 Saxitoxin (PSP) Elisa Kit with additional information.

Action by 2011 Laboratory Methods Review Committee	Recommended approval of Proposal 09-107 as an emerging method. NOTE: This approval is limited to the Abraxis Shipboard ELISA Method used in conjunction with the Extraction Method approved in Proposal 05-111.
Action by 2011 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 09-107 as an emerging method.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-107.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-107.

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	Saxitoxin (PSP) ELISA Kit	
Name of the Method Developer	Abraxis LLC	
Developer Contact Information	Fernando Rubio 54 Steamwhistle Drive Warminster, PA 18974 Phone: (215) 357-3911 FAX: (215) 357-5232	
Checklist	**Y/N**	**Submitter Comments**
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.		Shellfish are filter feeders that pump large quantities of water through their bodies when actively feeding. During this process, shellfish can concentrate toxigenic micro-algae and other substances from the water column when they are present. The ability of shellfish to concentrate chemical pollutants from water can lead to accumulation of these toxins to levels that constitute a public health hazard. Dinoflagellates producing Saxitoxin have caused mortality events in fish, and sea mammals. In humans, Saxitoxin (PSP) poisoning causes neurological symptoms that can lead to respiratory paralysis and even death. Some of the currently available methods used for the detection and monitoring of saxitoxin in water and shellfish are not conducive for the quick on-site or real time, dockside or ship board monitoring of this toxin. For example: 1) the mouse bioassay is labor intensive, requires the use and destruction of many vertebrate animals, analyses is only performed in a few laboratories with a low turn around time, 2) a lateral flow ELISA developed by Jellet Rapid Testing Ltd., however, this assay seems to produce a high degree of false positives. Therefore, faster, easier and/or more reliable methods are needed to satisfy the needs of the regulatory community and shellfish industry. The proposed ELISA method is a fast and easy to perform method with ready to use reagents i.e. analyst only needs to extract shellfish sample or dilute water sample before analysis. The proposed ELISA also provides a quantitative and/or semi-quantitative screening for shellfish extracts and/or water samples.

		This assay is part of Abraxis platform for marine toxin testing and complements the company's other offering for NSP, DSP, and ASP testing.
2. What is the intended purpose of the method?		The fast analysis of Saxitoxin (PSP) in shellfish extracts and/or water quality monitoring. The proposed ELISA can be used on-site (boat, dock) or established analytical laboratories.
3. Is there an acknowledged need for this method in the NSSP?		Yes. NSSP Guidance Documents, Chapter II Constitution by-laws and procedures of the Interstate Shellfish Sanitation Conference. Procedure XVI. Procedure for acceptance and approval of analytical methods for the NSSP. And: National Shellfish Sanitation Program 2003 Model Ordinance III. Laboratory @ 02 Methods C. Biotoxin. Methods for the analysis of shellfish and shellfish harvest waters shall be: 1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins
4. What type of method? i.e. chemical, molecular, culture, etc.		Immunochemical Method.
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title		Abraxis ELISA Kit for the Screening of Saxitoxin in Shellfish Extract and/or Harvest Waters.
Method Scope		A Method for the screening out negative saxitoxin samples in shellfish regulatory labs, to determine if shellfish are safe to harvest and or distribute. A method for water classification for saxitoxin around harvest areas and to screen for toxic phytoplankton in seawater to provide early warning. A method that provides multiple simultaneous results (depending on chosen cut-off values). This can be easily done because the assay is run with multiple STX concentrations.
References		Etheridge, S., Deeds, J, Easy, D., Laycok, M., Caulfield, C., Deardorff, D., Church, J., PSP & TTX Kits: Regulatory Perspectives. Satellite Workshop to the Gordon Conference on Mycotoxins and Phycotoxins 2007, Maine, USA, E. Hignutt, S.W. Longan, Environmental Health Laboratory, State of Alaska, Anchorage, AK; Comparison of HILIC/Tandem Mass Spectrometry, Abraxis ELISA and Mouse Bioassay for Determination of PSP in Shellfish. To be presented at the 2008 AOAC Annual Meeting, Dallas, Texas.
Principle		**The test is a direct competitive ELISA based on the recognition of Saxitoxin by specific antibodies. Saxitoxin, when present in a sample and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The**

		saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.
Any Proprietary Aspects		Immunoreagents and sample diluent.
Equipment Required		Pipettes and plate reader. Blender for shellfish extraction.
Reagents Required		Reagents provided in the ELISA kit. In addition diluted hydrochloric acid or vinegar and rubbing alcohol (depending on extraction procedure chosen by analyst).
Sample Collection, Preservation and Storage Requirements		Water samples need to be collected in glass vials and preserved according to users guide (attached). Diluted shellfish extracts should be stored in glass vials. All dilution should be done using provided sample diluent. If not analyzed promptly, samples should be stored refrigerated for up 2 days or frozen if longer periods are required.
Safety Requirements		As with any laboratory procedure, gloves and goggles should be used during the processing and analysis of samples.
Clear and Easy to Follow Step-by-Step Procedure		User's guide and an easy to follow flow chart are provided with each kit (attached).
Quality Control Steps Specific for this Method		As with any analytical procedure laboratory controls (positive and negative) are recommended.
C. Validation Criteria		
1. Accuracy / Trueness		Provided as an attachment.
2. Measurement Uncertainty		@ 0.046 ng/mL in water SD 0.004 CV 8.7% @ 0.087 ng/mL in water SD 0.004 CV 4.6% @ 0.227 ng/mL in water SD 0.008 CV 3.5%
3. Precision Characteristics (repeatability and reproducibility)		< 15%
4. Recovery		Average water recovery 112%; shellfish extract 96%.
5. Specificity		Saxitoxin (STX) 100% (per definition) Decarbamoyl STX 29% GTX 2 & 3 23% GTX-5B 23% Sulfo GTX 1 & 2 2.0% Decarbamoyl GTX 2 & 3 1.4% Neosaxitoxin 1.3% Decarbamoyl Neo STX 0.6% GTX 1 & 4 <0.2%
6. Working and Linear Ranges		0.02-0.4 ng/mL water or 20-400 ng/gm in shellfish extract or higher depending on dilution.
7. Limit of Detection		0.015 ng/mL
8. Limit of Quantitation / Sensitivity		0.02 ng/mL in water; 20 ng/gm in shellfish extract
9. Ruggedness		Since and analytical curve is run with each assay and the samples are compared to the standard curve, the proposed ELISA is rugged.
10. Matrix Effects		If used according to instructions (dilutions), none detected

11. Comparability (if intended as a substitute for an established method accepted by the NSSP)		Method is intended as a screening method to complement other accepted NSPP methods: i.e. mouse bioassay. Some comparison data is provided as an attachment.
D. Other Information		
1. Cost of the Method		As low as $15 per sample
2. Special Technical Skills Required to Perform the Method		Some technical skills are required. Familiarity with laboratory setting is adequate. Kit Manufacturer's on-site training is available.
3. Special Equipment Required and Associated Cost		As low as $1,800. Strip reader and pipette
4. Abbreviations and Acronyms Defined		ELISA: Enzyme linked immuno sorbent assay PSP: paralytic shellfish poisoning
5. Details of Turn Around Times (time involved to complete the method)		40 samples can be run in duplicate in approximately 2 hours. Shellfish sample extraction requires approximately 15 minutes
6. Provide Brief Overview of the Quality Systems Used in the Lab		The ELISA kits are manufactured following GMP and GLP procedures.
Submitters Signature	Date:	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	
Recommendations for Further Work	Date:	
Comments:		

DEFINITIONS

1. Accuracy/Trueness - Closeness of agreement between a test result and the accepted reference value.
2. Analyte/measurand - The specific organism or chemical substance sought or determined in a sample.
3. Blank - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. Comparability - The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. Fit for purpose - The analytical method is appropriate to the purpose for which the results are likely to be used.
6. HORRAT value - HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. Limit of Detection - the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. Limit of Quantitation/Sensitivity - the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. Linear Range - the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. Measurement Uncertainty - A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. Matrix - The component or substrate of a test sample.
12. Method Validation - The process of verifying that a method is fit for purpose.[1]
13. Precision - the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. Repeatability - the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. Reproducibility - the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. Quality System - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. Recovery - The fraction or percentage of an analyte or measurand recovered following sample analysis.
16. Ruggedness - the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. Specificity - the ability of a method to measure only what it is intended to measure.[1]
18. Working Range - the range of analyte or measurand concentration over which the method is applied.

REFERENCES:

1. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
2. IUPAC Technical Report, 2002. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem., Vol. 74, (5): 835-855.
3. Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-Laboratory Validation of Anilytical Methods for Trace-Level Concentrations of Organic Chemicals.
4. MAF Food Assurance Authority, 2002. A Guide for the Validation and Approval of New Marine Biotoxin Test Methods. Wellington, New Zealand.
5. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
6. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

ACCURACY OF PSP ELISA METHOD

PSP Analysis of Shellfish

Dockside Samples Analysis by: Jellet, Abraxis, Mouse Bioassay

Coordinates	SeaWatch #	Depth	Species	MBA result	Lab #	Jellett Result	Abraxis Result* (@40 ug/100g)	Abraxis Result ** (@80 ug/100g)
41 35 80 68 23 33	1	73	SC	40 ug/100g	22	Pos	Pos	Neg
41 34 77 68 23 43	2	105	SC	41	23	Pos	Pos	Neg
41 33 56 68 22 57	3	123	SC	45	24	Pos	Pos	Neg
41 32 65 68 21 19	4	110	SC	39	25	Pos	Pos	Neg
41 35 13 67 58 05	16	117	SC	<39	26	Pos	Neg	Neg
41 08 54 68 33 74	20	98	SC	<40	27	Pos	Neg	Neg
41 37 84 68 10 79	23	86	SC	<41	28	Pos	Neg	Neg
41 36 46 68 09 38	24	91	SC	<39	29	Pos	Neg	Neg
41 35 58 68 09 38	25	80	SC	<39	30	Pos	Neg	Neg
41 47 02 67 45 90	29	102	SC	74	31	Pos	Pos	Pos
41 46 85 67 47 23	30	106	SC	79	32	Pos	Pos	Pos

Abraxis cut-off for positive = can be chosen at 40 or 80 ug/100g simultenously.
other multiple cut-off values can also be chosen.

Data provided by Wallace and Associates

Saxitoxin in Freshwater
Sample Preparation

1. Intended Use
For the detection of Saxitoxin in freshwater samples: groundwater, surface water, drinking water, effluent.

2. Materials Required (Not Provided)
Pipettes capable of delivering 100 and 900µL
Glass sample collection vials with Teflon lined caps

3. Notes and Precautions
Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of Saxitoxin from the sample. This step is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation due to their naturally occurring salts.

4. Procedure
Add 100µL of 10X Concentrated Sample Diluent per 900µL of Sample. Cap container and invert several times to thoroughly mix.

The sample is now ready to analyze according to the procedure described in the Abraxis Saxitoxin Kit package insert.

5. Evaluation of Results
Results obtained with freshwater samples which have been preserved with 10X Concentrated Sample Diluent as described above must be multiplied by a factor of 1.1 to account for the initial dilution of samples with 10X Diluent.

6. Performance Data
Recovery
Four (4) freshwater samples were spiked with various levels of Saxitoxin, preserved as described above, and then assayed using the Saxitoxin Assay. The following results were obtained:

	Recovery				
Amount of Saxitoxin Added (ppb)	Mean (ppb)	48 Hours Mean (ppb)	1 Week Mean (ppb)	S.D. (ppb)	%
0.04	0.046	0.046	0.050	0.002	117.9
0.08	0.087	0.085	0.086	0.001	107.5
0.2	0.227	0.217	0.217	0.006	110.1
Average					111.8

7. Assistance

For ordering or technical assistance contact:
Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
Web: www.abraxiskits.com

082708

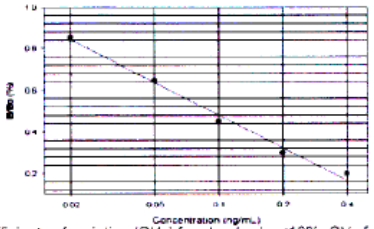
Importance of the Saxitoxin Determination
Saxitoxin is one of the "paralytic shellfish poisons" (PSP), produced by several marine dinoflagellates and fresh water algae. Contamination of shellfish with saxitoxin has been associated with harmful algal blooms throughout the world.

In man, PSP causes dose-dependent perioral numbness or tingling sensations and progressive muscular paralysis, which can result in death through respiratory arrest. The maximum tolerance levels established by the EU and FDA are 40-80 ug per 100 ug edible portion of fresh, frozen, or tinned shellfish.

The Saxitoxin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in about 1 hour.

Performance Data

Test sensitivity: The detection limit for Saxitoxin is 0.015 ng/mL (mean of 6 blank determinations minus 3 SD). The middle of the test (50% B/B_0) is at approximately 0.09 ng/mL. Determinations close to the middle of the tests gives the most accurate results.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: This ELISA recognizes Saxitoxin and other PSP toxins with varying degrees.

Cross-reactivities:		
	Saxitoxin (STX)	100% (per definition)
	Decarbamoyl STX	29%
	GTX 2 & 3	23%
	GTX-5B	23%
	Sulfo GTX 1 & 2	2.0%
	Decarbamoyl GTX 2 & 3	1.4%
	Neosaxitoxin	1.3%
	Decarbamoyl Neo STX	0.6%
	GTX 1 & 4	<0.2%

Cross-reactivities with other classes of algal toxins have not been observed.

Samples: Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in n accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose**

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R082508

Saxitoxin (PSP) ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Saxitoxin (PSP) in Water and Contaminated Samples

Product No. 52255B

1. General Description

The Saxitoxin ELISA is an immunoassay for the quantitative and sensitive detection of Saxitoxin. Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). This test is suitable for the quantitative and/or qualitative detection of Saxitoxin in water samples as well as other contaminated samples. For shellfish samples a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Saxitoxin. In addition the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Saxitoxin ELISA should to be stored in the refrigerator (4–8°C). The solutions have to be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Saxitoxin by specific antibodies. Saxitoxin, when present in a sample and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Saxitoxin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Saxitoxin ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (sheep anti-rabbit).
2. Standards (6): 0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL
3. Antibody Solution (rabbit anti-saxitoxin), 6 mL
4. Saxitoxin-HRP Conjugate, 6 mL
5. Sample Diluent Concentrate (10X), 2 X 25 mL. Use to dilute samples
6. Wash Solution (5X) Concentrate, 100 mL
7. Color Solution (TMB), 12 mL
8. Stop Solution, 12 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, the substrate solution and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microliter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer at a ratio of 1:5. If using the entire bottle (100 mL) then add to 400 mL of deionized or distilled water. Dilute the Sample Diluent at a ratio of 1:10 with deionized water.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.
6. Freshwater samples must be preserved immediately upon collection to prevent loss of saxitoxin from the samples. Please refer to the Saxitoxin in Freshwater Sample Preparation Bulletin for details.

C. Assay Procedure

1. Add 50 µL of the **standard solutions or the samples** (water) or sample extracts (shellfish) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of **enzyme conjugate** solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 µL of **antibody solution** to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 30 min at room temperature.
5. Wash the strips four times using the washing buffer solution. Please use at least a volume of 300 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of **substrate solution** to the wells. The strips are incubated for 30 min at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the %B/B_0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B_0 for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. %B/B_0 for samples will then yield levels in ppb of Saxitoxin by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing lower concentration of Saxitoxin compared to standard 1 (0.02 ng/mL) are considered as negative. Samples showing a higher concentration than standard 6 (0.4 ng/mL) must be diluted further to obtain more accurate results.

E. Additional Materials (not delivered with the test kit)

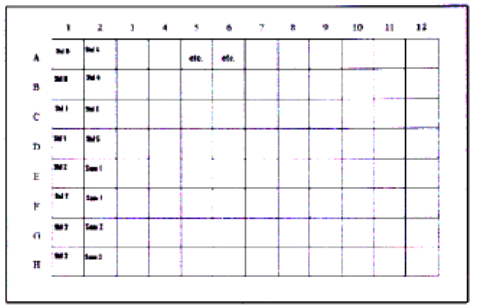
1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-300 µL) or stepper pipette with plastic tips (10-300 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microliter plates (optional)

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards have to be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
0; 0.02; 0.05; 0.10; 0.20; 0.40 ppb

Sam1, Sam2, etc.: Samples



G. Preparation of Sample (Mussels)

NOTE: If for regulatory purposes a 100 g sample is needed, extraction solution volume should be adjusted accordingly.

1. Mussels are removed from their shells, washed with deionized water and homogenized.
2. Mix 10 gm of homogenized mussels with 10 mL of 0.1M HCl and boil for 5 minutes while stirring.
3. Allow to cool and centrifuge for 10 minutes at approximately 3500 g.
4. Adjust pH to < pH 4.0 with 5 N HCl.
5. Remove 10 uL and dilute to 10 mL with Sample Dilution Buffer (1:1,000 dilution).
6. Run in the assay as sample (Assay Procedure step 1).

The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 2,000. Highly contaminated samples (outside the range of the curve), should be diluted further and re-analyzed, we recommend further dilutions of 1:10 with sample dilution buffer. The dilution factor will then be 20,000. Samples with low contamination of STX or samples that contain STX congeners with low cross-reactivity (see chart) can be detected in the assay by diluting samples 1:250 before analysis. The assay has low cross-reactivity against GTX 1 & 4, therefore food samples containing these congeners at low concentrations might be underestimated by this ELISA.

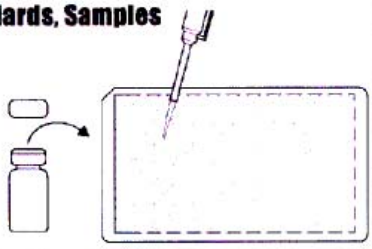
H. Alternative Sample Preparation

1. Mussels are removed from their shells, washed with deionized water, dried and homogenized using a Polytron or equivalent.
2. A 1.0 gm portion is then mixed with 6 mL methanol/DI water (80/20) using a Polytron or equivalent.
3. Centrifuge mixture for 10 minutes at 3000 g. Collect supernatant.
4. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge the mixture for 10 minutes. Add supernatant to first portion.
5. Bring the volume of the collected supernatant to 10 mL with methanol deionized water (80/20). Filter extract through a 0.45 um filter (Millex HV, Millipore).
6. Remove 10 uL and dilute to 1.0 mL with sample Dilution Buffer (1:100 dilution), then analyze as samples (Assay Procedure, step 1). The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 1,000.

Saxitoxin (PSP) Plate, Detailed ELISA Procedure

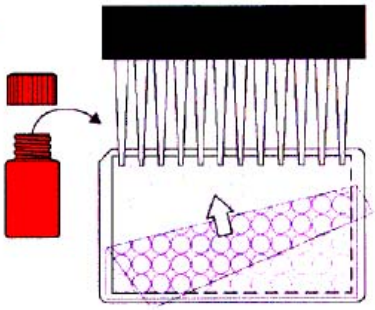
1. Addition of Standards, Samples

Add 50 uL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



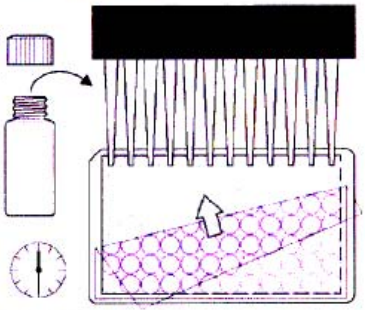
2. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



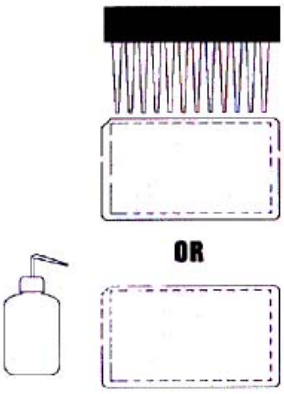
3. Addition of Antibody Solution

Add 50 uL of the Saxitoxin anitbody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



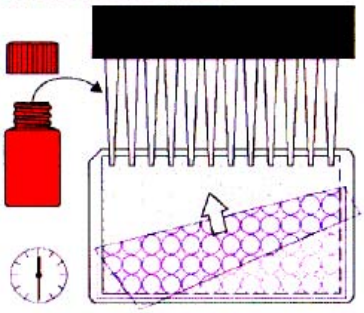
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 300 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels. Repeat steps an additional three times.



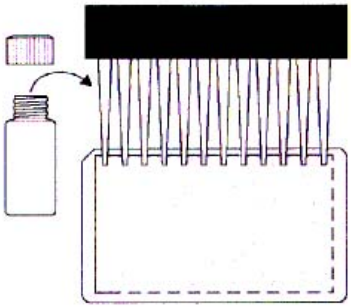
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



6. Addition of Stopping Solution

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

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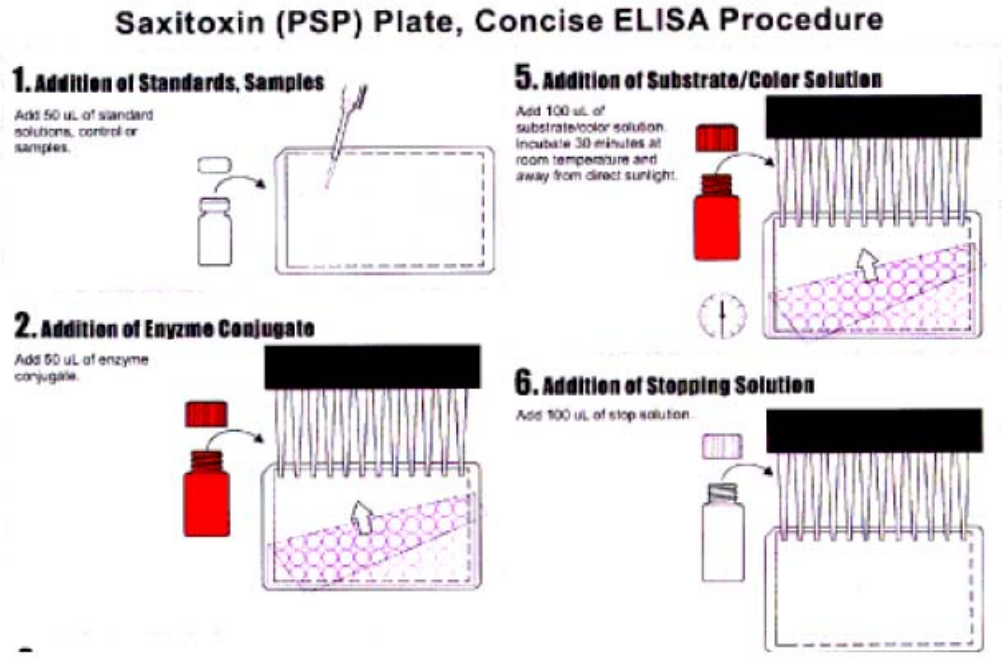
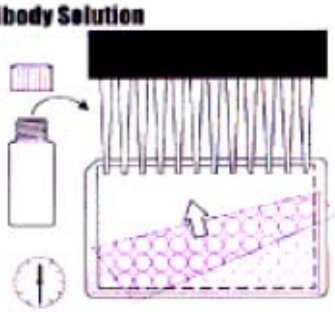
Saxitoxin (PSP) Plate Kit Part # 52255B


Saxitoxin (PSP) Plate, Concise ELISA Procedure
1. Addition of Standards, Samples
Add 50 uL of standard solutions, control or samples.
2. Addition of Enyzme Conjugate
Add 50 uL of enzyme conjugate.
5. Addition of Substrate/Color Solution
Add 100 uL of substrate/color solution. Incubate 30 minutes at room temperature and away from direct sunlight.
6. Addition of Stopping Solution
Add 100 uL of stop solution.

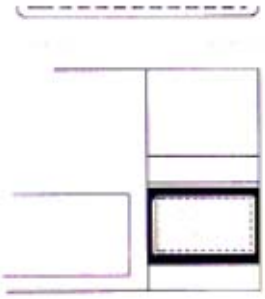
3. Addition of Antibody Solution

Add 50 uL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



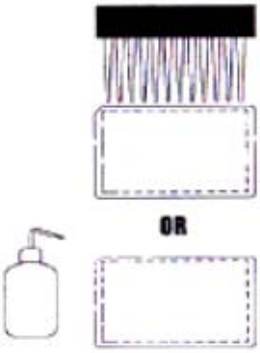
7. Measurement of Color

Measure color at 450 nm.
Calculate results.



4. Washing of Plates

Wash the plates four times with 300 uL of diluted 1X washing buffer.



OR

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Saxitoxin (PSP) Plate Kit Part # 522558