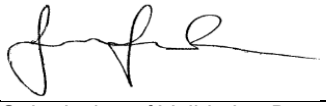
ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

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	Reveal 2.0 ASP (Domoic Acid)
Name of the Method Developer	Neogen Corporation
Developer Contact Information	Jennifer Rice 517-372-9200 Jrice@neogen.com

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.		There is a need for a simple, rapid screening method for domoic acid in shellfish, one that can be used in the field as well as in a laboratory setting.
2. What is the intended purpose of the method?		The method is designed for rapid qualitative screening of shellfish for domoic acid.
3. Is there an acknowledged need for this method in the NSSP?		Simple assays that provide rapid and accurate results are needed.
4. What type of method? i.e. chemical, molecular, culture, etc.		Lateral flow immunoassay in dipstick format.
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title		Reveal 2.0 ASP (Domoic Acid)
Method Scope		Qualitative detection of domoic acid in mussels, oysters and clams.
References		Study report and kit insert included in this submission.
Principle		Competitive lateral flow immunoassay in dipstick format. Water extraction of analyte from homogenized shellfish tissue.
Any Proprietary Aspects		Yes, commercial test kit.
Equipment Required		Extraction containers with lids (40 mL capacity), timer, bag roller, microwell holder, pipettes (0.1 mL), reader
Reagents Required (consumables)		Reveal ASP test devices, mesh filter extraction bags, microwells, distilled water.
Sample Collection, Preservation and Storage Requirements		Shellfish should be collected according to standard industry practices and stored at 2-8^{o}C before testing.
Safety Requirements		Used test devices, extraction bags, microwells, and pipettes should be treated as if contaminated with domoic acid and handled accordingly. Gloves and lab coats should be worn while performing the test.
Clear and Easy to Follow Step-by-Step Procedure		Step-by-step procedure in kit insert and study report.
Quality Control Steps Specific for this Method		Test device contains an internal control (control line) that confirms that the device is functioning properly. A domoic acid solution in buffer at a concentration 95.2 ng/mL can be used as an external positive control, if

		desired. This is the equivalent of a shellfish sample containing DA at a level of approx. 40 mg/kg (40 ppm).
C. Validation Criteria		
1. Accuracy / Trueness		>95% positive test results at or above 20 ppm DA, >95% negative test results at or below 10 ppm DA. (buffer, spiked and incurred matrices)
2. Measurement Uncertainty		Not applicable.
3. Precision Characteristics (repeatability and reproducibility)		Not applicable.
4. Recovery		Not applicable.
5. Specificity		No impact on test results by potentially interfering compounds – okadaic acid, glutamic acid, glutamine, saxitoxin.
6. Working and Linear Ranges		Not applicable.
7. Limit of Detection		17.5 ppm DA (positive cut-off)
8. Limit of Quantitation / Sensitivity		Not applicable.
9. Ruggedness		No impacts on performance observed with three lots or +/- 2 min variation in test incubation time
10. Matrix Effects		None observed.

11. Comparability (if intended as a substitute for an established method accepted by the NSSP)		The assay is comparable to LC-UV reference method in testing naturally incurred domoic acid samples
D. Other Information		
1. Cost of the Method		Approximately $17.00 per test (list price)
2. Special Technical Skills Required to Perform the Method		None
3. Special Equipment Required and Associated Cost		Reader (list price approximately $1,995)
4. Abbreviations and Acronyms Defined		ppm = parts per million, equivalent to mg/kg
5. Details of Turn Around Times (time involved to complete the method)		The test can be performed in approximately 20 minutes including sample preparation.
6. Provide Brief Overview of the Quality Systems Used in the Lab		
Submitters Signature 	Date: June 27, 2013	
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.

Laboratory Evaluation Checklist – Reveal 2.0 ASP

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
SHELLFISH PROGRAM IMPLEMENTATION BRANCH
SHELLFISH SAFETY TEAM
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. 301-436-2151/2147 FAX 301-436-2672

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:

ADDRESS:

TELEPHONE:	**FAX:**	**EMAIL:**
DATE OF EVALUATION:	**DATE OF REPORT:**	**LAST EVALUATION:**

LABORATORY REPRESENTED BY:	**TITLE:**

LABORATORY EVALUATION OFFICER:	**SHELLFISH SPECIALIST:** **REGION:**
OTHER OFFICIALS PRESENT:	**TITLE:**

Items which do not conform are noted by:

C - Critical **K** - Key **O** - Other **NA** - Not Applicable Conformity is noted by a **"√"**

PART I – QUALITY ASSURANCE		
Code		**Item Description**
		1.1 Quality Assurance (QA) Plan
K		1. Written Plan adequately covers all the following: (check √ those that apply) a. ☐ Organization of the laboratory. b. ☐ Staff training requirements. c. ☐ Standard operating procedures. d. ☐ Internal quality control measures for equipment, calibration, maintenance, repair and performance. e. ☐ Laboratory safety. f. ☐ Quality assessment. g. ☐ Proper animal care.
C		2. QA plan implemented.
		1.2 Work Area
O		1. Adequate for workload and storage.
O		2. Clean and well lighted.
O		3. Adequate temperature control.
O		4. All work surfaces are nonporous and easily cleaned.
C		5. A separate, quiet area with adequate temperature control for mice acclimation and injection is maintained.
		1.3 Laboratory Equipment
C		1. The balance provides a sensitivity of at least 0.1g at a load of 150 grams.
K		2. The balance calibration is checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent. Records maintained.
C		3. Refrigerator temperature is maintained between 0 and 4°C.
K		4. Refrigerator temperature is monitored at least once daily. Records maintained.
C		5. Freezer temperature is maintained at -20°C or below.
K		6. Freezer temperature is monitored at least once daily. Record maintained.
C		7. All glassware/plastic used with the high speed blender for homogenization is cleaned with water after each use.
C		8. Accuscan Pro Reader is calibrated before use
C		9. The correct QR code is scanned in the reader for the lot of strips that will be used
		1.4 Reagent and Reference Solution Preparation and Storage
K		1. Buffers are stored in plastic screw top vials at room temperature.
C		2. Buffers are within expiration date.
C		3. Make up water is distilled or deionized *(circle one)*
		1.5 Collection and Transportation of Samples
K		1. Shellstock are collected in clean, waterproof, puncture resistant containers.
K		2. Samples are appropriately labeled with the collector's name, harvest area and time and date of collection.
K		3. Immediately after collection, shellstock samples are placed in dry storage for transport (e.g. cooler) which is maintained between 0 and 10°C. Upon receipt at the lab, samples are placed under refrigeration.
K		4. The time from collection to completion of the assay does not exceed 48 hours if stored refrigerated. However, if there are significant transportation delays, then shellstock samples are processed immediately as follows *(circle the appropriate choice):* a. Washed, shucked, drained, frozen until extracted; b. Washed, shucked, drained, homogenized and frozen; c. The laboratory has an appropriate contingency plan in place to handle samples which can't be analyzed within 24 hours due to transportation issues.
K		5. Frozen shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed further.

PART II – EXAMINATION OF SHELLFISH FOR ASP TOXIN		
		2.1 Preparation of Sample
K		1. At least 12 animals are used per sample or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish.
K		2. The outside of the shell is thoroughly cleaned with fresh water.
K		3. Shellstock are opened by cutting adductor muscles.
K		4. The inside of the shell is rinsed with fresh water to remove sand or other foreign material.
K		5. Shellfish meats are removed from the shell by separating adductor muscles and tissue connecting at the hinge.
K		6. Damage to the body of the mollusk is minimized in the process of opening.
O		7. Shucked shellfish are drained on a #10 mesh sieve (or equivalent) without layering for 5 minutes.
K		8. Pieces of shell and drainage are discarded.
C		9. Drained meats or thawed homogenates are blended at high speed until a homogenous sample is obtained (time required is species dependent).
		2.2 Extraction
C		1. 1 gram of homogenized sample was weighed into a screw-cap leak-proof container (sample cup) capable of holding ~40mL-60mL volume
C		2. A volume of 30 mL (± 0.5 mL) of distilled water is poured into the sample cup containing the sample. Lid is secured.
C		3. Sample cup is shaken vigorously by hand for 30 seconds, until all shellfish tissue is in solution.
C		4. Both sides of the extraction bag are marked using a marker so there is a side labeled "1" and the other side labeled "2".
K		5. Solution/sample mixture is poured into side labeled "1".
K		6. The bag is sealed by holding the green straw approximately 2–3 inches down from the top of the bag. The upper edge of the bag is folded so that it covers the green straw and the white bag clip is applied.
K		7. Sample does not leak from the bag
C		8. Roller is firmly pressed onto the sample extraction bag and applied in a back and forth motion for 30 seconds to ensure a homogenous sample extract is obtained
K		9. The green straw is slid off the bag the white bag clip is removed.
K		10. All the bag contents from side "2" are poured back into the original sample cup. The extaction bag is discarded.
C		11. Sample cup is shaken vigorously by hand for 30 seconds
C		12. 100 µL of the sample extract is removed using a disposable pipette provided (or alternatively by use of a standard pipette), and added into an ASP buffer vial.

		2.3 Assay Procedure
O		1. The appropriate number of microwells are removed and place into the microwell holder.
C		2. The ASP buffer vial (containing diluted sample) is shaken vigorously by hand for 30 seconds.
C		3. 100 µL of diluted sample is transferred into each microwell using a new disposable pipette
O		4. The required number of test strips are removed from the lateral flow device container and the container is immediately closed.
C		5. The ASP test strips with the sample end down (Neogen logo on top) are placed into the microwells.
C		6. The strip is allowed to develop in the microwell for 10 minutes.
C		7. After 10 minute run time, the test strip is immediately removed and read using the AccuScan® Pro reader

		2.4 Reading Test Results
C		1. Test strips are read within 1 minute of completion of the 10 minute incubation.
K		2. The Reveal 2.0 ASP test strip is fully inserted into the black cartridge adapter with the sample end first and results facing out.
C		3. The cartridge with test strip side up is inserted in the AccuScan® Pro. The reader automatically begins analysis of the cartridge. The cartridge is not removed until the reader has completed the analysis
O		4. Results are displayed on the AccuScan® Pro reader and stored by the reader.
O		5. The reader reports Positive (20ppm DA or greater) or Negative (<20ppm).

LABORATORY:	DATE OF EVALUATION:

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
COMPNENT: PARTS I and II	
A. Results Total # of Critical (C) Nonconformities Total # of Key (K) Nonconformities Total # of Critical, Key and Other (O) nonconformities	 _____ _____ _____

B. Criteria for Determining Laboratory Status of the ASP Component

1. **Does Not Conform Status** The ASP component of this laboratory is not in conformity with NSSP requirements if:
   a. The total # of Critical nonconformities is ≥ 3 **or**
   b. The total # of Key nonconformities is ≥ 6 **or**
   c. The total # of Critical, Key and Other is ≥ 10

2. **Provisionally Conforms Status**: The ASP component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is ≥ 1 but < 3

C. Laboratory Status *(circle appropriate)*
Does Not Conform - Provisionally Conforms – Conforms

Acknowledgment by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____.

Laboratory Signature: _____ Date:_____

LEO Signature: _____ Date:_____

NEOGEN® CORPORATION

June 27, 2013

Laboratory Methods Review & Quality Assurance Committee
Interstate Shellfish Sanitation Conference
209-2 Dawson Road
Columbia, SC 29223-1740

Dear Members of the Committee:

Please find enclosed a validation study report and other supporting documentation for the Reveal 2.0 ASP (domoic acid) test kit. We respectfully request your review of this submission and consideration of the test for acceptance as an ISSC approved method for qualitative determination of domoic acid in molluscan shellfish. We believe that the test provides significant advantages in terms of time-to-result and ease of use, and we feel that acceptance of the method by ISSC will be of benefit to the shellfish industry and public health authorities.

I would be pleased to answer any questions or provide any further information that you may require. Thank you very much for your consideration.

Sincerely,

Jennifer Rice, DVM, MSc, Ph.D, MBA
Vice President and Senior Research Director
Neogen Corporation

Enclosures: Validation study report
Kit insert
MSDS
Single lab validation checklist
2013 ISSC conference proposal
Laboratory Evaluation Checklist - ASP

620 Lesher Place • Lansing, MI 48912-1595
(517) 372-9200 • (800) 234-5333 • Fax: (517) 367-0514
www.neogen.com • e-mail: jrice@neogen.com

Validation Study of the Reveal ® 2.0 ASP Test for the Qualitative Detection of Domoic Acid in Shellfish

Oscar Caballero, Karrie Melville, R. Lucas Gray, Waqass Jawaid, Mark Hooper, Paul Muirhead, Mark Mozola, and Jennifer Rice*
Neogen Corporation
620 Lesher Place, Lansing, MI 48912 USA
*corresponding author: phone 517-372-9200, email jrice@neogen.com
Submitted June 2013

1. Introduction

Domoic acid (DA), produced by certain species of the diatom *Pseudonitzschia*, is the primary toxin responsible for amnesic shellfish poisoning (ASP) associated with consumption of contaminated shellfish including oysters, clams, and mussels. Current methodologies for detection of DA in shellfish are laborious and time-consuming, consisting primarily of LC-UV, LC-MS, and immunoassay procedures. LC-UV methods [1, 2] have been accepted as quantitative reference methods in many parts of the world. Assays facilitating more rapid determination of DA with simplified procedures are needed by the shellfish industry and regulatory authorities.
In this report, we describe results of a validation study of the Reveal 2.0 ASP test for qualitative detection of DA in shellfish. Reveal 2.0 ASP is a lateral flow immunoassay designed for rapid determination of DA at a level of 20 ppm or greater. The test is easy to use and results can be obtained in less than 20 minutes, including sample preparation.

2. Principle of the Method

Reveal 2.0 ASP is a single-step, lateral flow immuno-chromatographic assay based on the principle of competitive immunoassay. Following a simple distilled water extraction of DA from homogenized shellfish tissue, the extract is then diluted in running buffer. The dipstick-format Reveal device is then placed into the diluted extract. The extract is wicked through a reagent zone containing antibodies specific for DA conjugated to colloidal gold particles. If DA is present, it will be captured by the labeled antibody. Migration of the sample continues through a membrane, which contains a zone of DA conjugated to a protein carrier. This zone captures any unbound antibody-gold conjugate, resulting in a visible line. With increasing amounts of DA in the test sample, less unbound conjugate is available for binding to the test line. Thus, intensity of the test line is inversely proportional to the amount of DA in the sample. The test device also incorporates a control conjugate, which binds to a second line. The control line will form regardless of the amount of DA present in the sample, ensuring that the test device is functioning properly. Results are analyzed as positive or negative using Neogen's AccuScan® Pro Reader.

3. Intended Use

Reveal 2.0 ASP is intended for the qualitative screening of shellfish for DA, by producing a positive result with samples containing 20 ppm or above. The test kit is designed for use by quality control personnel and other personnel familiar with handling shellfish possibly contaminated by DA toxins.

4.0 Reveal 2.0 ASP Method

The kit insert is included as Appendix I. An overview of the extraction method and how to perform the test are shown below.

4.1 Materials Provided

Reveal 2.0 ASP (Neogen item 9560)

1. 24 Reveal 2.0 ASP lateral flow test strips
2. 24 wells
3. 24 vials of ASP buffer
4. 25 extraction bags
5. 48 disposable 100µl pipettes

4.2 MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Marine biotoxins starter kit (Neogen item 9563)
   - Microwell holder
   - 1 roller
   - 1 bag clip (white clip with green straw)
2. Distilled water
3. Sample collection cups with lids (Neogen items 9428, 9428B)
4. Blender (Neogen items 9493, 9477 or 9495)
5. Scale capable of weighing 0.5 – 400g ±0.1g (Neogen item 9427)
6. Timer (Neogen item 9452)
7. Graduated cylinder, 50ml (Neogen item 9367) or bottle-top dispenser (Neogen item 9448)
8. AccuScan® Pro reader (Neogen item 9565)

4.3 PRECAUTIONS

1. The test strips must remain inside the stay-dry tube before use.
2. Do not use kit contents beyond expiration date.
3. Treat all liquids, including sample extract, and used components as if contaminated with toxin. Gloves and other protective apparel should be worn at all times.
4. To avoid cross-contamination, use clean pipettes, extraction bags and fresh extraction solutions for each sample.
5. A Material Safety Data Sheet (MSDS) is available from Neogen Corp.

4.4 Storage Requirements

Store Reveal 2.0 ASP kit components at controlled room temperature (18-30^{o}C, 64-86^{o}F). Do not freeze. Test strips should remain in their original sample tubes until use to maintain shelf life and ensure optimal performance.

4.5 AccuScan® Pro Reader Set up

Enter the lot-specific QR code by selecting the QR code icon on the reader. Place the QR code into the cartridge and insert the cartridge into the reader.

4.6 Sample Preparation and Extraction

The sample to be tested should be collected according to accepted sampling techniques.

1. Obtain a representative sample. Shell the samples.
2. Thoroughly rinse the samples with distilled or deionized water, and allow any excess water to drain.
3. Homogenize (e.g., blend, puree) the shellfish in a high-speed blender.
   NOTE: A good homogenate is essential in order to obtain an accurate result.
4. Weigh 1 g (± 0.05 g) of homogenized sample in a sample cup.
5. Pour 30 mL (± 0.5 mL) of distilled water into sample cup containing the sample and secure the lid.
6. Shake the sample cup vigorously by hand for 30 seconds, until all shellfish tissue is in solution (a cloudy appearance or bubbles may form, which does not affect the running of the test).
7. Number both sides of an extraction bag using a marker, so that there is a side labeled “1” and the other side labeled “2”. Pour solution/sample mixture into the side labeled “1.”
   NOTE: The extraction bag contains a mesh filter which allows for partial filtration of the sample. All samples/solutions should only be added to the side labeled “1”.
8. To seal the bag, position and hold the green straw approximately 2–3 inches down from the top of the bag, fold the upper edge of the bag so that it covers the green straw and firmly clip on the white bag clip. This prevents leakage of the sample.
9. Press the roller firmly on the sample extraction bag, pushing the roller back and forth for 30 seconds to aid in obtaining a homogenous sample extract.
10. Slide out the green straw and remove the white bag clip.
11. Pour all the bag contents from side “2” back into the original sample cup (there may be small pieces of shellfish remaining on side “1” of the bag). Discard the used extraction bag.
12. Cap and shake the sample cup vigorously by hand for 30 seconds (a cloudy appearance or bubbles may form, which does not affect the running of the test).
13. Remove 100 µL of the sample extract using a disposable pipette* provided (or alternatively by use of a standard pipette), and add into an ASP buffer vial.
    *To use the disposable pipettes, firmly press the top bulb of the pipette, insert the tip into the solution; slowly release the top bulb to draw up the sample extract. Excess volume (e.g., more than 100 µL) will overflow into the lower bulb, ensuring 100 µL is ready to dispense. Press the top bulb firmly and release slowly to dispense. Discard the used pipette.

4.7 Test Procedure

1. Remove the appropriate number of microwells and place into the microwell holder.
2. Shake the ASP buffer vial (containing diluted sample) vigorously by hand for 30 seconds.
3. Immediately transfer 100 µL of diluted sample into each microwell using a new disposable pipette
4. Remove the required number of test strips from the lateral flow device container and immediately close the container tightly.
5. Place the ASP test strips with the sample end down (Neogen logo on top) into the microwells.
6. Allow the strip to develop in the microwell for 10 minutes.
7. Immediately remove the test strip and read using the AccuScan® Pro reader (as described below)

4.8 Reading Test Results

1. Test strips should be read within 1 minute of completion of the 10 minute incubation. Refer to AccuScan® Pro Reader Set Up for test selection and set up information.
2. Fully insert the Reveal 2.0 ASP test strip into the black cartridge adapter with the sample end first and results facing out.
3. Insert the cartridge with test strip side up in the AccuScan® Pro. The reader will automatically begin analyzing the cartridge. CAUTION: Removing cartridge prior to completion can result in invalid readings.
4. The AccuScan® Pro reader will analyze the test strip and results will be displayed and stored in the reader.
5. The reader will report Positive with a result of 20ppm DA or greater. Any result of less than 20ppm will be reported as Negative.

   Notes:

   Ensure device is fully inserted into cartridge.

   The strips must be read using Neogen's AccuScan® Pro reader.

5. Single-Laboratory Validation Study

A single-laboratory validation study was conducted to measure accuracy/trueness, specificity, robustness and ruggedness of the Reveal 2.0 ASP method, as well as effects of potential interfering compounds. In addition, Reveal 2.0 ASP results were compared to those of an accepted LC-UV reference method [1]. Matrices tested were oysters, clams, and mussels.

5.1 Accuracy/trueness and specificity

Methods

Fresh pacific oysters (*Crassostrea gigas*), cherrystone clams (*Mercenaria mercenaria)*, and common mussels (*Mytilus edulis)* were obtained from a local retail market that receives fresh shellfish by air shipment daily. Shellfish were held at 2-8^{o}C before use. Shellfish were shucked and approximately 12-15 animals were combined and homogenized in a blender to produce a bulk sample. The bulk samples were separated into 8 portions of 1g each. Five served as unspiked controls. One each of the remaining 3 samples was spiked separately at 10, 17.5 and 20ppm DA. Certified reference material (CRM-DA-f), obtained from the National Research Council, Canada- Institute for Marine Biosciences (NRC- IMB), was used as the spiking material. The CRM consisted of 101.8 µg/mL of DA extracted from contaminated cultured blue mussels and dissolved in a solution of 5% acetonitrile/95% water.

Each sample was then prepared according to the procedures in Sample Preparation and Extraction outlined above, and tested with the Reveal 2.0 ASP assay. Twenty replicates of each extracted spiked sample and three replicates of each extracted un-spiked sample were tested.

Accuracy rates were calculated for each shellfish matrix separately and in combination. A dose-response curve was constructed using the combined data.

Results

Results of the accuracy study are shown in Table 1. Accuracy is defined as the level of agreement between the assay and the expected test results based on the DA spike level. Reveal 2.0 ASP was designed to signal 100% negative at 0 and 10ppm, partials at 17.5ppm (the majority will signal as positive) and 100% positives at 20ppm DA.

For oysters, accuracy of the assay for 0, 10, and 20ppm DA was 100%. All tests at 10ppm DA were negative, and all tests at 17.5ppm and 20ppm DA were positive. There were no false-positive results on un-spiked control samples.

For clams, accuracy of the assay for 0, 10, and 20ppm DA was 100%. All tests at 10 ppm DA were negative. Four of twenty tests at 17.5 ppm DA were negative and all 20ppm DA tests were positive. There were no false positive results on un-spiked control samples.

For mussels, accuracy of the assay for 0, 10, and 20ppm DA was 100%. All tests at 17.5ppm and 20ppm DA were positive. There were no false-positive results on un-spiked control samples.

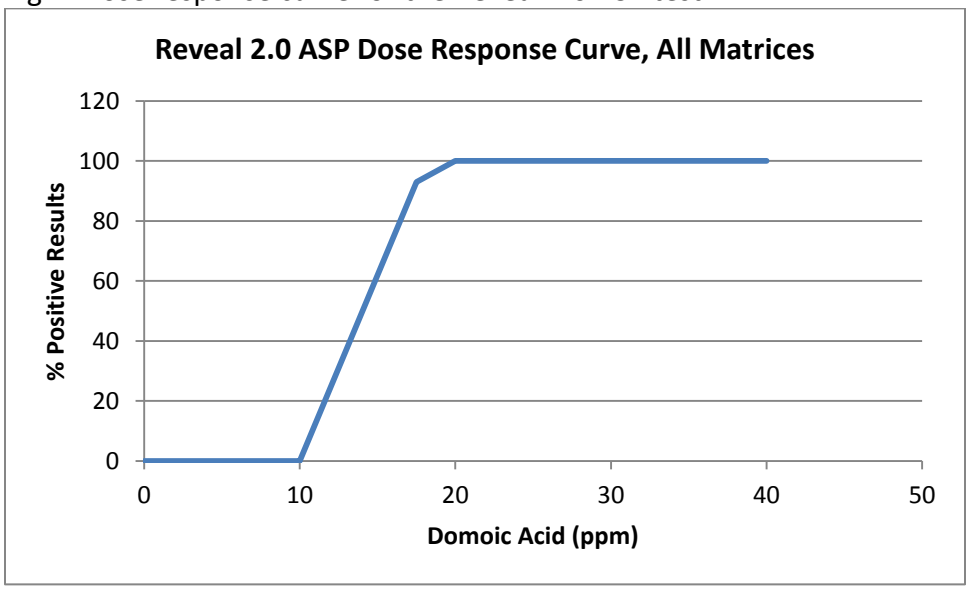
When excluding data from the 17.5ppm DA spike, overall accuracy of the Reveal 2.0 ASP test was 100%.

The dose response curve (Fig 1) shows a strong correlation between Domoic Acid levels and percentage of Reveal 2.0 ASP positive devices. As anticipated, 100% of all devices were positive by 20 ppm levels of DA.

Table 1: Results of accuracy study of the Reveal 2.0 ASP test

Sample Type	Level DA (ppm)	Number Tests	Number Positive	% Positive results
Oysters	0	15	0	0
	10	20	0	0
	17.5	20	20	100
	20	20	20	100
Clams	0	15	0	0
	10	20	0	0
	17.5	20	16	80
	20	20	20	100
Mussels	0	15	0	0
	10	20	0	0
	17.5	20	20	100
	20	20	20	100
All Data	0	45	0	0
	10	60	0	0
	17.5	60	56	93
	20	60	60	100

Fig 1: Dose response curve for the Reveal 2.0 ASP test



5.2 Interfering compounds

Methods

Fresh oysters, clams, and mussels were obtained as described above. Approximately 12-15 animals were combined and homogenized in a blender to produce a bulk sample. The bulk samples were separated into 12 portions of 1g each. The 12 portions were separated into 4 groups each containing three 1g samples. Samples in each group were spiked individually with one of the following potentially interfering compounds: okadaic acid, 10 ppm; glutamic acid, 100 ppm; glutamine, 100 ppm; or saxitoxin, 5 ppm. One sample in each group was spiked with 10 ppm DA, one sample was spiked with 20 ppm DA, and one sample was left unspiked. All interfering compounds were obtained from Sigma, except for saxitoxin, which was obtained from NRC-IMB. DA CRM, described above, was used as the spiking material.

Sample preparation and testing were performed as described above. Five replicates of each extracted sample were tested with the Reveal 2.0 ASP assay.

Results

Results of testing for effects of potentially interfering compounds on performance of the Reveal 2.0 ASP assay are shown in Table 2. There was no evidence of interference by okadaic acid, glutamic acid, glutamine, or saxitoxin on assay performance in any of the three shellfish matrices. All tests produced expected results at levels of 0, 10, and 20 ppm DA.

Table 2: Results of interference study for the Reveal 2.0 ASP test.

Sample Type	Interfering Compound and Level	Level DA (ppm)	Number Tests	Number Positive
Oysters	Okadaic acid 10 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamine 100 ppm	0	5	0
		10	5	0
		20	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	0
		20	5	5
Clams	Okadaic acid 10 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamine 100 ppm	0	5	0
		10	5	0
		20	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	0
		20	5	5
Mussels	Okadaic acid 10 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	0
		20	5	5
	Glutamine 100 ppm	0	5	0
		10	5	0
		20	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	0
		20	5	5

5.3 Ruggedness

Methods

Fresh oysters, clams, and mussels were obtained as described above. Approximately 12-15 animals were combined and homogenized in a blender to produce a bulk sample. The bulk samples were separated into 3 portions of 1 g each. One portion was spiked at 10 ppm, one at 20 ppm, and the remaining sample left unspiked. DA CRM, described above, was used as the spiking material.

Each sample was tested using devices from three test kit lots (LFD-001, LFD-002, and LFD-003). The devices were interpreted after 8, 10 and 12 minutes of incubation.

Results

Results of assay ruggedness trials with respect to Reveal 2.0 ASP kit lot and assay incubation period are shown in Table 3. There was no difference in the number of positives obtained at incubation times of 8, 10 and 12 minutes at any spike level in any shellfish matrix (Table 3). The number of replicates for each condition varied from 13 to 15 strips. This is due to lost or mis-read strips.

Table 3: Assay ruggedness trials for the Reveal 2.0 ASP test – effect of incubation time.

Sample Type	Level DA (ppm)	Number Tests	Number Positive 8 min.	Number Positive 10 min.	Number Positive 12 min.
Oysters	0	15	0	0	0
	10	13	0	0	0
	20	14	14	14	14
Clams	0	14	0	0	0
	10	15	0	0	0
	20	15	15	15	15
Mussels	0	15	0	0	0
	10	13	0	0	0
	20	14	14	14	14
All Data	0	44	0	0	0
	10	41	0	0	0
	20	43	43	43	43

*As the numbers of positive results are the same, no statistical test is required.

5.4 Robustness

Methods

Two certified reference materials (CRM's) from the National Research Council were purchased and used for this study.

- CRM Mus D containing 49ppm DA (*Mytilus edulis)*
- Mus zero containing 0ppm DA (*Mytilus edulis)*

The CRM Mus D was blended with Mus zero to give a final concentration of DA at 10, 15, 17.5 and 20ppm as outlined below. Blends were then aliquoted into 1g amounts. Three randomly chosen aliquots at each level were analyzed by LC-UV [1] 24 hours before day one of methods robustness testing.

Total weight required to fulfill robustness (g)	Final DA concentration required (ppm)	CRM Mus D (at 49ppm) required (g)	CRM Mus zero required (g)
23	20	9.388	13.612
23	17.5	8.214	14.786
23	10.0	4.694	18.306
23	0.0	0	23.000

Testing took place over two days with three operators. Samples were presented to operators randomized and blind. Three samples at each level were extracted by each operator on each day. One strip from each lot of lateral flow devices (LFD) was then tested with every extract.

Results

Method robustness showed that results were consistent across kit lot, operator and day. All strips were negative at 0ppm and 10ppm DA, and positive at 20ppm DA for all lots, with all operators on both days. At 17.5ppm DA, one negative result was obtained by operator 1, day 1, lot 3 (Table 4a and 4b). HPLC results correlated well with expected levels of DA (Table 4c).

Table 4a: Results of assay method robustness trials for the Reveal 2.0 ASP test – Day 1

Day 1		Number of positives (n = 3LFDs)		
Level DA (ppm)	Operator	LFD-001	LFD-002	LFD-003
0	1	0	0	0
	2	0	0	0
	3	0	0	0
10	1	0	0	0
	2	0	0	0
	3	0	0	0
17.5	1	3	3	2
	2	3	3	3
	3	3	3	3
20	1	3	3	3
	2	3	3	3
	3	3	3	3

Table 4b: Results of assay method robustness trials for the Reveal 2.0 ASP test – Day 2

Day 2		Number of positives (n = 3LFDs)		
Level DA (ppm)	Operator	LFD-001	LFD-002	LFD-003
0	1	0	0	0
	2	0	0	0
	3	0	0	0
10	1	0	0	0
	2	0	0	0
	3	0	0	0
17.5	1	3	3	3
	2	3	3	3
	3	3	3	3
20	1	3	3	3
	2	3	3	3
	3	3	3	3

Table 4c: HPLC Results on blinded samples used for methods robustness

Theoretical DA level (ppm)	HPLC Result	HPLC Mean DA level (ppm)	% Recovery
Negative	<LOD	<LOD	NA
	<LOD		
	<LOD		
10	9.87	10.27	103
	9.98		
	10.97		
17.5	17.86	17.81	102
	17.32		
	18.26		
20	19.21	19.39	97
	19.19		
	19.78		

5.5 **Comparison with Reference Method**

Methods

Incurred Mussels (wild bay mussel, wild sea mussel or cultured bay mussel) and Oysters (cultured pacific oyster) were obtained from Canadian Food Inspection Authority (CFIA). A total of 22 samples were tested. Five of these samples were divided into four 1g samples (A, B, C, and D). Each sample was extracted and tested on 5 devices. The remaining samples were extracted once and run on 5 LFD devices. All samples were also analyzed via LC-UV [3].

Results

Data presented in Table 5 from naturally contaminated mussels and oysters show strong correlation between the lateral flow results and analytical analyses. All samples containing 1.9-12.6ppm DA gave 100% negative results on Reveal 2.0 ASP. Samples containing 28.2-30.6ppm DA gave 100% positive results. Oysters and mussels containing 16-17.6ppm DA gave a mix of positive and negative results.

Table 5: Incurred mussel and oyster tissue tested with Reveal 2.0 ASP and LC-UV reference method

Sample ID	Species	Reference method result (ppm)	Extract (A, B or C)	LFD rep	LFD result
M11P00774	Mussel	30.6	A	1	+
				2	+
				3	+
				4	+
				5	+
			B	1	+
				2	+
				3	+
				4	+
				5	+
			C	1	+
				2	+
				3	+
				4	+
				5	+
			D	1	+
				2	+
				3	+
				4	+
				5	+
M11P00775	Oyster	28.2	A	1	+
				2	+
				3	+
				4	+
				5	+
			B	1	+
				2	+
				3	+
				4	+
				5	+
			C	1	+
				2	+
				3	+
				4	+
				5	+
			D	1	+
				2	+
				3	+
				4	+
				5	+

Table 5 continued: Incurred mussel and oyster tissue tested with Reveal 2.0 ASP and LC-UV reference method

Sample ID	Species	Reference method result (ppm)	Extract (A, B or C)	LFD rep	LFD result
M11P00989	Mussel	17.6	A	1	-
				2	-
				3	-
				4	-
				5	-
			B	1	+
				2	+
				3	+
				4	+
				5	-
			C	1	+
				2	+
				3	+
				4	+
				5	+
			D	1	+
				2	-
				3	+
				4	+
				5	+
M11P00529	Oyster	29.6	A	1	+
				2	+
				3	+
				4	+
				5	+
			B	1	+
				2	+
				3	+
				4	+
				5	+
			C	1	+
				2	+
				3	+
				4	+
				5	+
			D	1	+
				2	+
				3	+
				4	+
				5	+

Table 5 continued: Incurred mussel and oyster tissue tested with Reveal 2.0 ASP and LC-UV reference method

Sample ID	Species	Reference method result (ppm)	Extract (A, B or C)	LFD rep	LFD result
M11P00560	Oyster	17.6	A	1	-
				2	+
				3	+
				4	+
				5	+
			B	1	+
				2	+
				3	+
				4	+
				5	-
			C	1	+
				2	+
				3	+
				4	+
				5	+
			D	1	+
				2	+
				3	+
				4	+
				5	+

Table 5 continued: Incurred mussel and oyster tissue tested with Reveal 2.0 ASP and LC-UV reference method

Sample ID	Species	Reference method result (ppm)	LFD Rep	LFD result
M11P00719	Oyster	3.0	1	-
			2	-
			3	-
			4	-
			5	-
M1100764	Mussel	12.6	1	-
			2	-
			3	-
			4	-
			5	-
M11P00804	Oyster	11.4	1	-
			2	-
			3	-
			4	-
			5	-
M11P00805	Mussel	10.1	1	-
			2	-
			3	-
			4	-
			5	-
M11P00888	Mussel	4.7	1	-
			2	-
			3	-
			4	-
			5	-
M11P00964	Mussel	6.1	1	-
			2	-
			3	-
			4	-
			5	-
M11P01003	Mussel	8.1	1	-
			2	-
			3	-
			4	-
			5	-
M11P01027	Mussel	16.0	1	-
			2	+
			3	+
			4	-
			5	-
M11P01087	Mussel	5.4	1	-
			2	-
			3	-
			4	-
			5	-

Table 5 continued: Incurred mussel and oyster tissue tested with Reveal 2.0 ASP and LC-UV reference method

Sample ID	Species	Reference method result (ppm)	LFD Rep	LFD result
M11P01138	Mussel	16.8	1	-
			2	-
			3	-
			4	+
			5	-
M11P00510	Mussel	2.3	1	-
			2	-
			3	-
			4	-
			5	-
M11P00514	Oyster	1.9	1	-
			2	-
			3	-
			4	-
			5	-
M11P00528	Oyster	3.3	1	-
			2	-
			3	-
			4	-
			5	-
M11P00529	Mussel	29.6	1	+
			2	+
			3	+
			4	+
			5	+
M11P00542	Oyster	5.1	1	-
			2	-
			3	-
			4	-
			5	-
M11P00550	Oyster	7.0	1	-
			2	-
			3	-
			4	-
			5	-
M11P00561	Oyster	8.9	1	-
			2	-
			3	-
			4	-
			5	-

6.0 **Reveal 2.0 ASP Stability**

A stability trial of the Reveal 2.0 ASP assay is in process. Briefly, test strips from three lots were incubated at 25°C and 37°C. Strips were tested at Day 0, weeks 1, 2, 3, 4 and month 2, 3, 4, 6 and 8. Further testing time points are planned for month 10, 12, 14, 16 and 18. Results from month 8 testing of strips held at 25°C are comparable to those obtained on Day zero; therefore qualifying a shelf life of 8 months at room temperature. However, using the Arrhenius equation* to predict the shelf life of an assay using accelerated data, the estimated shelf life to date of Reveal 2.0 ASP is 12 months. Stability trials of all lots will continue until a final shelf life can be assigned.

*Arrhenius equation:
For every 10^{o}C temperature rise above the normal storage temperature, the stability at the higher temperature is multiplied x 2^{x} to give the predicted stability where x is the number of 10^{o}C increments above the planned storage temperature. Therefore if the planned storage temperature is 25°C, accelerated testing at 45^{o}C allows a predicted shelf life of x 2^{2} = 4.

7.0 **Quality Control Testing**

Quality control testing of manufactured lots of the Reveal 2.0 ASP assay is performed at both in-process and finished product stages. In-process QC consists of testing every card with negative extracted sample to check for line intensity and line position on both the test and control lines. For finished product testing, bulk QC samples have been produced by blending CRM Mus-D and CRM Mus-zero material to concentrations of 0, 10, 17.5 and 20 ppm DA. These bulk QC samples have been extracted, aliquoted and stored at -20°C and have been made available to QC as single use vials. This is to further ensure consistent samples are used for each batch of devices made. Multiple cards are selected for testing across the batch. For example, in a 40 card batch, card 1, 10, 20, 30 and 40 are tested using all standards with n = 10 LFDs per level. For acceptance of the lot, all tests at 0 and 10 ppm must be negative and all tests at 20 ppm must be positive. In addition to using extracted frozen samples, one negative and one positive check matrix sample is freshly extracted and tested to further confirm batch performance.

QC standards are prepared in bulk and frozen. Domoic acid levels in standard aliquots are confirmed by HPLC. In addition, freeze-thaw studies have also been completed to ensure that aliquots of QC standards remain consistent throughout lifespan. Stability of standards and extracts has also been validated to ensure consistent performance during testing runs.

8.0 Discussion

Results of the validation study showed that the Reveal 2.0 ASP test is an effective procedure for qualitative determination of DA in oysters, clams, and mussels. In the accuracy study, all tests at the accepted action level of 20 ppm were positive. There were no false-positive results on unspiked control samples or those with DA at 10ppm.

Four compounds, okadaic acid, glutamic acid, glutamine, and saxitoxin, were tested for potential interference with the Reveal 2.0 ASP assay. None was noted, as all samples produced the expected results at 0, 10, and 20 ppm DA.

Results of ruggedness trials indicated that there was no difference in performance between three Reveal 2.0 ASP kit lots, nor was there any difference in performance in assays conducted with variation of +/- 2 minutes around the specified run time of 10 minutes. Methods robustness confirmed lot-to-lot consistency, and also showed that there was no variation between operators or when testing was repeated on a second day.

Results of testing of mussel and oyster tissue samples containing incurred DA showed agreement between the Reveal 2.0 ASP and reference LC-UV methods.

Reveal 2.0 ASP can be used as an accurate screening test for the rapid determination of DA in shellfish. The test requires little equipment, uses water for sample extraction, and can be performed by personnel with minimal training. The test can be used in a field or laboratory setting, with results available within 20 minutes of sample receipt.

Based on the results presented here, it is recommended that the Reveal 2.0 ASP test be approved by the Interstate Shellfish Sanitation Conference as a screening method for qualitative determination of DA in oysters, clams, and mussels.

9.0 References

1. Quilliam, M.A., Xie, M., & Hardstaff, W.R. (1995) Rapid extraction and cleanup for liquid chromatographic determination of DA in unsalted seafood. *J. AOAC Intl.* **78**,543-554.
2. Lawrence, J.F., Charbonneau, C.F., & Menard, C. (1991) Liquid chromatographic determination of DA in mussels, using AOAC paralytic shellfish poison extraction procedure: collaborative study. *J. Assoc. Off. Anal. Chem*. **74**,68-72.
3. Siegel, S. (1956) *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill Book Co., New York, NY.

10. Acknowledgements

We thank Brian Bammert, Kayla Walton, Nate Banner, Sharon Graham, James Clarke, Steve Schadler and Frank Kleinof Neogen for all their help throughout this study.

SAMPLE PREPARATION AND EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques.

1. Obtain a representative sample. Shell the samples.
2. Thoroughly rinse the samples with distilled or deionized water, and allow any excess water to drain.
3. Homogenize (e.g., blend, puree) the shellfish in a high-speed blender.
   NOTE: A good homogenate is essential in order to obtain an accurate result.
4. Weigh 1 g (± 0.05 g) of homogenized sample in a sample cup.
5. Pour 30 mL (± 0.5 mL) of distilled water into sample cup containing the sample and secure the lid.
6. Shake the sample cup vigorously by hand for **30 seconds**, until all shellfish tissue is in solution (a cloudy appearance or bubbles may form, which does not affect the running of the test).
7. Number both sides of an extraction bag using a marker, so that there is a side labeled "1" and the other side labeled "2". Pour solution/sample mixture into the side labeled "1."
   NOTE: The extraction bag contains a mesh filter which allows for partial filtration of the sample. All samples/solutions should only ever be added to the side labeled "1".
8. To seal the bag, position and hold the green straw approximately 2–3 inches down from the top of the bag, fold the upper edge of the bag so that it covers the green straw and firmly clip on the white bag clip. This prevents leakage of the sample.
9. Press the roller firmly on the sample extraction bag, pushing the roller back and forth for **30 seconds** to aid in obtaining a homogenous sample extract.
10. Slide out the green straw and remove the white bag clip.
11. Pour all the bag contents from side "2" back into the original sample cup (there may be small pieces of shellfish remaining on side "1" of the bag). Discard the used extraction bag.
12. Cap and shake the sample cup vigorously by hand for **30 seconds** (a cloudy appearance or bubbles may form, which does not affect the running of the test).
13. Remove 100 µL of the sample extract using a disposable pipettor* provided (or alternatively by use of a standard pipettor), and add into an ASP buffer vial.
    *To use the disposable pipettors, firmly press the top bulb of the pipettor, insert the tip into the solution, slowly release the top bulb to draw up the sample extract. Excess volume (e.g., more than 100 µL) will overflow into the lower bulb, ensuring 100 µL is ready to dispense. Press the top bulb firmly and release slowly to dispense. Discard the used pipettor.

TEST PROCEDURE

1. Remove the appropriate number of microwells and place into the microwell holder.
2. Shake the ASP buffer vial (containing diluted sample) vigorously by hand for **30 seconds**.
3. Immediately transfer 100 µL of diluted sample into each microwell using a new disposable pipettor
4. Remove the required number of test strips from the lateral flow device container and immediately close the container tightly.
5. Place the ASP test strips with the sample end down (Neogen logo on top) into the microwells.
6. Allow the strip to develop in the microwell for **10 minutes**.
7. Immediately remove the test strip and read using the Accuscan Pro reader (as described below).

READING TEST RESULTS

Test strips should be read within **1 minute** of completion of the 10 minute incubation. Refer to **AccuScan Pro Reader Set Up** for test selection and set up information.

1. Fully insert the Reveal 2.0 for ASP test strip into the black cartridge adapter with the sample end first and results facing out.

2. Insert the cartridge with test strip side up in the AccuScan Pro. The reader will automatically begin analyzing the cartridge. **CAUTION:** Removing cartridge prior to completion can result in invalid readings.

3. The AccuScan Pro reader will analyze the test strip and results will be displayed and stored in the reader.

NOTES

1. Ensure device is fully inserted into cartridge.
2. Readings should be made between **10–11 minutes**. Readings after 11 minutes may be inaccurate due to overdevelopment of the device.
3. The strips must be read using Neogen's AccuScan Pro reader.

PERFORMANCE CHARACTERISTICS

The AccuScan Pro reader will report **Positive** with a result of 20 ppm or greater. Any result of less than 20 ppm will be reported as **Negative**.

VALIDATED MATRICES

Mussels, scallops, oysters, clams and cockles.
NOTE: Neogen continues to validate new commodities. Please contact a representative for the latest validated commodity list.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins
- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus*, *Salmonella enteritidis*

Sanitation
- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
- Almonds, crustacea, eggs, gliadin, hazelnut, lupine, milk, mustard, peanut, sesame, soy, walnut

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

NEOGEN® CORPORATION

North America
Neogen Headquarters
620 Lesher Place, Lansing, MI 48912
USA
800/234-5333 (USA/Canada) or
517/372-9200
Fax: 517/372-2006 •
foodsafety@neogen.com
www.neogen.com

Mexico
Neogen Latinoamérica
Darwin No. 83, Col. Anzures, México,
11590 D.F.
(55) 5254-8235, (55) 5203-0111,
(55) 5531-2837
Fax: (55) 5531-1647
informacion@neogenlac.com •
www.neogen.com

Europe, Middle East and Africa
Neogen Europe
The Dairy School, Auchincruive, Ayr
KA6 5HW Scotland, UK
+ 44 (0) 1292 525 600
Fax: + 44 (0) 1292 525 601
info_uk@neogeneurope.com
www.neogeneurope.com

Brazil
Neogen do Brasil
Rua: Alberto Guizo 760, Distrito Indus-
trial João
Narezzi, Indaiatuba – SP Brasil,
Cep: 13.347-402
Tel: +55 19 3935.3727
info@neogendobrasil.com.br •
www.neogen.com

NE1559 R2.0_ASP_0213

Read instructions carefully before starting test

Reveal® 2.0 for ASP

For use with the AccuScan® Pro reader.

Store at 18–30°C (64–86°F) • Do not freeze.

THE TOXINS

Toxins that cause amnesic shellfish poisoning (ASP) are produced by toxigenic diatoms of the genus *Pseudo-nitzschia*. ASP toxins primarily include domoic acid (DA). In addition to contamination of seafood, these marine biotoxins can result in human and marine wildlife mortality. The clinical toxicological effects attributed to DA can include: permanent loss of short-term memory, nausea, vomiting, headache, disorientation and loss of balance. Action limits for DA were established soon after the 1987 domoic acid/mussel crisis in Canada which included more than 100 cases of human illnesses and also several mortalities as a direct result of contaminated shellfish consumption. Most countries have currently established a maximum permitted level of 20 mg DA per kg whole shellfish (20 ppm).

INTENDED USE/USER

Reveal 2.0 for ASP is intended for the qualitative screening of shellfish for DA, which produces a positive result with samples containing 20 ppm or above. The test kit is designed for use by quality control personnel and other personnel familiar with handling shellfish possibly contaminated by DA toxins.

ASSAY PRINCIPLES

Reveal 2.0 for ASP is a single-step lateral flow device based on a competitive immunoassay format. Shellfish extract is wicked through a reagent zone containing antibodies specific for DA which have been conjugated to colored particles. If DA is present in the sample, the toxin will be captured by the particle-antibody complex. The complex then is wicked onto a membrane, which contains a stationary capture zone of a toxin-protein conjugate. This zone captures any uncomplexed DA particle-antibody. Therefore, as the concentration of DA in the sample increases, the test line intensity decreases. The membrane also contains a stationary control zone which will always form regardless of the level of DA.

STORAGE REQUIREMENTS

Store kit components at room temperature (18–30°C, 64–86°F) to ensure full shelf life. Test strips should remain capped in their original sample tubes until used to ensure optimal performance.

MATERIALS PROVIDED

Reveal 2.0 for ASP (Neogen item 9560)
1. 24 Reveal 2.0 for ASP lateral flow test strips
2. 24 microwells
3. 24 vials of ASP buffer
4. 25 extraction bags
5. 48 disposable 100 µL pipettors

MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Marine biotoxins starter kit (Neogen item 9563)
   - Microwell holder
   - 1 roller
   - 1 bag clip (white clip and green straw)
2. Distilled water
3. Sample collection cups with lids (Neogen items 9428, 9428B)
4. Blender (Neogen items 9493, 9477 or 9495)
5. Scale capable of weighing 0.5–400 g ± 0.1 g (Neogen item 9427)
6. Timer (Neogen item 9452)
7. Graduated cylinder, 50 mL (Neogen item 9367) or bottle-top dispenser (Neogen item 9448)
8. AccuScan Pro reader (Neogen item 9565)

PRECAUTIONS

1. The test strips must remain inside the stay dry tube before use.
2. Store test kit at room temperature (18–30°C, 64–86°F) when not in use. Do not freeze.
3. Do not use kit contents beyond expiration date.
4. Treat all liquids, including sample extract, and used components as if contaminated with toxin. Gloves and other protective apparel should be worn at all times.
5. To avoid cross-contamination, use clean pipettors, extraction bags and fresh extraction solutions for each sample.

ACCUSCAN PRO READER SET UP

1. Enter the lot-specific QR code by selecting the QR code icon on the reader. Place the QR code into the cartridge and insert the cartridge into the reader.
2. Return to the home screen and select the test strip icon. Touch the **Marine Biotoxins** category, then select the **ASP** test type.

NEOGEN CORPORATION

MATERIAL SAFETY DATA SHEET

Section 1. Company Identification and Product Information			
Product Name or Identity:	**Reveal® 2.0 for ASP**		
Manufacturer's Name:	**Neogen Europe, Ltd.**	**Fax No.:**	UK, 01292 525 601 International: ++44 (0) 1292 525 601
	The Dairy School	**Phone No.:**	UK, 01292 525 600 International: ++44 (0) 1292 525 600
	Auchincruive, Ayr, KA6 5HW, Scotland, UK	**e-mail:**	info@neogeneurope.com
Date Prepared or Revised: February 2013		**Chemtrec: (800) 424-9300** **Outside US and Canada: (703) 527-3887**	

Section 2. Composition / Information on Hazardous Ingredients			
This product is a mixture of the substances listed below with the addition of nonhazardous materials.			
Hazardous Components Specific Chemical Identity:	**CAS-No.**	**%**	**Hazard Symbol**
This product contains no hazardous constituents, or the concentration of all chemical constituents are below the regulatory threshold limits described by Occupational Safety Health Administration Hazard Communication Standard 29 CFR 1910.1200 and the European Directive 91/155/EEC, and 93/112/EC.	NA	NA	NA

Section 3. Health Hazard Identification	
Health Hazards: *(Acute and Chronic)*	**Information pertaining to particular dangers for man and environment.** When used and handled according to specifications, the product does not have harmful effects according to the information provided to us. May cause minor irritation of the eyes and skin.

Section 4. First Aid Measures	
Emergency / First Aid Procedures:	**Ingestion:** If swallowed, seek medical attention immediately. Wash out mouth with water, provided person is conscious. Show physician product label.
	Inhalation: If inhaled, supply fresh air or oxygen. Seek medical attention if breathing is labored or becomes difficult. If not breathing, apply artificial respiration.
	Eye Contact: Rinse opened eye for at least 15 minutes under running water, lifting lower and upper eyelids occasionally. Seek medical attention.
	Skin Contact: Remove contaminated clothing. Immediately wash with plenty of soap and water for at least 15 minutes. Seek medical attention if irritation develops. Wash clothing before reuse.

Section 5. Fire and Explosion Hazard Data	
Flash Point (Method Used): N/A	**Flammable Limits**: LEL – N/A UEL – N/A
Extinguishing Media: Use alcohol foam, dry chemical, or carbon dioxide. Water may be ineffective.	
Protective Equipment: Firefighters should wear protective equipment and self-contained breathing apparatus.	
Unusual Fire and Explosion Hazards: During heating or in case of fire, poisonous gases are produced. Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source, is a potential dust explosion hazard.	

NEOGEN CORPORATION

Section 6. Accidental Release Measures
Personal Precautions: Wear self-containing breathing apparatus, rubber boots, and heavy rubber gloves. Place contaminated material in a chemical waste container.
Environmental Precautions: Prevent dispersion of material. Wipe up with damp sponge or mop.
Clean-up Methods: Contact safety officer if questions arise and ventilate area.

Section 7. Handling and Storage
Handling: Protect against physical damage. Ensure good ventilation / exhaustion and do not breathe vapor. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.
Storage: Keep container tightly closed. Keep away from heat, sparks, flame and incompatible material. Storage area should be cool, dry, and away from incompatible materials. Containers of this material may be hazardous when empty since they retain product residues. Store at 18 - 30˚C.
Other Precautions: N/A

Section 8. Exposure Controls / Personal Protection	
Components with limit values that require monitoring: Not Applicable	
OSHA–PEL: N/A	**TLV:** N/A
Additional Information: Personal Protection listed below are general requirements for laboratory personnel. Follow the usual precautionary measures for handling chemicals / powder. Avoid contact with eyes, skin, and clothing.	
Personal Protective Equipment: Keep away from food, beverages, and feed. Wash hands before and after entering laboratory. **Breathing Equipment:** In case of brief exposure, use a chemical fume hood or a NIOSH/MSHA-approved respiratory. **Hand Protection:** Use chemical resistant gloves. **Eye Protection:** Wear safety glasses. **Body Protection:** Wear lab coat or other protective work clothing.	

Section 9. Physical and Chemical Properties
Appearance and Odor: N/A
Boiling Point: Not determined
Melting Point: Not determined
Density: Not determined

Section 10. Stability and Reactivity			
Stability:	Unstable		
	Stable	X	Conditions to Avoid: Stable under normal storage conditions.
Incompatibility *(Materials to Avoid)*: None known.			
Hazardous Decomposition or Byproducts: Carbon dioxide (CO_2), Carbon monoxide (CO), or Nitrogen oxides (NOx).			
Hazardous Polymerization:	May Occur		Conditions to Avoid: No dangerous reactions known.
	Will Not Occur	X	

NEOGEN CORPORATION

Section 11. Toxicological Information

LD/LC50 values that are relevant:
Acute Toxicity: When used and handled according to specifications and according to information provided for us, this product is not known to be toxic or hazardous at use concentrations.
Carcinogenicity Classification: Not Applicable
IARC (International Agency for Research on Cancer) – Not Listed
NTP (National Toxicology Program) Not Listed

Chronic: Prolonged or repeated skin contact may cause dermatitis.
Additional toxicological information: Any toxin(s) present in this kit are at concentration levels below the regulatory threshold limits which require registration under the Select Agent Program in as detailed in 42 CFR Part 73, 9 CFR Part 121, and 7 CFR Part 331.

Section 12. Ecological Information

Ecotoxicity Tests: The ecological effects have not been thoroughly investigated, but currently none have been identified.

Section 13. Disposal Considerations

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local environmental regulations.
RCRA P-Series: None listed.
RCRA U-Series: None listed.
Contact a licensed professional waste disposal service to dispose of this material if questions arise.

Container Information: Do not remove labels from containers until they have been cleaned.

Section 14. Transport Information

DOT Regulations: Not Regulated

Land Transport ADR/RID (cross-border): Not Regulated

Maritime Transport IMDG: Not Regulated

Air Transport ICAO-TI and IATA-DGR: Not Regulated

Section 15. Regulatory Information

EU Regulations, Hazard Symbol(s): N/A

Section 16. Other Information

This document is believed to be correct, but does not purport to be all inclusive and shall be used only as a guide. Neogen Corporation shall not be held liable for any damage resulting from handling or from contact with the above product. These suggestions should not be confused with state, municipal or insurance requirements, and constitute NO WARRANTY.