# Appendix 2

# RAPID SCREENING METHODS FOR HARMFUL ALGAL BLOOM TOXINS

Donald M. Anderson[1], Maurice Laycock[2], and Fernando Rubio[3]

[1]Woods Hole Oceanographic Institution, Woods Hole, MA USA
[2]Scotia Rapid Testing Ltd, Chester Basin, Nova Scotia Canada
[3]Abraxis LLC, Warminster, PA USA



## 1 INTRODUCTION

At the core of all national harmful algal bloom (HAB) programs are the monitoring programs needed to detect HAB toxins in shellfish, fish, water, or other resources sufficiently early to take management actions (Anderson et al. 2001). These programs measure toxins produced by multiple species of algae, with the methods used varying dramatically in scope and complexity due to the types of toxins that need to be detected, the nature of the affected resource, and regulatory requirements.

Some of the methods developed for analysis of shellfish tissues and algal blooms can be of direct use in desalination plants for analysis of toxins in water – both the raw, untreated water before desalination, and the treated, fresh water. A major concern, however, are the detection limits of the assays. All analytical methods have limits of detection (LODs) and the choice of a method should be consistent with potential bloom concentrations and possible toxin levels. With desalination plants, toxins need to be measured at exceedingly low levels in water, whereas shellfish concentrate toxins to much higher levels. A recent study summarized the epidemiological data for four common algal toxins (Laycock et al. 2010) and estimated the potential contamination of water that might enter a desalination plant during major blooms. The assessment was based on a hypothetical (and dense) bloom of toxic algae consisting of $10^7$ cells/L with a toxin cell quota of 40 pg toxin/cell. If all of that toxin were released from the cells into the water, that would give a concentration in

seawater of 400 µg/L. An alternative approach to estimating the total amount of toxin present in a bloom is given in Chapter 1 (Table 1.4), where the amounts of toxin contained in hypothetical blooms of various common HAB species are presented. The values range from a few hundred to 1,000 µg/L. Given that 99% or more of a toxin is likely to be removed by thermal or reverse osmosis desalination (Chapter 10), the sensitivity of an analytical method must therefore be at least 0.1 – 1.0 µg/L or 0.1 – 1.0 ng/mL. Therefore, analysis of water samples for dissolved or particulate toxins (i.e., inside algal cells) will require high sensitivity methods, such as enzyme-linked immunosorbent assays (ELISAs). For example, the LOD for saxitoxin (STX) using the Abraxis STX ELISA kit is 0.02 ng/mL and there is similar sensitivity for domoic acid.

This appendix presents details on simple screening methods for HAB toxins. More complex analytical methods are described or cited in Chapter 2. The screening methods are presented here as a guide to desalination plant staff who wish to conduct on-site analyses. These analyses could be of raw intake water, treated water, or algal cell extracts from monitoring programs (Chapter 3).

The example assays are restricted to four HAB toxins i.e., saxitoxins, domoic acid, microcystins/nodularins, and anatoxin-a. Although sample preparation procedures may differ for the other HAB toxins not include here, the commercial ELISA kit protocols are similar to each other. Sample preparation procedures, however, vary depending on solubility of the toxins, source (e.g., phytoplankton, shellfish, or cyanobacteria) and method of analysis. Sample preparation methods will be described in detail, as will procedures used to obtain samples. Methods of analysis other than ELISA are also presented.

Lateral flow tests (such as the Scotia tests) are described as simpler alternatives to the ELISA kits. The advantages and disadvantages of both tests will be discussed.

## 2 ELISA METHODOLOGY

For the purpose of demonstrating antibody methods that are potentially useful for monitoring HAB toxins in water, four commercial ELISA kits are discussed here: saxitoxins (STXs), microcystins/nodularins (MCT/NOD), anatoxin-a (ATX) manufactured by Abraxis, and the domoic acid (DA), manufactured by Biosense Laboratories. Other kits are commercially available – these specific kits are presented as examples, not as endorsements.

Antibodies are especially useful for analysis of HAB toxins because of their high sensitivity and specificity. Since the introduction of the ELISA format in 1971 by Engvall and Perlmann (1971), this microtitre plate format has been used for thousands of clinical applications. For a review of ELISAs for shellfish toxins, see Usleber et al. (2001). Antibodies to the common HAB toxins are relatively easy to prepare using purified toxins conjugated to carrier proteins that are injected into rabbits or other animals from which crude serum can be used. Most other components for this technology are commercially available. Nevertheless, the basic ELISA requires a well-equipped laboratory and skill in performing the numerous steps involved. Equipment required includes a plate reader (Figure 1) and multi-channel pipettes.

There are several ELISA plate readers available commercially at a cost of approximately $10,000 (Figure 1). A plate reader also requires a computer to record and plot data. A simple reader is available from Abraxis for ~ $1,200 and is described below.

Aside from being very sensitive, antibody methods such as ELISA are also very specific to a compound or group of closely related compounds that occur as mixtures. For the STXs, of which there are more than 20 different derivatives or congeners (Chapter 2), anti-STX antibodies have different affinities for the individual congeners, i.e., different cross-reactivities. The congeners also have different specific toxicities that vary over a wide range. For example, the Abraxis antibodies have poor cross-reactivities for $GTX_{1,4}$. The limitation of antibody methods for saxitoxins is thus that quantitative test results can be misleading for both concentration and toxicity. The net result is that antibody methods for naturally occurring mixtures of saxitoxins tend to *underestimate* toxicity.

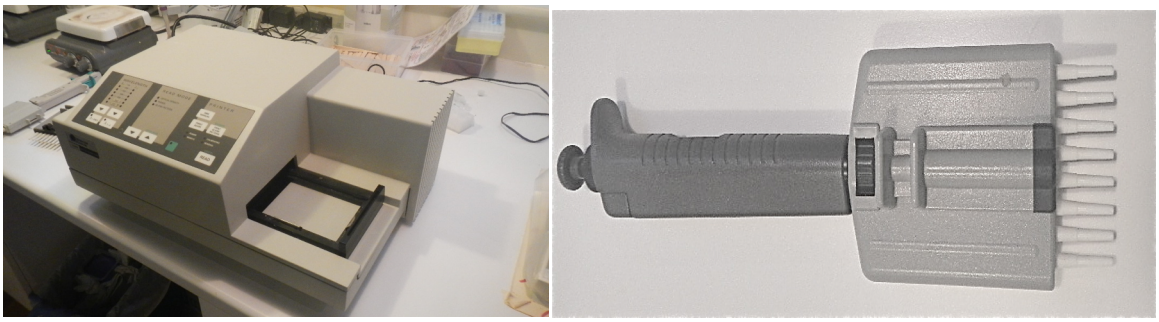

**Figure 1.** An ELISA plate reader (left) and a multi-channel pipette.

The standard ELISA is an *indirect* method and is therefore a more lengthy procedure than that used in the commercial kits described below where a *direct* method is used. Fewer steps are involved in using kits because a solution of toxin-enzyme conjugate or antibody-enzyme conjugate (e.g., STX-HRP (horse radish peroxidase) or anti-domoic acid-HRP conjugate) are provided and competition occurs directly with free toxin. In an indirect method, the enzyme is linked to a second antibody (sheep anti-rabbit) that becomes attached to wells that have bound primary (rabbit) antibody on their surfaces. Wells coated with a toxin conjugate (as in the DA kit) would not bind the primary antibody if there were sufficient toxin in the sample to outcompete the toxin conjugate. The net result is *less* color with free toxin and *more* color with less toxin.

## 2.1 Abraxis ELISA kit for saxitoxin

### 2.1.1 Principle

Competition occurs between STX in samples and a STX-HRP conjugate. With no STX in the sample, all STX-HRP conjugate binds to the rabbit anti-STX antibody that becomes bound to the sheep anti-rabbit antibody well coating. With excess STX in the sample, no STX-HRP conjugate binds to the rabbit anti-STX antibody. There is thus no HRP attached to the well coating, resulting in no color when the enzyme substrate (TMB) is added. The steps of the procedure can be seen in Figure 2.

This kit has been developed for testing shellfish, as well as drinking and fresh water. To obtain accurate results when analyzing seawater samples using the Abraxis Saxitoxin ELISA Kit, Seawater Matrix Standards and an alternate testing procedure are necessary. Seawater samples which exceed the calibration range of the assay must be diluted using the Seawater Matrix Sample Diluent and re-analyzed.

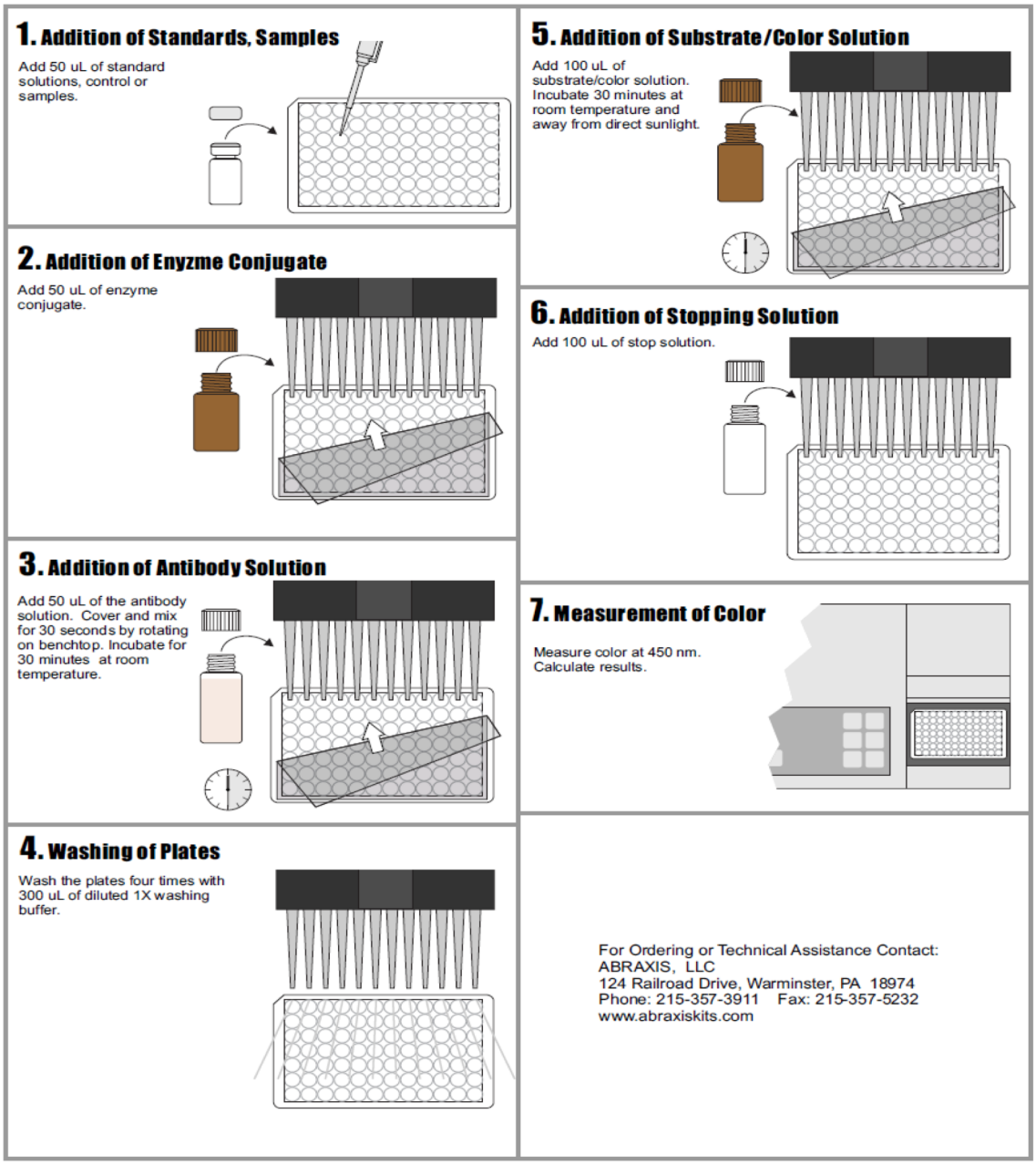


**Figure 2.** Schematic of the Abraxis saxitoxin ELISA procedure.

Twelve strips of eight micro-titer wells pre-coated with sheep anti-rabbit antibody are provided with the kit. The coating procedure is equivalent to steps 1-5 in the standard ELISA protocol given above.

| | |
|---|---|
| Well coating | Sheep anti rabbit antibody |
| Well solution | STX solution 50$\mu$L +STX-HRP 50$\mu$L + STX antibody 50$\mu$L |
| Incubate | 30 min |
| Wash 4x | 300$\mu$L |
| TMB solution | 100$\mu$L |
| Incubate | 30 min |
| Stop | 100$\mu$L |
| Read | 450 nm |
| Limit of detection | 0.02 ng/mL |

## 2.2 Abraxis ADDA-DM ELISA kit for microcystins/nodularins

### 2.2.1 Principle

There are over 140 microcystins/nodularins congeners known. The antibody used in ADDA-DM ELISA has been designed for congener-independent detection of both toxin families. The antibody binds the ADDA part of the molecule which is common to all toxic congeners.

This kit is an example of a direct ELISA in which competition occurs between microcystins/ nodularins (MCT/NOD) in samples and a MCT-HRP conjugate. With no MCT/NOD in the sample, all MCT-HRP conjugate binds to the mouse anti-ADDA antibody that becomes bound to the goat anti-mouse antibody well coating. With excess MCT/NOD in the sample, no MCT-HRP conjugate binds to the mouse anti-ADDA antibody so there is no HRP attached to the well coating resulting in no color when the enzyme substrate (TMB) is added.

Twelve strips of eight micro-titer wells pre-coated with goat anti-mouse antibody are provided with the kit. The coating procedure is equivalent to steps 1-5 in the standard ELISA protocol given above. The steps of the procedure can be seen in Figure 3.

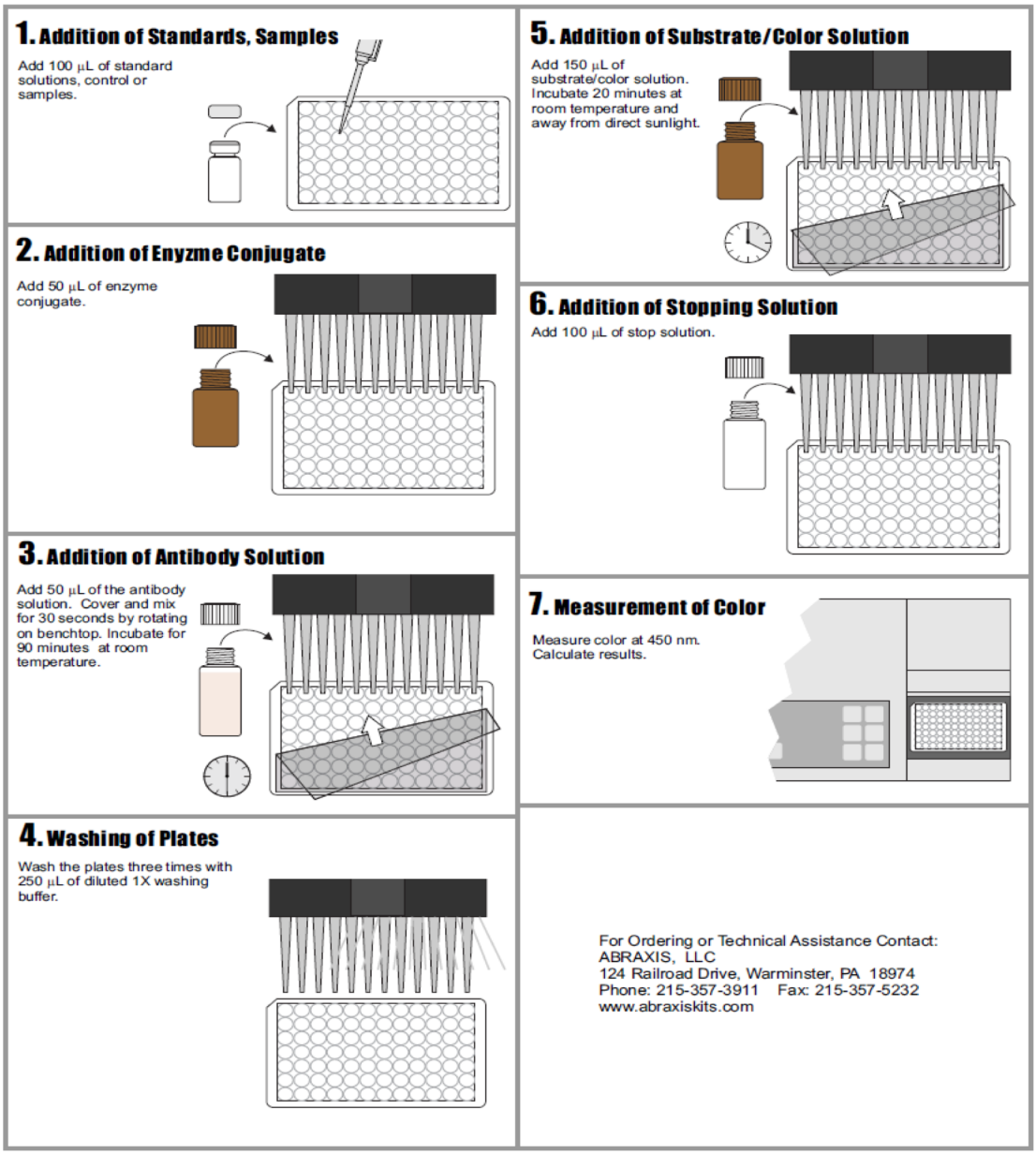


**Figure 3.** Schematic of the Abraxis microcystins ELISA procedure.

This kit has been developed for testing drinking and fresh water. The manufacturer provides a technical bulletin for testing brackish and sea water which involves the addition of one reagent to the sample to remove salt.

| | |
|---|---|
| Well coating | Goat anti mouse antibody |
| Well solution | MCT solution 100$\mu$L +MCT-HRP 50$\mu$L + MCT antibody 50$\mu$L |
| Incubate | 90 min |
| Wash 4x | 300$\mu$L |
| TMB solution | 150$\mu$L |
| Incubate | 20-30 min |
| Stop | 100$\mu$L |
| Read | 450 nm |
| Limit of detection | 0.15 ng/mL |

## 2.3 Abraxis ELISA kit for Anatoxin-*a* (ATX)

### 2.3.1 Principle

This is a direct ELISA. Competition occurs between ATX in samples and ATX-HRP conjugate. With no ATX in the sample, all ATX-HRP conjugate binds to the mouse anti-ATX antibody that becomes bound to the goat anti-mouse antibody well coating. With excess ATX in the sample, no ATX-HRP conjugate binds to the mouse anti-ATX antibody so there is no HRP attached to the well coating resulting in no color when the enzyme substrate (TMB) is added.

Twelve strips of eight micro-titer wells pre-coated with goat anti-mouse antibody are provided with the kit. The coating procedure is equivalent to steps 1-5 in the standard ELISA protocol given above. The steps of the procedure can be seen in Figure 4.

This kit has been developed for testing drinking, fresh water and sea water. Brackish and sea water can be analyzed without any sample treatment.

| | |
|---|---|
| Well coating | Goat anti mouse antibody |
| Well solution | ATX solution 50 $\mu$L +ATX-HRP 50$\mu$L + ATX antibody 50$\mu$L |
| Incubate | 60 min |
| Wash 4x | 300 $\mu$L |
| TMB solution | 100 $\mu$L |
| Incubate | 20-30 min |
| Stop | 100 $\mu$L |
| Read | 450 nm |
| Limit of detection | 0.10 ng/mL |

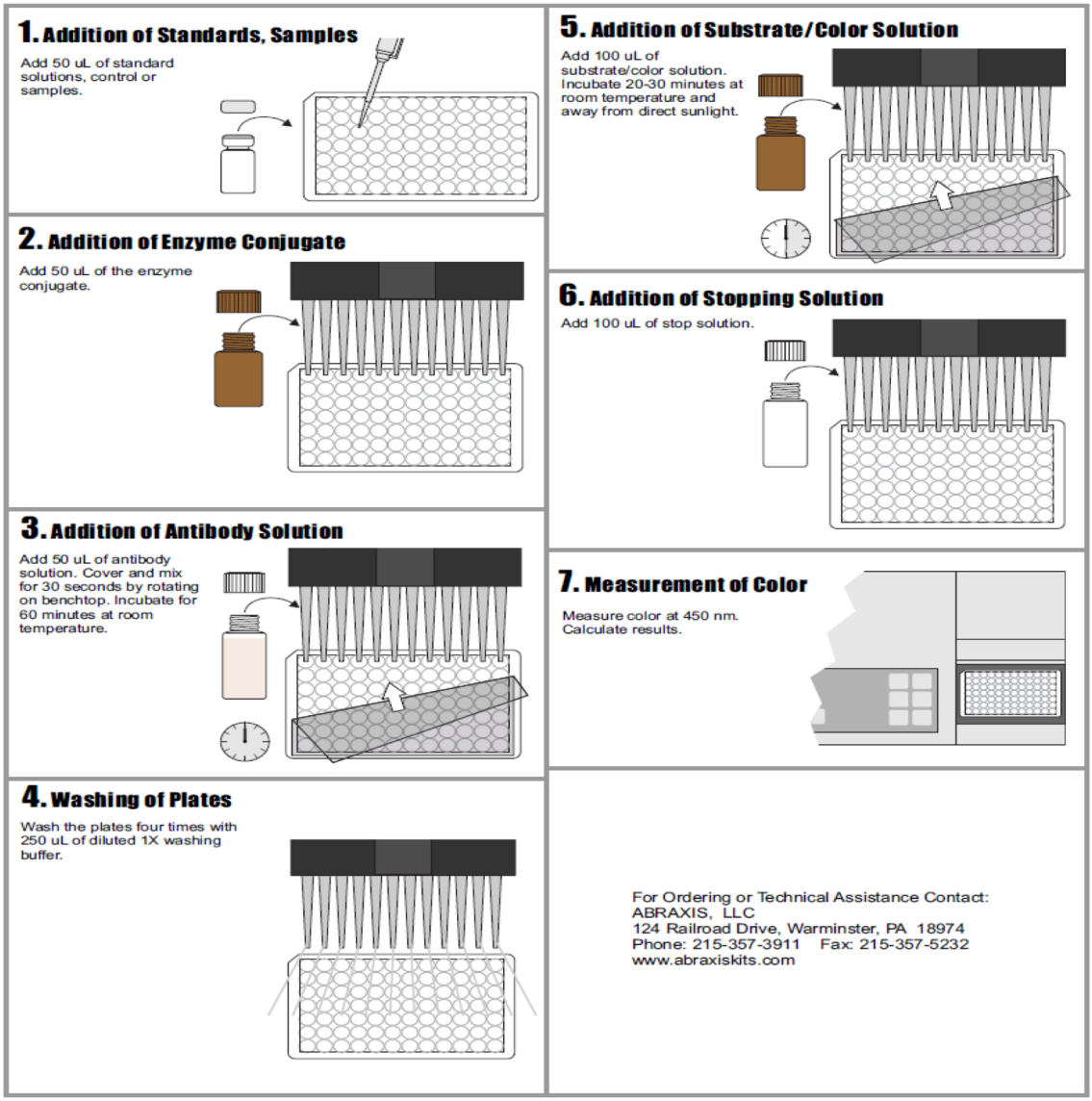


**Figure 4.** Schematic of the Abraxis anatoxin-*a* ELISA procedure.

## 2.4 Biosense ELISA Kit for Domoic acid (DA)

### 2.4.1 Principle

This is an example of an indirect ELISA. Competition occurs between DA in samples and the well coating in the presence of an antibody-HRP conjugate. With no DA in solution, all antibody-HRP binds to the well giving strong color. With excess DA in solution no antibody-HRP binds to the well and no color develops.

| | |
|---|---|
| Well coating | DA-protein conjugate |
| Well solution | Sample 50 $\mu$L + Antibody-HRP conjugate 50$\mu$L |
| Incubate | 60 min |
| Wash 4X | 300 $\mu$L |
| TMB solution | 100 $\mu$L |
| Incubate | 15 min room temperature |
| Stop with 0.3M $H_2SO_4$ | 100 $\mu$L |
| Read | 450nm after 2-5 min |

## 3 IMUNOCHROMATOGRAPHIC OR LATERAL FLOW FORMAT

These are simple devices intended to detect the presence/absence of a target analyte in a sample without the need for specialized or costly equipment. The lateral flow format for these tests is simple and fast compared to ELISA, and makes them suitable for field-work without laboratory equipment. They are useful when it is only necessary to show the presence of a toxin and not suitable to try to measure toxin concentrations. They are less sensitive than ELISA and only semi-quantitative in that toxin concentration can only be estimated from the sensitivity of the test. They are not deemed suitable for testing of low levels of toxins in water samples, but can be easily used to test for toxins in concentrated plankton net tow extracts (Chapter 3).

The benefits of immunochromatographic tests include:

- User-friendly formats
- Short time to get results
- Long-term stability over a wide range of climates
- No need for specialized or costly equipment
- No need for experienced lab personnel
- Can be run on-site or in a laboratory setting

### 3.1 The Scotia tests

Scotia Rapid Testing Ltd. manufactures lateral flow immunochromatographic (LFI) tests for three HAB toxins: the saxitoxins, domoic acid, and okadaic acid. These kits were formerly known as Jellett Rapid Test Kits, but are now called Scotia Rapid Test Kits.

#### 3.1.1 Principle

In a similar manner to the ELISA kits, immnunochromatographic tests are competition assays. Free toxin competes with bound toxin for specific antibodies. Anti-toxin antibodies are attached to colloidal gold particles (diameter 40 nm) that are dried during manufacture onto a fiberglass pad on the test strip. When a sample is added, the red colored gold particles are carried by capillary action along a nitrocellulose membrane where they interact with a line consisting of a toxin-protein conjugate known as the test or T-line. If there is no toxin in the sample, the antibodies interact with bound toxin forming a red line of colloidal gold particles. If, on the other hand, toxin is present in the sample, it competes with bound toxin on the test line and fewer gold particles stick to the line resulting in no red color or a fainter line depending on the concentration of toxin in the sample. Further along the membrane, the particles cross another line that consists of a protein recognizable by other antibodies on the particles. This is a control line that should bind particles when the test is working properly.

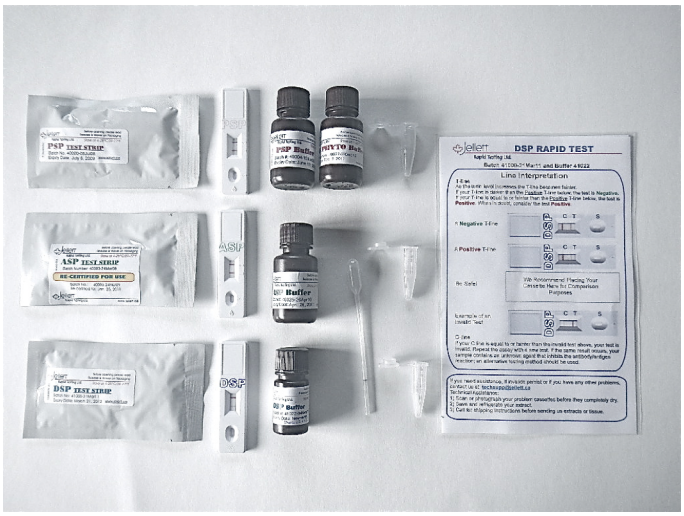


**Figure 5.** The Scotia rapid test kits.

The Scotia tests for shellfish toxins can be used as spot tests on a single sample with minimal and unsophisticated equipment as

shown in Figure 5. Figure 6 shows a schematic of the lateral flow process, and Figure 7 an example of the results.

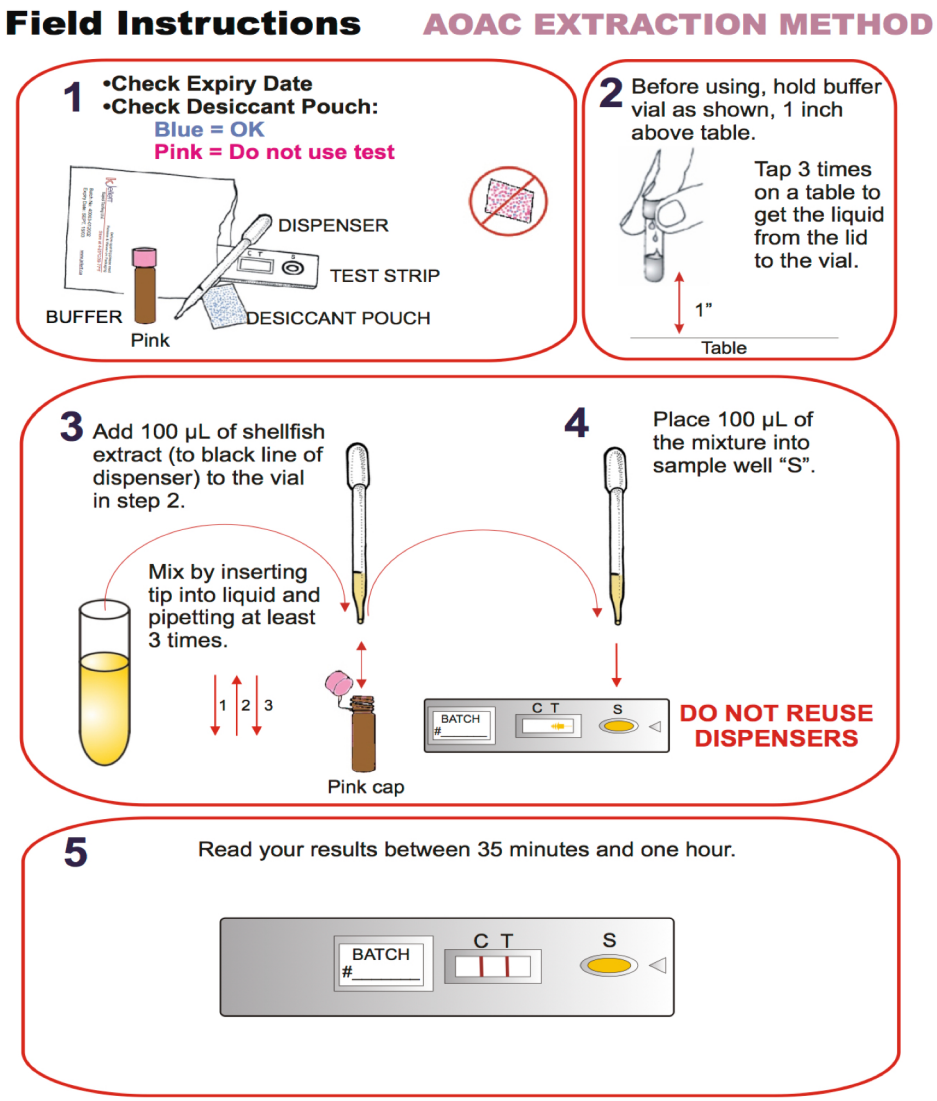


**Figure 6.** Instructions for using the Scotia STX lateral flow assay. Source: Scotia Rapid Testing.

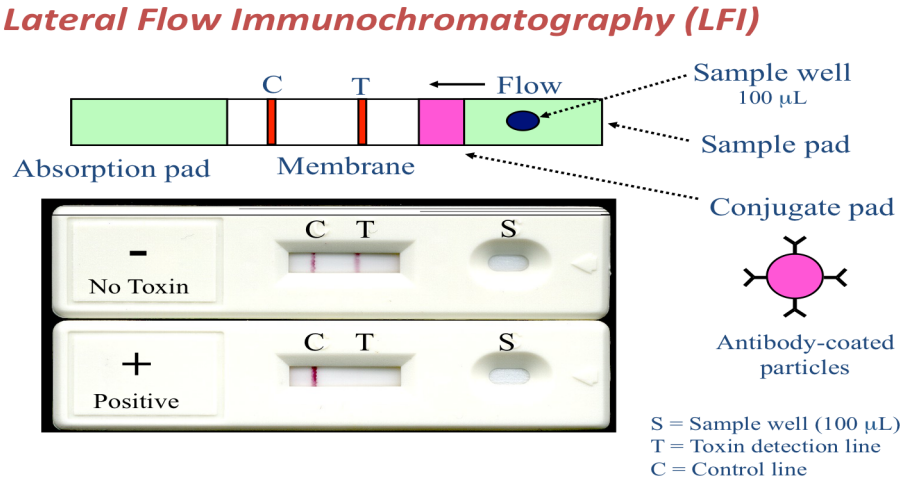


**Figure 7.** Lateral flow immnochromatography (LFI).

### 3.2 Abraxis microcystins finished drinking water immunochromatographic tests

Abraxis offers several immunochromatographic tests for microcystins/nodularins.The antibody used is the same as in the ELISA test kit, and therefore offers a broad reactivity for congener detection. Available test kits are for source waters, recreational waters, and finished drinking water. The differences are detection limits and the option to lyse the cyanobacterial cells. The drinking water, source water, and recreational water test kit are not recommended for estuarine or sea water because of salt interference with the test. The drinking water test can be used to analyze finished drinking water from desalination plants, and it has a sensitivity of 0.5 ng/mL. Chlorinated finished drinking water must be quenched immediately with sodium thiosulfate.

#### 3.2.1 Principle

The test is based on the recognition of microcystins, nodularins, and their congeners by specific antibodies. The toxin conjugate competes for antibody binding sites with microcystins/nodularins that may be present in the water sample. The test device consists of a vial with specific antibodies for microcystins and nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of microcystins in the water sample, and therefore, should be present in all reactions. In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized microcystins conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area**.**

The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect the toxin at or above the cut-off point established for the assay.

If microcystins are present in the water sample, they compete with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate, therefore preventing the development of a colored line. If a colored line is not visible in the Test Line Region, or if the Test Line is lighter than the negative Control Line, microcystins are present at the levels of concern (>1 ppb). Semi-quantitative results can be obtained by comparing the test line intensity to those produced by solutions of known microcystin concentrations (control solutions). The steps of the procedure can be seen in Figure 8.

#### 3.2.2 Sample preparation methods

There is no general method for sample preparation for all HAB toxins from a variety of sources. The same methods can be used for both Abraxis and Scotia tests except for solutions used to dilute samples. These solutions are provided with the kits. Methods depend on the following considerations.

> *Type of organism*. Shellfish have fairly uniform extractability, whereas some algal cells with rigid cell walls are difficult to extract in plankton samples. These cells can be most easily disrupted by sonication or freeze-thaw cycles.
>
> *Solubility*. Molecular characteristics such as charge distribution determine the degree of water solubility. Saxitoxins and domoic acid are readily soluble in aqueous solutions. For less polar compounds like okadaic acid, methanol is a useful solvent as it can be easily removed by evaporation, and at low concentrations (<10%) does

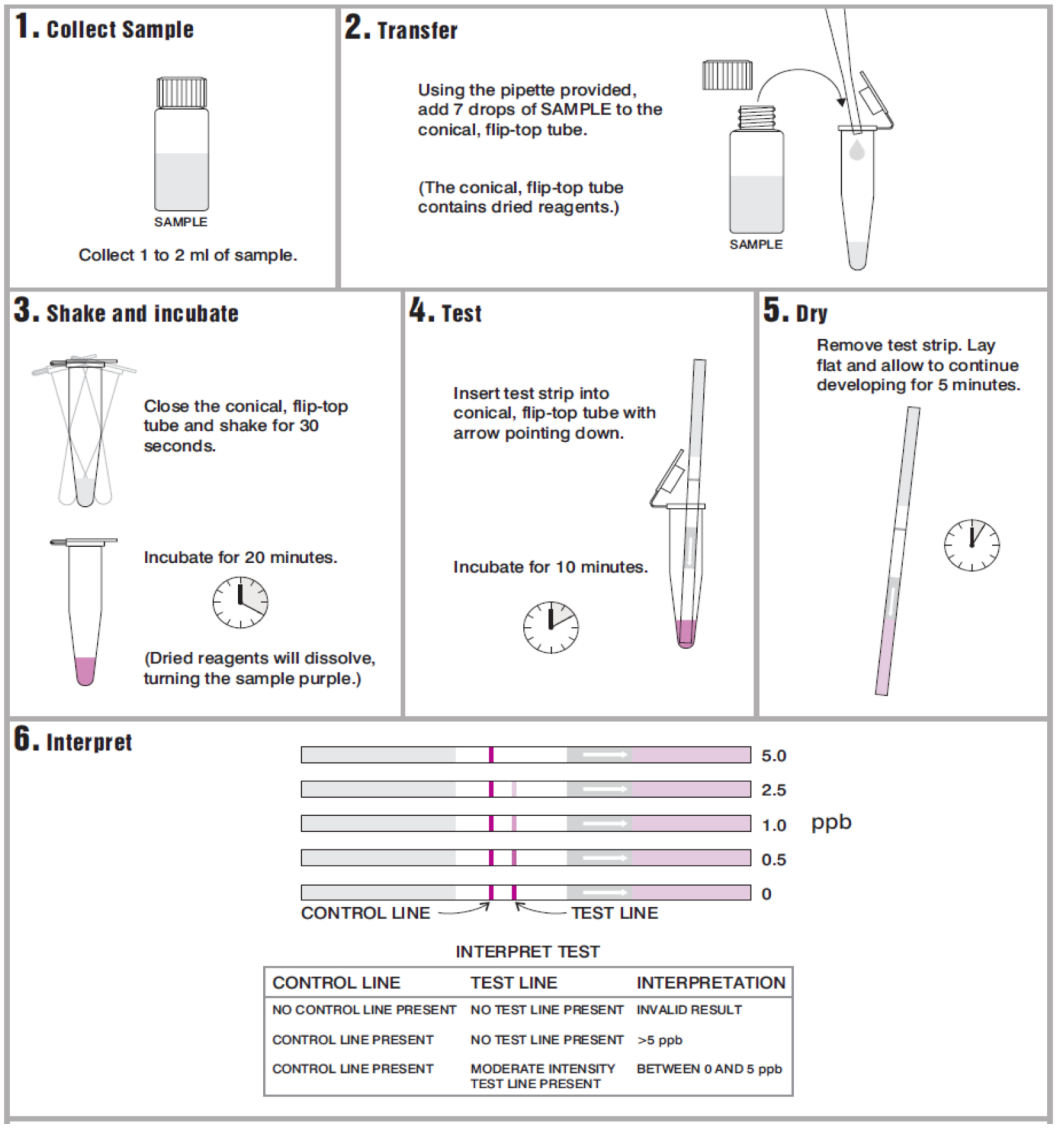


**Figure 8.** Schematic of the Abraxis Microcystins Finished Drinking Water Lateral Flow procedure.

not interfere with antibody reactions. An additional problem when working with hydrophobic compounds (e.g., brevetoxins) is that they are readily adsorbed onto surfaces; plastic containers should therefore be avoided.

*Method of analysis.* The final steps in sample preparation provide a solution that is compatible with the analytical procedure. Antibodies are susceptible to organic solvents, pH, and salt concentrations that can interfere with immuno-reactions or alter their protein folding.

### *3.2.2.1 Toxins from phytoplankton*

Initial water sampling can be done with a plankton net (see Chapter 3). Because cells occur at various depths, the net can be raised through a column of water to obtain an integrated sample. Alternatively, if a bloom is visible and at the surface, a known volume of water can be filtered through a 20-cm diameter Nitex sieve and further concentrated onto a 1-cm diameter filter using a syringe. (Figure 9). For saxitoxins, the filter can be transferred to a 4-mL glass tube for extraction with 0.1M hydrochloric acid. The extract is heated at 100 °C for

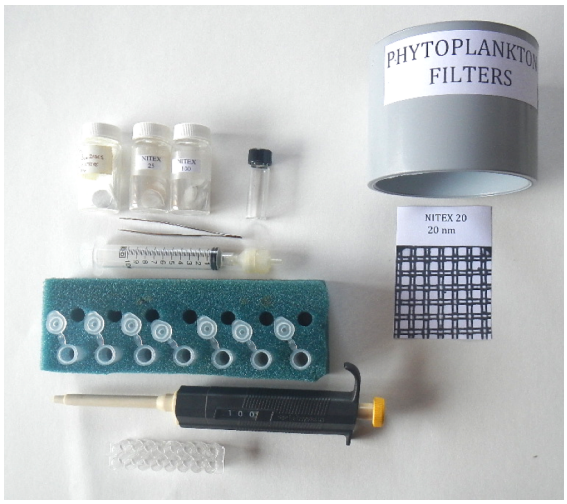


**Figure 9.** Tools needed to filter and concentrate a water sample for analysis.

10 min. This step is used to convert C toxins to gonyautoxins which are more readily detected.

Cells from a plankton net and Nitex filters can be concentrated on a 1-cm diameter fiberglass filter pad which is transferred to a 4-mL glass tube for extraction with 0.5 mL dilute hydrochloric or acetic acid.

The Biosense method for extracting *Pseudo-nitzschia* cells taken from Fehling et al. (2004) involves sonicating in sample buffer (PBS: phosphate buffered saline). If an ultrasonic apparatus is not available, freezing and thawing several times should be effective.

The Biosense protocol recommends extracting 4 g homogenate with 10 mL 50% methanol followed by centrifuging. There is no heating step. For more details see the Biosense website http://www.biosense.com.

The following is an example modified from the Biosense manual. The original example was for shellfish tissue, but similar procedures would apply to concentrated plankton net tow samples. Because ELISA kits are very sensitive and quantitative, a series of dilutions of both calibration standards and extracts is necessary to obtain data in the dynamic range (i.e., on the slope of the calibration graph). For Scotia tests, the ASP buffer provided with the tests is used for diluting samples.

*3.2.2.2 Preparation of shellfish samples*

Extraction of DA from plankton samples

1) Accurately weigh a known mass of sample into a 50 mL centrifuge tube.
2) Add 16 mL of Extraction solution (50% methanol).
3) Mix well by vigorous shaking for 1 min.
4) Centrifuge at 3000 x g for 10 minutes at room temperature.
5) Retain the supernatant for further dilution prior to analysis. The extracts can be stored at -20˚C for up to 14 days, although with a possible reduction in DA content.

Dilution of extracts

1) Prepare dilutions of the shellfish extract in Standard/Sample buffer (10% methanol in PBS-T) as follows:
   1:20 dilution: 50 µL shellfish extract + 950 µL buffer
   1:200 dilution: 50 µL of the 1:20 dilution + 450 µL buffer
   1:2000 dilution: 50 µL of the 1:200 dilution + 450 µL buffer
   1:20 000 dilution: 50 µL of the 1:2000 dilution + 450 µL buffer
   1:200 000 dilution: 50 µL of the 1:2000 dilution + 450 µL buffer
   Cap and vortex each dilution before proceeding to the next dilution step.
2) Analyze the sample dilutions according to the DA concentration range of interest (see Table 1), to give absorbance values within the calibration curve working range.

**Table 1.** Shellfish extract dilution for quantification of domoic acid.

| DA concentration range of interest (mg/kg) | Corresponding sample extract dilution to be analyzed |
|---|---|
| 0.01 - 0.25 | 1:200 (minimum dilution) |
| 0.1 - 2.5 | 1:2,000 dilution |
| 1.0 - 25 | 1:20,000 dilution |
| 10 - 250 | 1:200 000 dilution |

### 3.3 Obtaining quantitative data with Abraxis ELISA reader

Results obtained using ELISA can be measured using a laboratory ELISA reader. There are several such instruments available which cost between $5,000 and $25,000. They are not designed to be portable, weighing around 10 kg. Abraxis has, however, developed a relatively simple portable instrument for field use, such as on survey vessels.

#### 3.3.1 Essential steps to obtain data with the Abraxis + reader

Detailed instructions and software are provided with the Abraxis reader (Figure 10). A simplified step-by-step guide to using the instrument is provided below.

1. Load the software from the disc to a computer.

3. Connect the reader and computer.

3. Plug the 12 volt DC supply into the reader.

4. Click on the M6+ icon to launch the computer software.

5. Set up a COM port (see operation manual).

6. Make sure the M6+ is showing "Abraxis LLC" before selecting the COM port for the connection between M6+ and the computer. If not it may be necessary to restart the computer.

7. In the "add a parameter" function (Green button A) choose a name, concentration units, concentration range, filter wavelength and curve type. (The default parameter is for microcystins). If this has already been done for saxitoxin and domoic acid, click on the one for the current analysis.

*Note: 1. The displayed **data will be lost message** is OK. The loss is temporary.*

*Note: 2. The parameter window is 'greyed out'. This is normal.*

8. Click 'Set up' (top row middle on screen). Select OK in comport setting window if the correct comport is showing (COM5). This connects the M6+ and PC (showing "on line").


**Figure 10.** Portable Abraxis plate reader.

9. Click on the third green button (capture data) to take readings from a strip of wells. In the capture data window on the PC select "Clear sample data and get real time reading then OK. A blank must be taken at this stage.

10. The M6+ window should show: Abraxis LLC. If not, scroll round the options by pressing SEL/ESC several times until it appears.

11. Insert a strip with water (blank) in a well until it clicks into position in the light path then press READ/ENTER on the M6+.

12. The reading will be 0.000 ABS., then repeat before going to the next level

13. On M6+ press SEL/ESC twice to get to level [2] PARAMETER. Here the number of standards is shown, e.g. 6, but should correspond with those listed in the parameter with their respective concentrations. Press READ/ENTER to select the number of replicates.

14. In the screen on the M6+ the number of replicates can be selected. Unlike the **number of standards** which corresponds to the pre-determined PARAMETER any number of replicates can be chosen for the standards at this stage up to a maximum of 5 by pressing SEL/ESC repeatedly then press READ/ENTER.

15. Now the number of **sample** replicates can be chosen up to a maximum of 5 by pressing SEL/ESC repeatedly (usually 2).

16. Press SEL/ESC to get to level [3] STANDARD. Here the standards can be read. Press READ/ENTER to read the first Standard. The M6+ screen shows S1-1 Standard. Read Standard? Press READ/ENTER. The screen will show S1-1 and the absorbance (eg 0.528A). After S1-2 the PC screen will show the two values and the average. To move to standard number two press SEL/ESC. Move the strip to the next well and press READ/ENTER. Press SEL/ESC. The M6+ screen will ask if you want to continue. Press READ/ENTER to continue.

17. Move the strip of wells ahead after the replicates have been read for the **standard**. The readings and averages will appear on the PC screen under the Data tab. If they do not, repeat steps 9-17. After the second replicate of standard 6 the M6+ screen will show END.

18. To read the samples press SEL/ESC to go to level [4] SAMPLE.

19. Press READ/ENTER. M6+ screen shows T01-1 Sample and Read Sample? Press READ/ENTER repeatedly to read the replicates of the first sample. The PC screen will show the absorption values for each replicate and their average.

18. Continue through all samples, then click on the green button to stop reading.

19. To see the calibration graph and sample concentrations click on the quantitative tab to show the calibration graph and sample concentrations.

20. To print the quantitative results click **File** on the top line then print. If no printer is attached to the PC save as a pdf to an external memory (flash drive).

20. To save the data so that this existing calibration graph can be used for new samples without generating another graph each time samples are analyzed (and using up the limited number of wells) scroll up to [6] RS232 to PC in the M6+ window followed by READ/ENTER to M6 -OK->PC. Then capture data (third green button) by selecting **upload batch data** in the capture data window ("not clear sample data and get real-time reading" as before). Click OK in message window "Please load STD data".

21. Samples should be read using a calibration graph prepared at the same time. However, for approximate values calibration data in the memory can be used.

22. Select [6] RS232 to PC then enter to show M6->PC in the M6 window. Then Capture Data in the PC screen.

23. Select Upload batch data in the PC screen to show the data for calibration.

24. To read new samples using the uploaded calibration click on Capture Data button and select "Clear sample data and get real time reading, then OK. The sample data will be erased from the table under data in the PC screen.

25. Select [4] SAMPLE. The M6 screen will show "Rewrite Memory-are you sure?

26. Press read/enter on M6 and read the first new sample. If two replicates were chosen for samples - read again.

27. The new sample data will be shown in the Table in the Quantitative tab on the PC.

28. To save data after obtaining sample concentrations

    a. Under File select Load data
    b. Select the protocol, eg Saxitoxin
    c. Click on the data capture icon
    d. Select Real Time Reading
    e. Select Read Sample on M6+
    f. Stop reading
    g. Save and give a new name

## 4 SUMMARY AND CONCLUSIONS

Desalination plants should establish a capability for screening samples for HAB toxins. This would not be a routine analysis, but instead an opportunistic approach triggered by identification of toxic cells in plankton samples, or other indications that toxic species are entering the plant. It is advisable for operators in areas subject to toxic HABs to have some lateral flow or ELISA kits on site, as it can take days or even weeks to get new kits during a crisis. ELISA kits are suitable for use when an estimate of toxin concentration is necessary. Lateral flow tests are more suitable than ELISA for routine monitoring of phytoplankton and shellfish extracts, but are not sensitive enough for water analysis, nor are they quantitative.

If a positive result is indicated by the ELISA or the lateral flow tests, samples should be collected and evaluated with more sophisticated analytical instruments. A fully equipped analytical laboratory capable of measuring algal toxins is unrealistic at a desalination plant, but capabilities for HAB toxin analysis exist within many countries or regions at government agencies or universities. Operators should have contact details and sampling protocols prepared as part of their Alert Level Framework actions (Chapter 8) in the event that this type of external or outsourced analysis is required. Local or regional HAB experts should also be identified and contacted for guidance during potentially dangerous or damaging bloom events.

## 5 REFERENCES

Anderson, D. M., Andersen, P., Bricelj, V. M., Cullen, J. J., and Rensel, J. E. 2001. *Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters*. Asia Pacific Economic Program, Singapore, and Intergovernmental Oceanographic Commission, Paris. 268 pp.

Engvall, E. and Perlmann, P. 1971. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry* 8(9), 871-874.

Fehling, J., Green, D. H., Davidson, K., Bolch, C. J., and Bates, S. S. 2004. Domoic acid production by *Pseudo-nitzschia seriata* (Bacillariophyceae) in Scottish waters. *Journal of Phycology* 40, 622-630.

Laycock, M. V., Donovan, M. A., and Easy, D. J. 2010. Sensitivity of lateral flow tests to mixtures of saxitoxins and applications to shellfish and phytoplankton monitoring. Toxicon 55, 597-605.

Usleber, E., Dietrich, R., Burk, C., Schneider, E., and Martlbauer, E. 2001. Immunoassay methods for paralytic shellfish poisoning toxins. Journal of AOAC International 84, 1649-1656.