EVALUATION OF FIELD TEST KITS TO DETECT MICROCYSTINS: 2010 STUDY

FINAL REPORT

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Introduction

Cyanobacteria blooms may present a public health concern in sources of drinking water and recreational water, as certain cyanobacteria species can produce toxins. These cyanotoxins fall in the categories of hepatotoxic (microcystins, cylindrospermopsin), neurotoxic (anatoxin-a, saxitoxin) or cytotoxic (cylindrospermopsin) based on the target tissues. Within the hepatotoxins, microcystins are the most commonly produced toxin by cyanobacteria worldwide. Over 90 different variants have been identified, but the predominant variant found is microcystin-LR. Not all cyanobacteria blooms produce toxins, and there is no visual indication of toxin presence so accurate, rapid methods to determine or estimate microcystin levels in a water source are necessary to facilitate quick risk assessment decisions during cyanobacteria bloom events. In Canada, the drinking water quality guideline for microcystin-LR (MC-LR) is 1.5 µg/L (or ppb) [1]. The guideline for microcystin-LR is considered protective of other microcystin variants (total microcystins) which may be present in the water. The new Canadian recreational water quality guidelines are 100,000 cells/mL for cyanobacteria and 20 µg/L of total microcystin [2]. The World Health Organization established a drinking water guideline of 1 µg/L of total microcystin. Due to a lack of toxicological and occurrence data on which to assess potential health risks, there are currently no guidelines for any other cyanotoxins including cylindrospermopsin and anatoxin-a.

In Canada, detection of cyanobacteria blooms in water bodies has been steadily increasing. Some provinces have established monitoring programs whereas others deal with them on a case by case basis. Although laboratory techniques to detect microcystins are available, the analysis per sample is costly and in places with high demand, the time for analysis and reporting is lengthy (days). However, authorities must respond quickly when a cyanobacteria bloom appears, in either a drinking water source or in recreational water bodies.

For water sources affected by cyanobacteria blooms, the following flowchart (Figure 1) is suggested for monitoring purposes [1] in water bodies used for human consumption. When a bloom event is observed, where possible, raw water (collected at the water intake) and treated water are collected simultaneously (step 2). Raw water is analyzed either by sending samples to a laboratory for testing or using a field test kit on site. If the results show microcystin levels of more than 1 µg/L (step 4), treated water is then analyzed (step 5). If the results show microcystin levels of more than 1.5 µg/L in the treated water, appropriated agencies and communities are notified, in addition to continuing sampling of treated water (step 7 and 9).

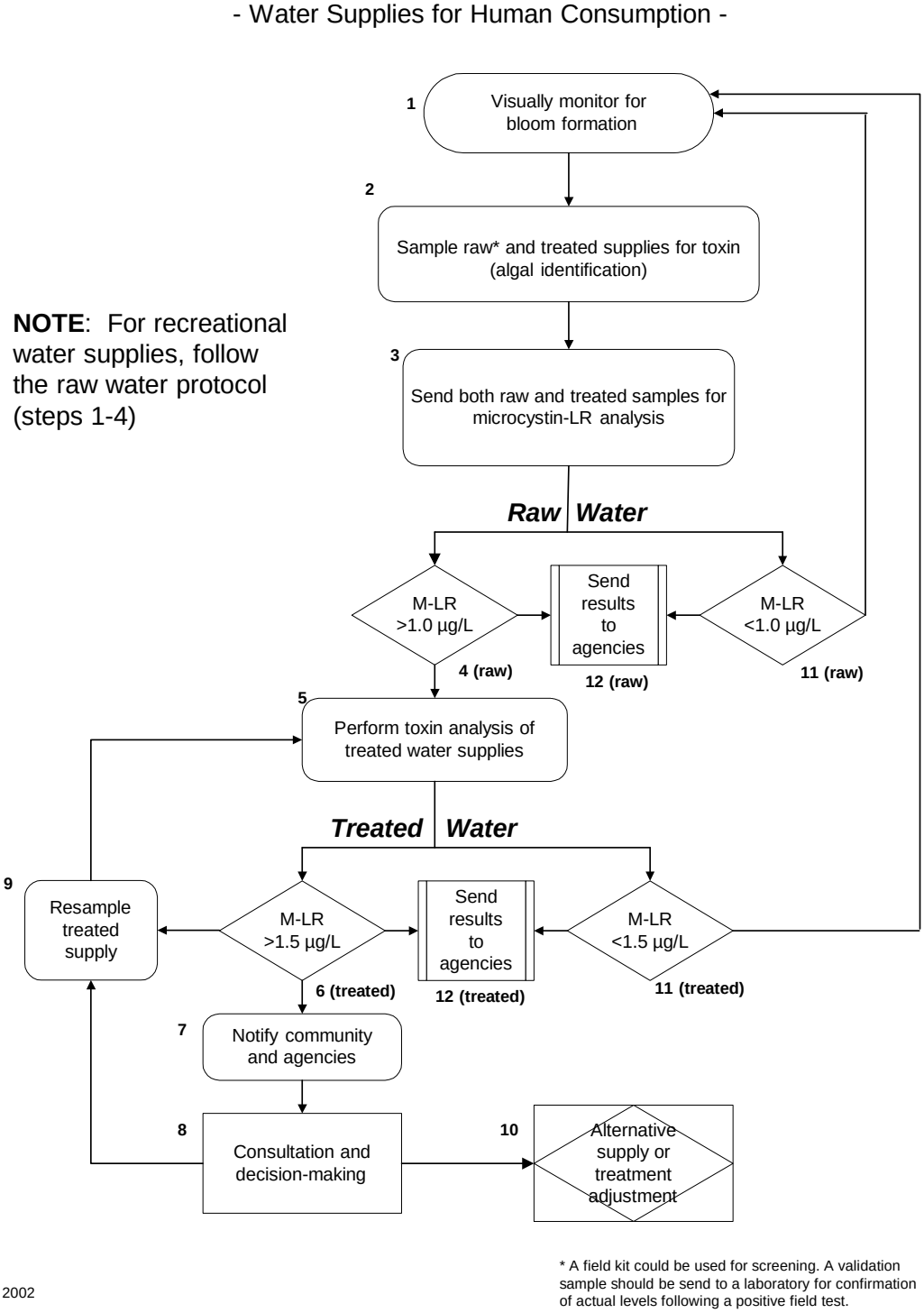

Figure 1. Flow chart from the Guidelines for Canadian Drinking Water Quality: Supporting Documentation. Cyanobacterial Toxins-Microcystin-LR. Annex A. Water Supplies for Human Consumption [1]

As shown in Figure 1, the threshold values of 1 µg/L and 1.5 µg/L are crucial to deciding on the course of action during a bloom event. The use of field test kits may provide quicker results at a lower cost and may be used to screen the water samples for toxin formation throughout a bloom episode which is important since as previously noted, not all blooms produce toxin. Therefore, it is important to evaluate the performance of these commercially available kits to understand their limitations. It is understood that the field test kits under evaluation may provide qualitative results or a semi-quantitative result at best which should be confirmed by laboratory analysis. However, the results may be used to provide preliminary evidence for possible action by the responsible authorities. Only a few studies have evaluated the kits in the laboratory setting using model solutions (standards in water or cyanobacteria cultures) and using experienced laboratory personnel [3,4]. In real situations, there was a broad range of end-users with variable laboratory experience. In this project, it was important to see how different end-users interpreted the results of the field test kit from real samples and conditions.

The main objectives of the project were:

- To assess the usefulness of the commercially available field test kits for quick, on-site analysis of microcystins by the end-user
- To compare the results obtained with the kits to the laboratory results (accuracy)
- To evaluate the possible presence of other toxins in Canadian water supplies: anatoxin-a, cylindrospermopsin and saxitoxin (data not shown here).

Methodology

1) Participants recruitment

Information regarding the project was presented to members of the Federal-Provincial-Territorial Committee on Drinking Water as well as the Regional Health Managers from First Nations and Inuit Health Branch (FNIHB) who were asked to identify municipalities/communities which might be willing to participate. Once participants were identified, they were contacted to discuss the project and arrangements were made to send the sampling kits.

2) Field test kits

The following test kits are currently available commercially:

a) Abraxis LLC. This company offers two products for testing drinking water sources.

a.1) Immunochromatographic Strip Test for the Detection of Microcystins and Nodularins in Source Drinking Water at 1 ppb (PN 520019) (herein named as strip test) [5].

a.2) Microcystin Tube kit. (PN 520012) (herein named as Abraxis tube) [6].

b) Envirologix Qualitube Kit for Microcystin (#ET022) (herein named as Envirologix) [7]. The test does not contain the lysing agent, therefore only detects free, not total, toxin.,

c) Zeu-Inmunotec S.L. MicroCystest tube kit (ZE/CCT32) (herein named as Zeu) [8]. This kit is a protein phosphatase inhibition assay. The test does not contain the lysing agent, therefore only detects free, not total, toxin. The manufacturer provides a protocol to lyse the cell consisting of filtration, extraction and dilution steps.

The Abraxis and Envirologix kits are based on the recognition of microcystins by specific antibodies (immunoassay), therefore the results of these tests is the sum of all microcystins that cross react with the antibodies. The Zeu-Inmunotec kit is based on the inhibition of protein phosphatase 2A and the results represent the presence of all compounds that inhibit the enzyme.

Table 1. List of field test kits available in the market

Manufacturer	**Format**	**Principle**	**Standards**	**Interpretation**	**Time of analysis**
Abraxis LLC	Strip Test	Inmunochromatography	None (graph for 0.5 to 5 ppb)	Visual interpretation	~ 40 min
Abraxis LLC	Tube	ELISA	0.15, 0.4, 1.0, 2.0, 5 ppb	Visual or photometer	~ 50 min
Envirologix	Tube	ELISA	0.5 and 3 ppb	Visual or photometer	~ 50 min
Zeu-Inmunotec S.L.	Tube	Phosphatase inhibition	0.5, 1.0 and 2.5	Photometer (405nm)	~ 65 min

As the Canadian Drinking water guideline for microcystin-LR is based on the total microcystin (dissolved/free and cell bound) a step to lyse the cyanobacterial cells is necessary. Abraxis LLC manufactures a lysing product named QuikLyse™ [9] which can be purchased individually and is part of the strip kit. In order to compare the results obtained by all the kits and because all participants received the strip test kit containing the QuikLyse™, each participant was asked to follow the methodology depicted in Figure 2. The amount of sample obtained after using the reagents in the QuikLyse™ protocol was enough to perform the strip test kit and one additional kit.

All the participants received the strip test kit plus an additional kit (either Abraxis Tube, Envirologix or Zeu).

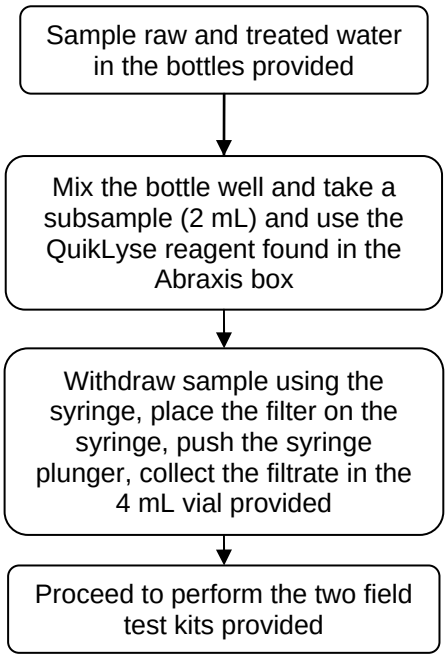

Figure 2. Flow chart of the procedure followed in the field.

3) Sample collection and use of the field test kits

Sampling kits and protocols were provided to participants in advance. Each sampling kit (cooler) included: Abraxis strip test kit which included the lysing reagent, one of the other field test kits, sampling bottles, questionnaires related to sample collection/site identification/the treatment process, a field test kit data report form and an evaluation form on the use of the kits.

Water samples were collected in duplicate. Participants were asked to take an aliquot of one of the collection bottles and proceed with the flowchart as described in Figure 2.

End-users tested the samples using the field test kits according to the manufacturer's instructions and the results were recorded on the forms provided. In order to assist in the interpretation of the results, a negative (deionized water) and positive control (1.5 µg/L microcystin-LR) were also sent to each participant. The duplicate collection bottle(s) were sent to the Exposure and Biomonitoring Division (EBD) laboratory in Health Canada for further confirmation with instrument analysis (LC-MS/MS and ELISA).

4) Laboratory Analysis

Sample preparation

Samples were received in 120 mL sampling bottles within two days of collection. Upon arrival to the EBD laboratory, samples were tested using a randomly selected field test kit following the same protocol as shown in Figure 2. A small aliquot (~4 mL) was taken from each bottle for analysis of saxitoxin and cylindrospermopsin.

Once the field test in the lab was completed, the contents of each bottle were filtered with a 47 mm Whatman GF/C glass microfiber filter under vacuum. The volume of filtrate (water) was recorded and the samples were stored at 4°C until analysis. The filters were wrapped with aluminum foil and stored at -20°C until analysis.

The filters underwent three freeze-thawing cycles prior to extraction. They were extracted with 75% methanol [10]. The extracts were evaporated, reconstituted with 50% methanol and analysed as described below.

Analysis

The filtrates (water) were analyzed using Envirologix ELISA quantitative plate without further concentration or clean up. The extracts obtained from the extraction of the filters were analyzed following the protocol described below.

Calibration standard mixtures containing anatoxin-a and microcystin variants (MC-RR, MC-LR, MC-LF, MC-LW, MC-LA and Mc-YR) were prepared by diluting secondary stock solution (1ppm in 50% methanol /water) to construct a calibration curve with concentrations of 500 ppb, 100 ppb, 50 ppb, 10 ppb, 5 ppb, 1 ppb and 0.5 ppb.

Standards and extracts (10µL) were injected onto a Liquid Chromatograph (Thermo Accela) coupled to a Mass Spectrometer (Thermo TSQ Quantum). The analytical column was

the Hypersil Gold 50mm x 2.1 mm, 1.9 µm from Thermo Scientific. The mobile phase was A: H_2O with 0.1% formic acid and 0.5% methanol; B: acetonitrile with 0.1% formic acid. Flow rate was 0.2 mL/min. The gradient method started with 95%A and held for 1min. It changed to 70%A at 2min, then changed to 10% A at 4min and held at 10%A until 5min. Then it changed back to 95%A at 5.2 min until 7 min for column equilibration. The switching valve switched from detector to waste at 5.5 min of the run.

There were two segments with the duration time of 2.48 min and 4.52 min in the MS method. For both segments the Q2 Gas Pressure was 1.5, scan width 0.01, scan time 0.1s and peak width for both Q1 and Q3 were 0.7. Table 2 shows all microcystins included in the analysis and their transitions.

Table 2. MS/MS transitions of anatoxin-a and microcystins

Name	Parent *m/z*	Product *m/z*	CE	Tube Lens
MC-RR	520.040	135.177	26	130
MC-LA	910.400	135.114	52	172
	910.400	776.600	20	172
MC-LF	986.360	375.297	36	190
	986.360	478.400	26	190
MC-LR	995.600	134.953	63	189
	995.600	213.096	59	189
MC-LW	1025.390	288.151	42	169
	1025.390	375.114	36	169
MC-YR	1045.460	134.973	61	199
	1045.460	212.947	58	199

Quality Control

For each batch, one filter was spiked with 100 uL of 10 ppm working standard mixture of anatoxin-a, MC-LR, MC-YR, MC-RR and MC-LA. Because of the limited amount of MC-LF and MC-LW available, they were not included in this step. In addition, three blanks (filters free of analyte placed in the extraction cell) were included in the batch to evaluate the potential for carry-over in the ASE system during the run: pre-run blank, mid-run blank and post-run blank. All controls were processed in the same way as the samples. During the LC-MS/MS run, the calibration curve standards were run at the beginning and at the end of the batch to monitor the deviation of the instrument system. QC low (1 ppb) and QC high (100 ppb) were injected with every batch. All the samples in the batch were injected in triplicate.

Results

During this study, samples were collected in four provinces in Canada at 20 sites; the description of the participants per province and the field test kit used are found in Table 3. The samples were collected between July and October 2010 from surface water with a history of cyanobacteria blooms. The water bodies selected were categorized as source drinking water, drinking/recreational or recreational waters. In some of the source drinking water, samples were collected at the intake of the water treatment plant (raw) and at the treatment plant (treated). A total of 153 samples (including duplicates) were received.

Table 3. Description of the participants, test used and site type.

		Site/Surface water	Use	Sample type	Test used
P 1	End-user 1*	Site 1/Lake	Drinking/recreational	Raw	Strip
		Site 2/Lake	Recreational	Raw	Strip
		Site 3/Reservoir	Drinking/recreational	Raw	Strip
	End-User 2	Site 1/Lake	Drinking/recreational	Raw & Treated	Strip & Envirologix
		Site 2/Lake	Recreational	Raw & Treated	Strip & Envirologix
		Site 3/Reservoir	Drinking/recreational	Raw & Treated	Strip & Envirologix
		Site 4/Lake	Drinking	Raw & Treated	Strip & Envirologix
		Site 5/Lake	Drinking	Raw & Treated	Strip & Envirologix
P2		Site 1/Lake	Drinking/recreational	Raw & Treated	
	End-user 3	- Sampling 1			Envirologix
	End-user 4	- Sampling 2			Abraxis
	End-user 5				Strip
	End-user 4	- Sampling 3			Strip
P3	End-user 6	Site 1/Lake	Recreational	Raw	Strip & Abraxis
		10 locations in the lake			
		6 of them were sampled three times in the summer.			
P4	End-user 7	Site 1/ Lake- (11 sampling/times)	Recreational	Raw	Strip
		Site 3/Lake	Recreational	Raw	Strip
		Site 4 /lake	Drinking	Raw & Treated	Strip
	End-user 8	Site 1/lake	Recreational	Raw	Strip
		Site 2/ Lake- (2 times)	Recreational	Raw	Strip
		Site 2 /lake	Recreational	Raw	Strip
		Site 3/lake (8 times)	Recreational	Raw	Strip
	End-user 9	Site 1/river (3 times)	Drinking/recreational	Raw	Strip
		Site 2/reservoir (7 times)	Drinking	Raw	Strip
		Site 3 Lake (2 times)	Recreational	Raw	Strip

* Results from 2009

P2 received all the field test kits

P4 received strip and Zeu

Analysis of microcystins

Analysis of free microcystins was performed using the Envirologix Plate ELISA kit. The kit provides the sum of all microcystins that cross react with the antibody. The detection limit of the Envirologix plate is 0.147 ppb (provided by manufacturer). Water samples were analyzed without further concentration because it was expected that in most cases, the majority of microcystins would have been present inside the cyanobacteria cells (cell bound).

Figure 3 shows all microcystins included in the LC-MS/MS method: MC-LR, MC-RR, MC-YR, MC-LA, MC-LW and MC-LF. It is important to remember that although more than 90 microcystin variants have been identified, including a number of MC-LR homologues, only six of them are commercially available and are included in our LC-MS/MS method. From previous studies, where all the extracts were analyzed by LC-MS/MS and ELISA, ELISA results were generally higher as some samples contained additional microcystins that were not accounted for in the LC-MS/MS method. In this study, the majority of the cyanobacteria cell extracts were only analyzed by LC-MS/MS. Overall, MC-LR was detected in 95% of the cyanobacteria cells and accounted for 10% to 100 % of total microcystin. In addition to MC-LR some samples contained MC-RR and/or MC-LA. In few samples, the predominant variant was MC-LA, accounting for almost 100% of total microcystin in the cyanobacterial cell.

Microcystins were not detected in 16% of the samples analyzed (neither in cyanobacteria cells nor water). In samples with very low levels of microcystins, they were detected in the cyanobacteria cells but not the sample filtrate (as free microcystin). Approximately 68% of the samples contained total microcystins at a concentration below 1 ppb.

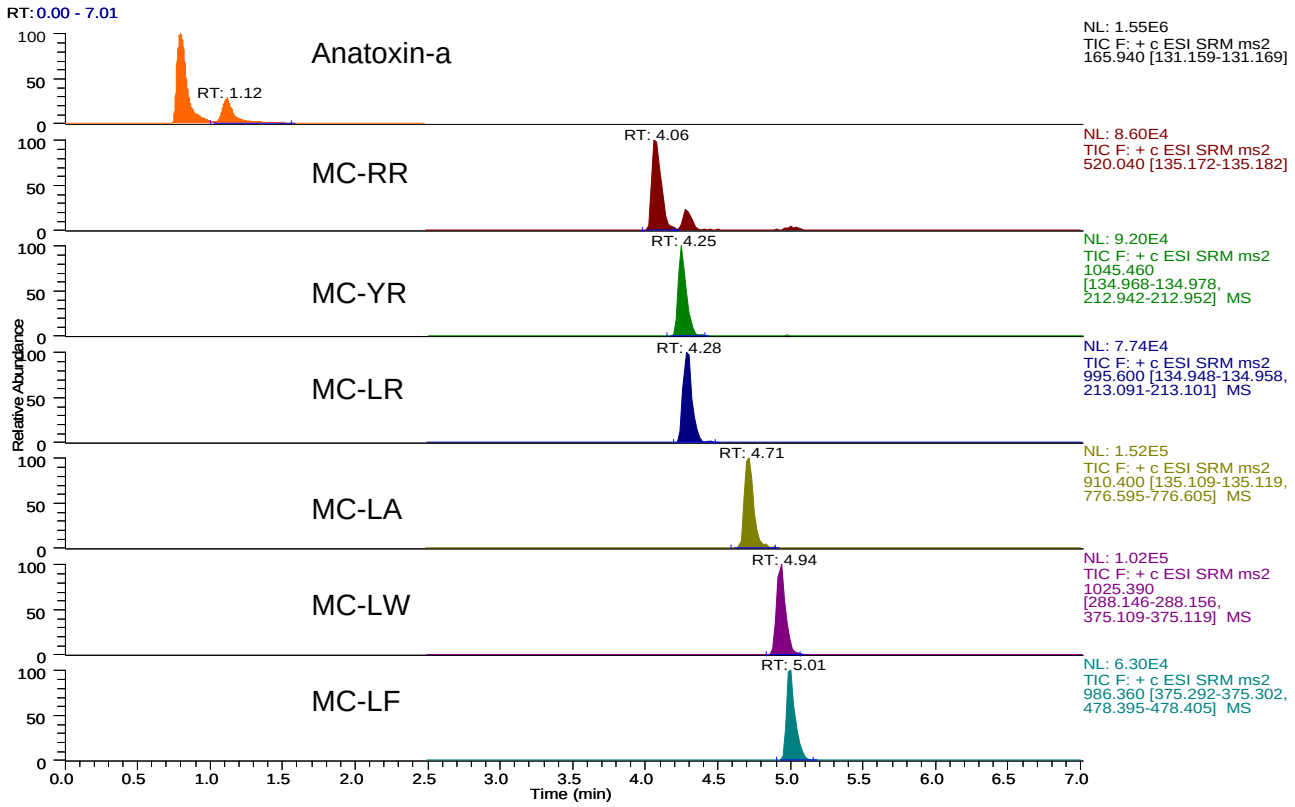

Figure 3. MRM chromatograms of the cyanotoxins (anatoxin-a and microcystins) analyzed in this study (100 ppb).

Field test kits

In this study, three Canadian provinces and 2 First Nations regions participated in the study. As part of the letter of agreement, on-site training on the use of all the kits was provided to First Nations personnel only; attendees included drinking water treatment plant operators, Circuit riders, Environmental Health Officers and managers. Because of travel and budget restrictions, training could not be provided to the other participants.

Table 3 shows the description of which kits were used by the end-users.

The results of the field test kits will be discussed below and are compared with the total amount of microcystins present in the sample sent to EBD corresponding to the bound (amount of microcystins found in the extracts (LC-MS/MS)) plus free (the amount of microcystins found in water (ELISA)). The field test results obtained by the EBD staff (2 chemists) are also included in the overall results.

Strip test kit

The Abraxis strip test kit is described by the manufacturer as "a rapid immunochromatographic test, designed solely for the use in the qualitative screening of Microcystins and nodularis in source water". The strip kit does not include microcystin standards, only a picture for comparison depicting the estimated concentration present based on the colour of the test line. A control line on the strip test also develops and indicates the kit is working. According to the manufacturer, a noticeable change in the test line will begin at 0.5 ppb and it will fade completely at 5 ppb. If the colour on the test line is similar to the control line, microcystins are either not present or at a level not detected by the kit (i.e., <0.5 ppb). The test line colour will fade with increasing amounts of microcystin present in the sample (no colour on the test line at >5ppb).

All the participants received the strip test kits. A total of 110 samples were tested by 10 end-users (EBD staff included). Eighty percent of the samples contained microcystin levels below 5 ppb (range of the kit) and 20 % contained high levels of microcystins which were beyond the working range of the kit (6-2000 ppb). Three respondents did not provide an interpretation of the kit, only the description of the test line results/colour.

Figure 4 shows the overall results of the strip test kits and the corresponding result obtained by in the EBD laboratory and Figure 5 shows the results with the X-axis corresponding to the working range of the kit (0-5 ppb). The results were grouped according to the results reported by the users: as 0 or less than 0.5 ppb (n=54); between 0.5-1 and ~1 ppb (n=16); between 1 to 2.5 ppb (n=17), between 2.5 and 5 ppb (n=7) and finally higher than 5 ppb (n=13).

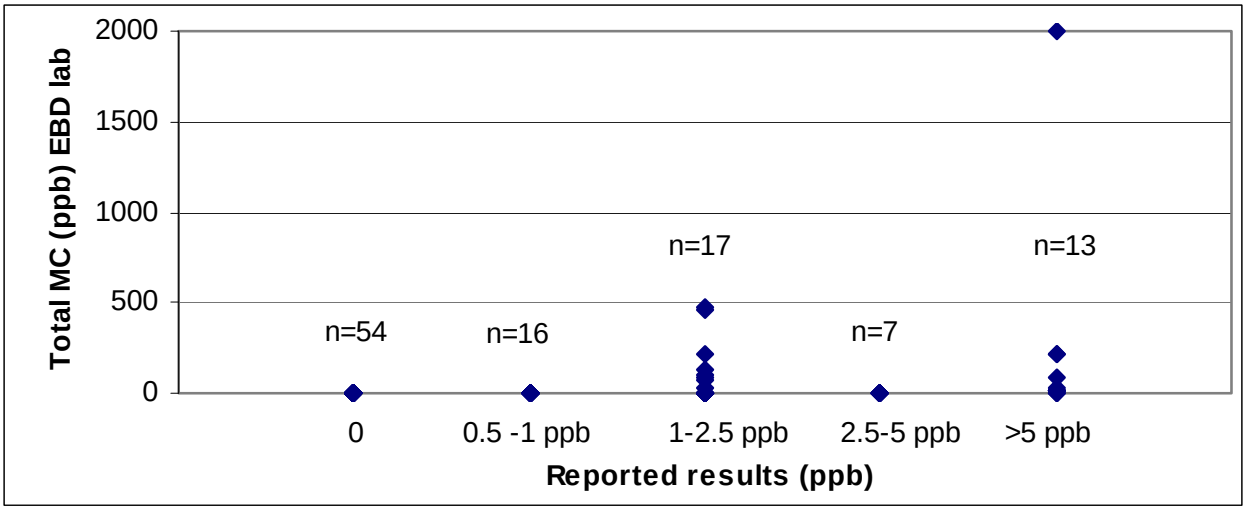

Figure 4. Overall results of the strip test kit compared to LC-MS/MS.

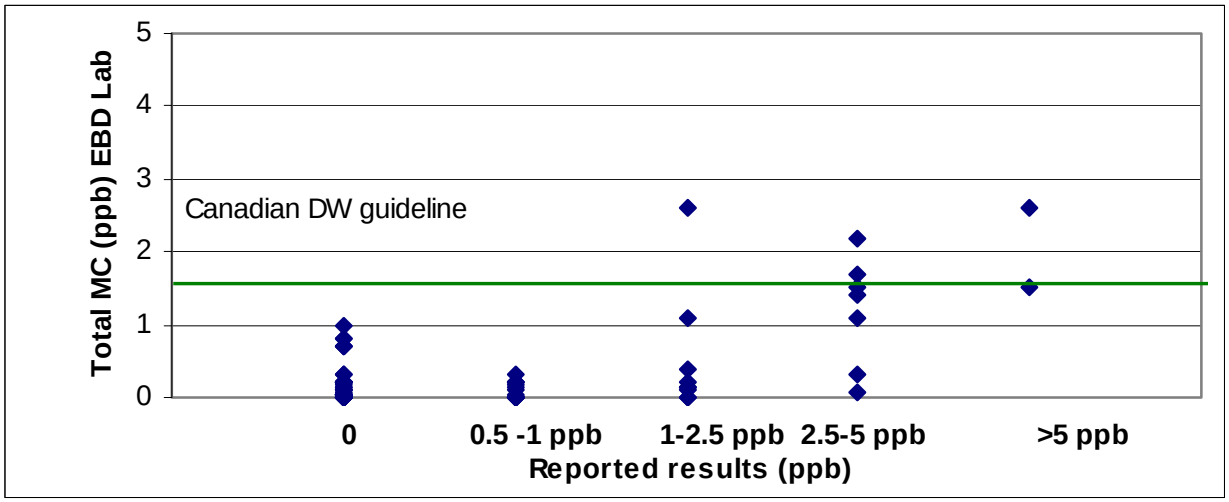

Figure 5. Results of the strip test kit compared to the LC-MS/MS. The Y axis only shows the range of the strip test kit (0-5 ppb)

Table 4 summarizes the agreement between the strip test and the LC-MS/MS results. The false negative rate is low as 11 % of the samples contained microcystins between 0.5 and 0.8 ppb. The false positive rate was high in all the ranges of the kit; results in the laboratory showed that microcystins were not detected in 94% of samples where the end-users reported levels between 0.5-1, 41% were below 1 ppb when end users reported between 1-2.5 ppb, and MCs were not detected in 29% of the samples when end-users reported between 2.5-5 ppb. In all cases, the reported results would have triggered further testing. For samples containing more than 5 ppb total microcystins, 84% of kit results were in agreement with laboratory results.

Table 4. Agreement between the strip test kits reported result and the range of concentration

Strip test results (ppb)	Results obtained in the laboratory						
			0	0.5-1	1-2.5	2.5-5	>5
	0.00		89%	11%			
	0.5-1		94%	6%			
	1-2.5		41%		12%	47%	
	2.5-5		29%		71%		
	>5				8%	8%	84%

Each user was asked to fill out a questionnaire regarding the use of the kits. Seven out of 9 users responded to the questionnaire. Those who used the kit more than once only responded once. The responses are summarized in Table 5.

Table 5. Users' responses to the use of the strip test kit

	Very easy/fast	Somewhat easy/fast	Difficult/ slow	Very difficult/ slow
The instructions were___ to follow	6		1	
The kit was___ to use	4	2	1	
The results were ___ to interpret	1	2	4	
The time to get the result was__	5	2		

These results suggest that

1) The percentage of false negative is very low 11% (reported 0 when there is more than 0). However, microcystin levels in the samples were below 0.8 ppb.
2) Interpretation of the results is challenging in the lower level of the kit (0-1 ppb); all the samples reported to have microcystin levels ~1ppb, had microcystin less than 0.5 ppb. However, at these levels, the concentration of microcystins is not of concern and the course of action would still have been to continue monitoring the water intake.
3) At levels between 1-2.5 ppb (when action is required), the interpretation is also challenging. However, according to the results reported by the users, the course of action would be to send the samples to the laboratory for confirmation and to test the treated water, which still protects the public.
4) At levels above 2.5 ppb, interpretation varies, however a "presence" interpretation can be given with certain confidence resulting in further monitoring.
5) The result of the kits is very obvious when the amount of microcystins is above the 5 ppb upper limit of the kit.
6) The strip kits are useful when microcystins are in the range where decisions should be made.
7) As specified by the manufacturer, the test provides preliminary qualitative results, and another quantitative analytical method should be utilized to confirm the results.

Envirologix

The Envirologix QualiTube™ kit is an enzyme immuno-sorbent assay without a lysing agent designed for semiquantitative field screening. Similar to the Envirologix ELISA quantiplate, the results represent all the microcystins that can cross react with the antibody used. Aliquots from the filtrate obtained using the QuikLyse™ reagent found in the Abraxis box (Figure 2) were used to perform the test as per manufacturer's instructions. In this kit, two standards are included (0.5 and 3 ppb) and the interpretation is done by comparing the colour of the samples to the two standards either visually or with the aid of a spectrophotometer. Three interpretations are possible: less than 0.5 ppb MC, between 0.5 and 3.0 ppb, and more than 3 ppb microcystin. According to the manufacturer, the limit of detection of the kit is 0.3 ppb, although it was calculated with the aid of a spectrometer. Therefore, it is expected that this limit may not be visually achievable in the field.

During this study, the Envirologix QualiTube was used to test a total of 50 samples by 4 end-users (including EBD staff). In all cases, a photometer was not used and all the results were interpreteted by visual comparison of the samples with the standards.

Figure 6 shows the results as reported by the participants and the results obtained in the EBD laboratory. Ranges are divided according to the possible interpretation and the standards included in the kit described above.

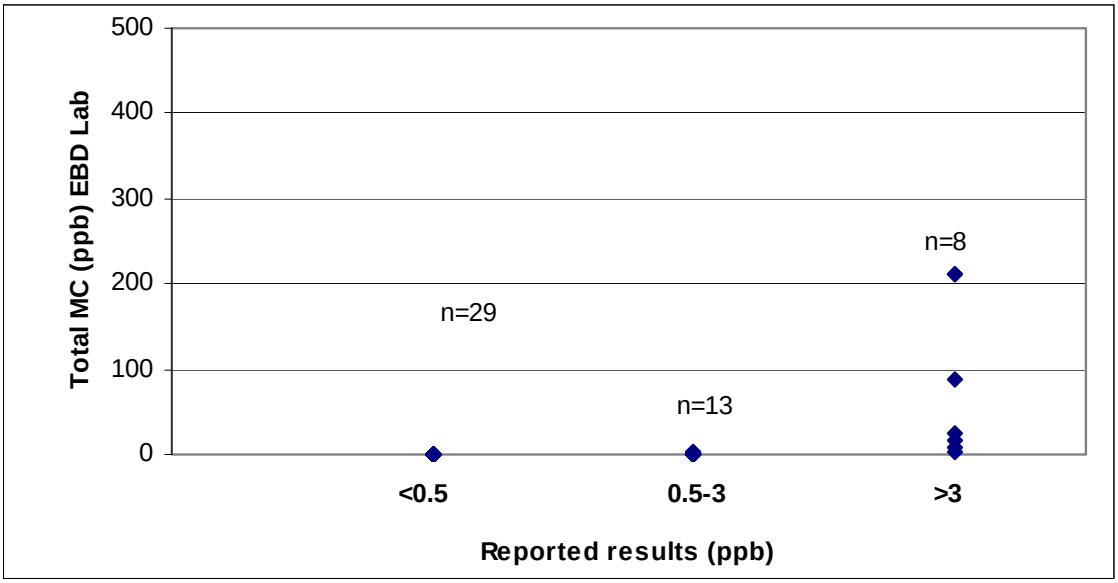

Figure 6. Overall results of the Envirologix kit compared to LC-MS/MS.

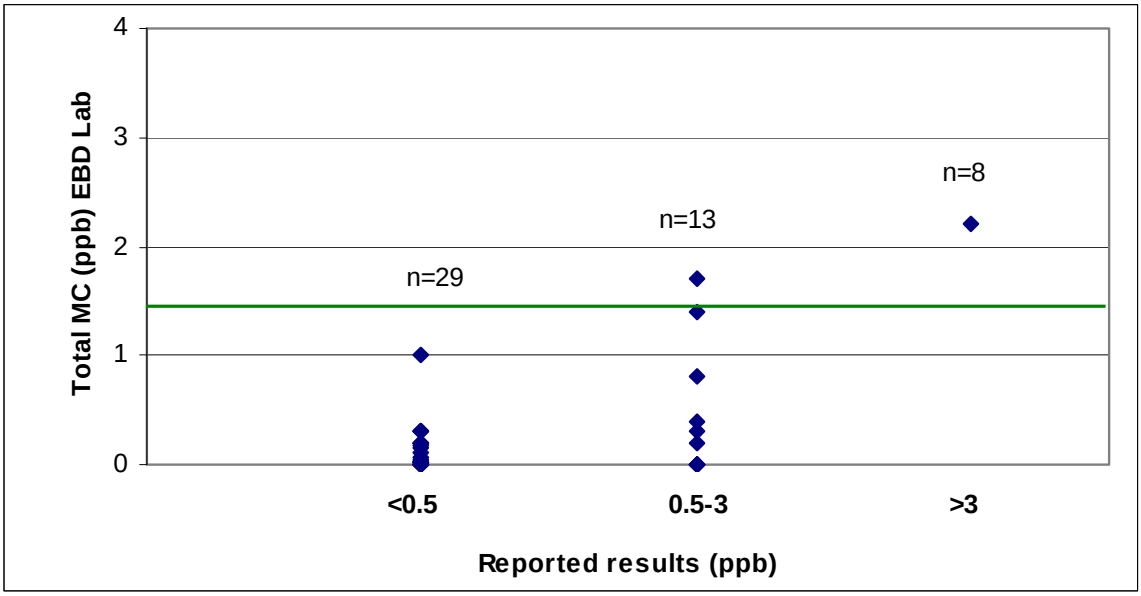

Figure 7. Results of the Envirologix kit compared to the results obtained in the laboratory. The Y axis only shows the range of kit (0-3 ppb)

Only 3 % of the samples were reported as less than or equal to 0.5 ppb when the laboratory results showed total microcystin of 1 ppb which suggests a very low false negative rate. Whereas end-users reported concentrations between 0.5-3 ppb in 69% of the samples when laboratory results indicated these samples actually contained less than 0.5 pbb or no microcystin (false positive rate). End-users accurately reported levels in 31% of the samples. All the samples containing microcystins above the range of the kit were reported as >3 ppb.

Below are the replies to the questionnaire from 2 of 3 users who used the Envirologix kit.

Table 6. Users' responses to the use of the Envirologix kit

	Very easy/fast	Somewhat easy/fast	Difficult slow	Very difficult/slow
The instruction were___ to follow		2		
The kit was___ to use		2		
The results were ___ to interpret		1	1	
The time to get the result was__		2		

The limited results suggest (visual comparison only)

1) The use of QuikLyse™ reagents does not appear to interfere with the Envirologix test kits.
2) The false negative rate is very low. Samples that contain microcystin levels less than 0.5 ppb were verified by LC-MS/MS to contain less than 0.5 ppb.
3) In the range between 0.5 and 3 ppb, the visual comparison between the sample and the standards is not clear, resulting in a false positive rate of 69%. However, the interpretation is still protecting the public as, given this result, additional samples would have been sent to an accredited laboratory for confirmation and treated water would have been tested.
4) At concentrations above the upper limit of the kit (3 ppb) the interpretation is accurate.
5) Comparison of the results of the samples with the standards contained in the Envirologix kit allows better interpretation of the result, but this is limited if solely using visual comparison.

Abraxis tube

Similar to Envirologix ELISA kits, the Abraxis tube kit uses polyclonal antibodies that bind to all microcystins that cross react with the antibody. This kit contains a wider range of standards: 0.15, 0.4, 1.0, 2.0, and 5 ppb. Interpretation was done with the aid of a spectrophotometer or tube reader at 450 nm as described by the manufacturer. Once the optical density (OD) is obtained, the results are calculated by plotting %Bo against the standards (semi-exponential plot).

Two participants used this kit. One used it on three on occasions for a total of 22 samples tested. The other participant used it only once and according to the reported results, the results were not considered (the OD reported for the standards and samples were similar).

Following the procedure described in Figure 2, an aliquot was taken after using the QuikLyse™ reagent found in the Abraxis box and the Abraxis tube test was performed according to the manufacturer's instructions. In most of the cases, the OD values were reported in the field and calculations were done using Excel in the EBD laboratory.

Depicted in Figure 8 are the results reported using the Abraxis tube and the results obtained in the laboratory. By LC-MS/MS all samples had concentrations of microcystin

below 1ppb. The Abraxis tube agreed with the laboratory results for all but three samples. One sample was reported to contain 3.2 ppb of microcystin using the Abraxis tube kit, but laboratory results showed that it was below 1 ppb. For the second sample the field test kit showed total microcystin as 0.3 ppb as opposed to the laboratory result of 0.7 ppb and the third sample was reported to have 0.9 ppb with the field test kit while the laboratory result showed 0.1 ppb.

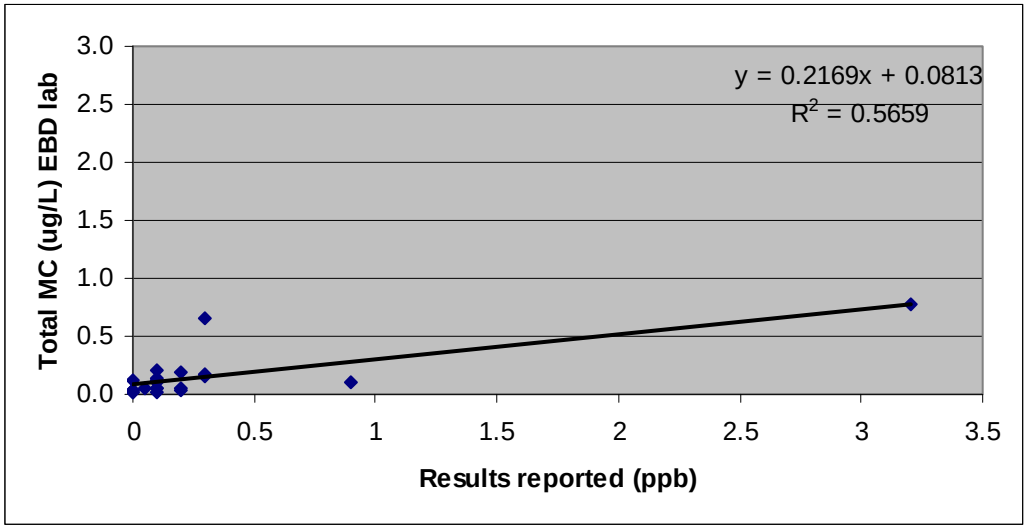

Figure 8. Comparison of the results obtained by LC-MS/MS and the results reported using the Abraxis Tube kit.

Table 7. Users' responses related to the use of the Abraxis kit

	Very easy/fast	Somewhat easy/fast	Difficult/ slow	Very difficult/slow
The instruction were___ to follow		2		
The kit was___ to use		2		
The results were ___ to interpret		1	1	
The time to get the result was__	fast	Fast 2	slow	Very slow

Zeu-Inmunotec S.L.

The MicroCystest tube kit is described as a "test for the detection of microcystins and nodularins in water. A simple and rapid method that allows end-users to determine whether the toxin concentration is over the maximum allowed levels (1 µg/L)".

The test is based on phosphatase activity inhibition. Under normal conditions the phosphatase is able to hydrolyze a specific substrate that can be detected at 505 nm. Samples containing microcystins will inhibit the enzyme activity proportionally to the amount of toxin contained in the sample. The kit contains 3 standards at 0.5, 1 and 2.5 µg/L and the protocol is simple although it requires an incubation period at 37°C as well as the use of a spectrometer with a 405 nm filter. The absorbance of standards and samples are recorded in duplicate and absorbance of the sample can be compared with the standards. The manufacturer also provides an Excel worksheet to aid in the calculation.

The kit was sent to one participant. However, during the course of the study it was found that the QuikLyse™ lysing reagent interfered with the assay. The interferences were not resolved on time and additional testing of the kit was not possible. Further testing is required to compare the results of this kit with laboratory methods.

Conclusions

In general, commercially available field test kits appear to be a simple and inexpensive way to screen water samples, both raw and treated, for the presence of microcystin toxins. However, it is important that the end-user understands the scope of each kit; free toxin vs. bound, qualitative vs. semiquantitative, concentration range of the various kits, concentration at which a response is observable and more importantly the interpretation of the kit.

All of the kits are able to detect free microcystins; total microcystins can be detected by adding a lysing agent (QuikLyse™), although the effect of the reagent with kits produced by other manufacturers must be evaluated. Semi-quantitative results can only be obtained when MC-LR standards are used and samples compared to those standards.

The concentration ranges for the majority of the kits are relevant for use in surface drinking water (0-5 or 0-3 ppb). For recreational uses the Abraxis strip test kit can be used (0-10 ppb) however, samples above the range of the kit must be diluted as the guideline is 20 µg/L.

Interpretation of results will be fundamental during decision making processes in drinking water monitoring policies. Overall the kits can provide presence/absence response with a certain confidence. In cases where false positives are observed, the interpretation still protects the public as the results trigger additional testing/monitoring in an accredited laboratory.

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