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Evaluation of field test kits to detect microcystins

Rocio Aranda-Rodriguez
Exposure and Biomonitoring Division

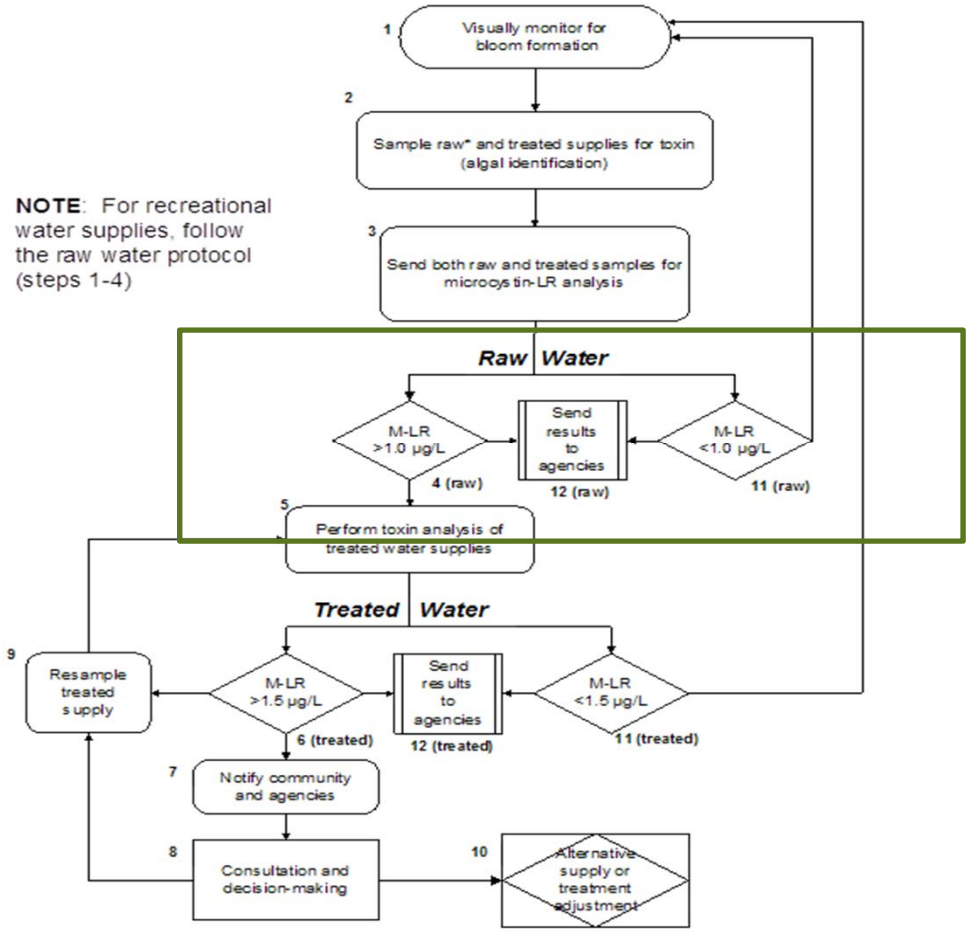
Canadian guidelines

- Drinking water quality guideline is 1.5 µg/L (ppb) total MCYST-LR
- Proposed guideline for recreational water is 20 µg/L or 100,000 cells/mL

- **DWG Monitoring Policy**
- Provincial/municipal decision
  - visual monitoring in known bloom areas
  - sampling near bloom, raw water and treated water
- Field-test kit
  - Laboratory analysis for confirmation by an accredited laboratory for monitoring/regulatory purpose

Cyanobacterial Toxins -- Microcystin-LR
Flow Chart
- Water Supplies for Human Consumption -

NOTE: For recreational water supplies, follow the raw water protocol (steps 1-4)



April, 2002

* A field kit could be used for screening. A validation sample should be send to a laboratory for confirmation of actual levels following a positive field test.

Objectives of the study

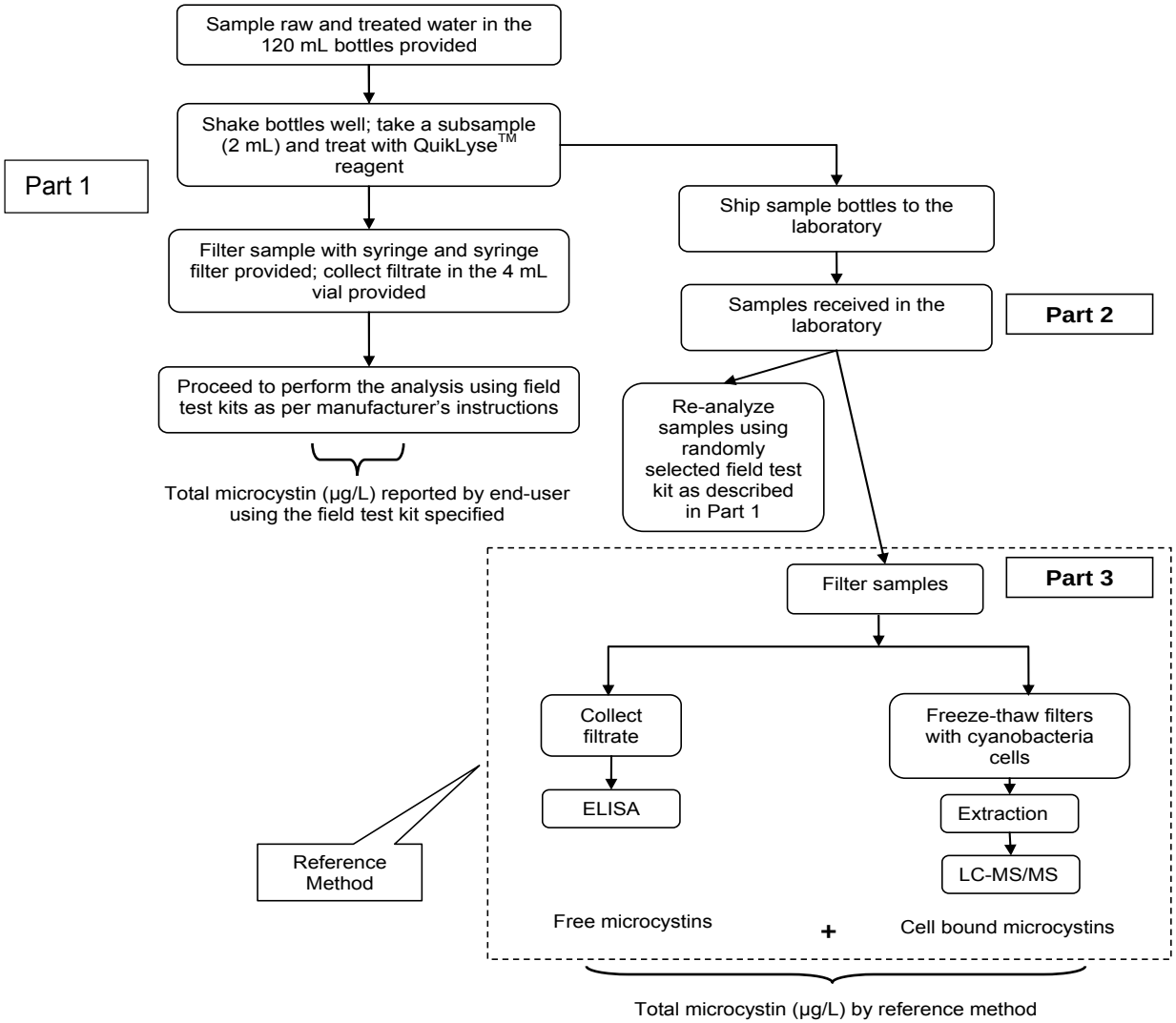
To evaluate commercially available field test kits for on-site analysis of microcystins by the end-user, based on the threshold value of 1 µg/L as well as product specifications

Methodology

1) Participant recruitment
2) Sampling kits and protocols were provided to the participants in advance. Each sampling kit (cooler) included: two field test kits, sampling bottles, questionnaires related to sample collection and a field test kit data report
3) Results reported by participants were compared to results using the reference method

Field test kits

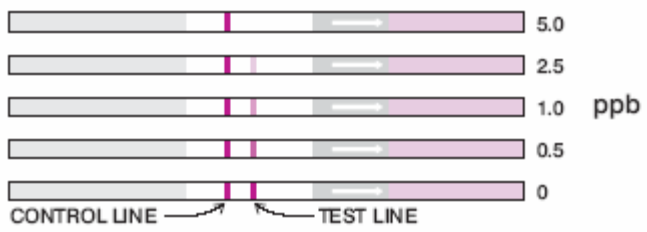
Manufacturer	Format	Principle	Standard Concentrations (µg/L)	Interpretation	Time of analysis	# of users
Abraxis LLC	Strip Test	Immuno-chromatography	None (graph for 0.5 to 5)	Visual interpretation	~40 min	10
Abraxis LLC	Tube	ELISA	0.15,0.4,1.0, 2.0, 5.0	Visual or photometer	~ 50 min	1
Envirologix	Tube	ELISA	0.5 and 3	Visual or photometer	~ 50 min	2
Zeu-Inmunotec S.L.	Tube	Phosphatase inhibition	0.25, 0.5, 1.0 and 2.5	Photometer	~ 95 min	1


Sample raw and treated water in the 120 mL bottles provided
Shake bottles well; take a subsample (2 mL) and treat with QuikLyse™ reagent
Part 1
Filter sample with syringe and syringe filter provided; collect filtrate in the 4 mL vial provided
Proceed to perform the analysis using field test kits as per manufacturer's instructions
Total microcystin (µg/L) reported by end-user using the field test kit specified
Ship sample bottles to the laboratory
Samples received in the laboratory
Part 2
Re-analyze samples using randomly selected field test kit as described in Part 1
Filter samples
Part 3
Collect filtrate
ELISA
Freeze-thaw filters with cyanobacteria cells
Extraction
LC-MS/MS
Reference Method
Free microcystins
+
Cell bound microcystins
Total microcystin (µg/L) by reference method

Abraxis Strip test
Prod. No. 520020

- "The test will detect microcystins and nodularins in Source water at **1 µg/L** or higher".

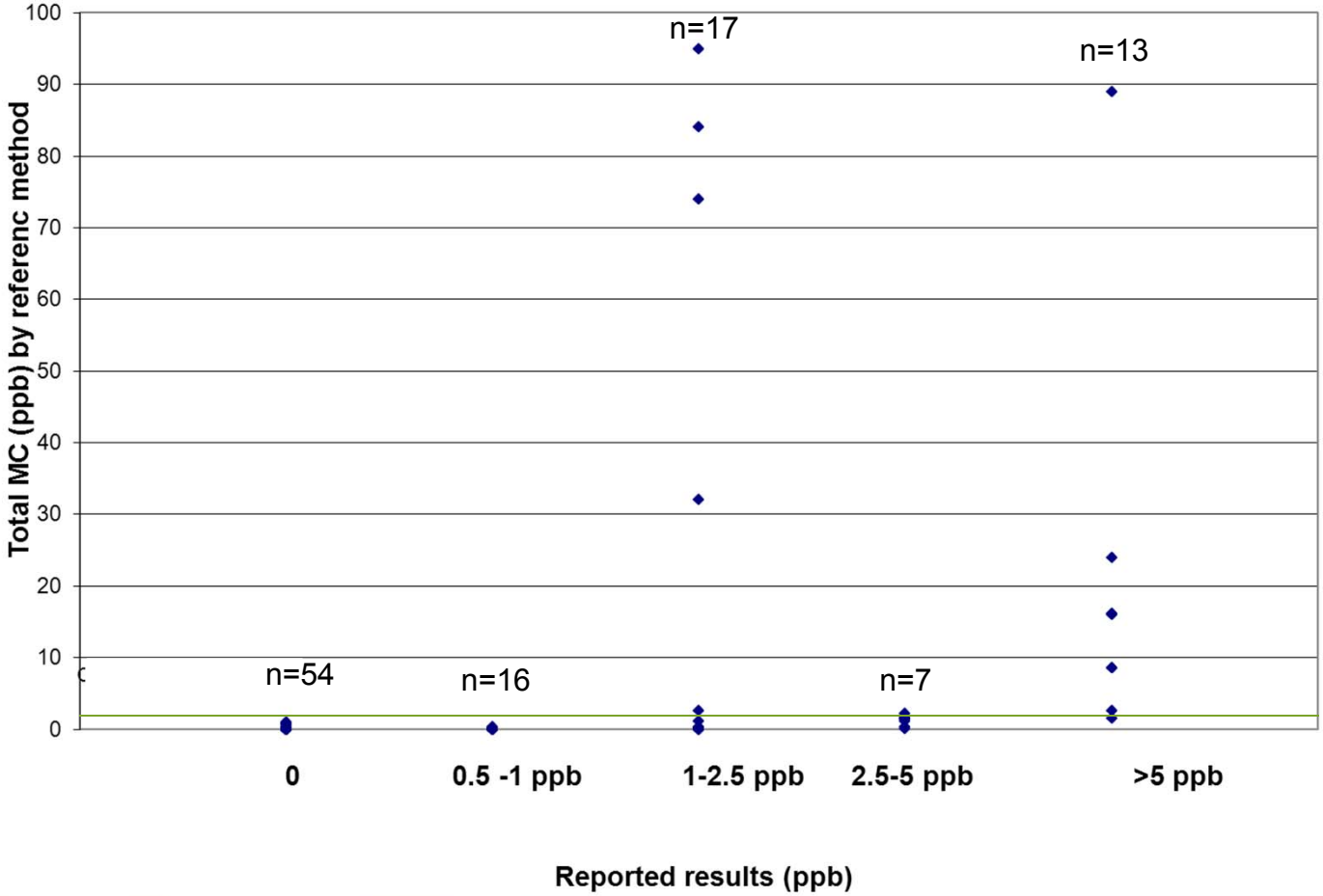
Interpretation of the Results



Test interpretation

Control Line	Test Line	Interpretation
No control line present	No test line present	Invalid result
Control line present	No test line present	>5 µg/L
Control line present	Moderate intensity	Between 0 and 5 µg/L

Abraxis strip test (surface water)



Performance evaluation based on threshold value of 1µg/L

		Total MC determined by reference method			
		≥1 µg/L	<1 µg/L	Total	
Total MC by Abraxis strip test	Positive (≥1 µg/L)	A True positive (28)	B False positive (17)	45	P.P.R. 0.62
	Negative (<1 µg/L)	C False negative (1)	D True negative (61)	62	N.P.R 0.98
	Total	29	78		
		Sensitivity 0.97	Specificity 0.78		

Abraxis Strip test continuation

The strip test is suitable for rapid screening at 1 µg/L

1. PPR 0.62 (false positive 38%) suggest challenges in the interpretation.

2. Good NPR 0.98, sensitivity 0.97and specificity 0.78.

3. All participants reported the results based on the illustration contained in the manufacturer's instruction.

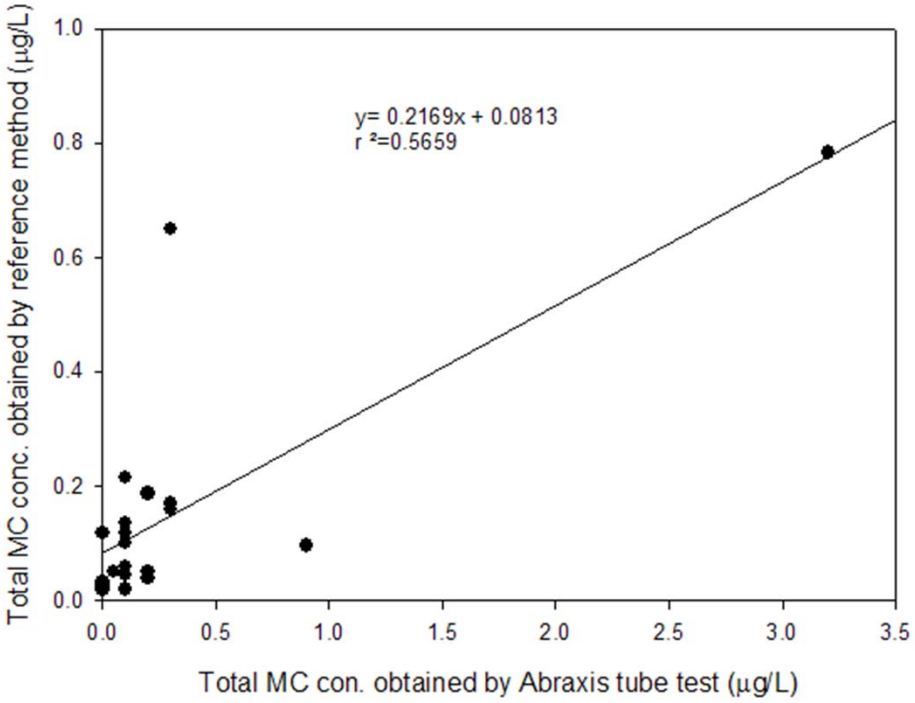
4. Several challenges with the interpretation of the kit were observed.

Abraxis tube
Prod. # 520012

- Standards contained in the kit: 0.15, 0.4, 1.0, 2.0 and 5 µg/L
- Negative and positive controls added
- Interpretation can be done visually or with a photometer
- Time to complete analysis ~50 min

- Current product (Prod. # 520036) contains the following standards: 0, 0.5 and 3 ppb

Results from Abraxis tube



Abraxis tube

- The kit could not be evaluated at the threshold value of 1 µg/L or over a wider range.
- Giving the number of standards and the use of spectrophotometer this kits could provide more quantitative results within the range of concern.
- During recruiting, participants hesitated to use the kit.

Note: the new product contains fewer standards than the kits used during the study

Other Abraxis Products

- Strip test:
  - 1ppb Source drinking water (with QuikLyse option)
  - 1 ppb Finished drinking water (up to 5 ppb)
  - 10 ppb Recreational water (with QuikLyse option)
- Tube:
  - Microcystin, field screen ELISA kit

- Other formant 96 ELISA plates and PP2A kit
- ELISA other toxins: Cylindrospermopsin, saxitoxin and BMAA

Abrascan dipstick reader

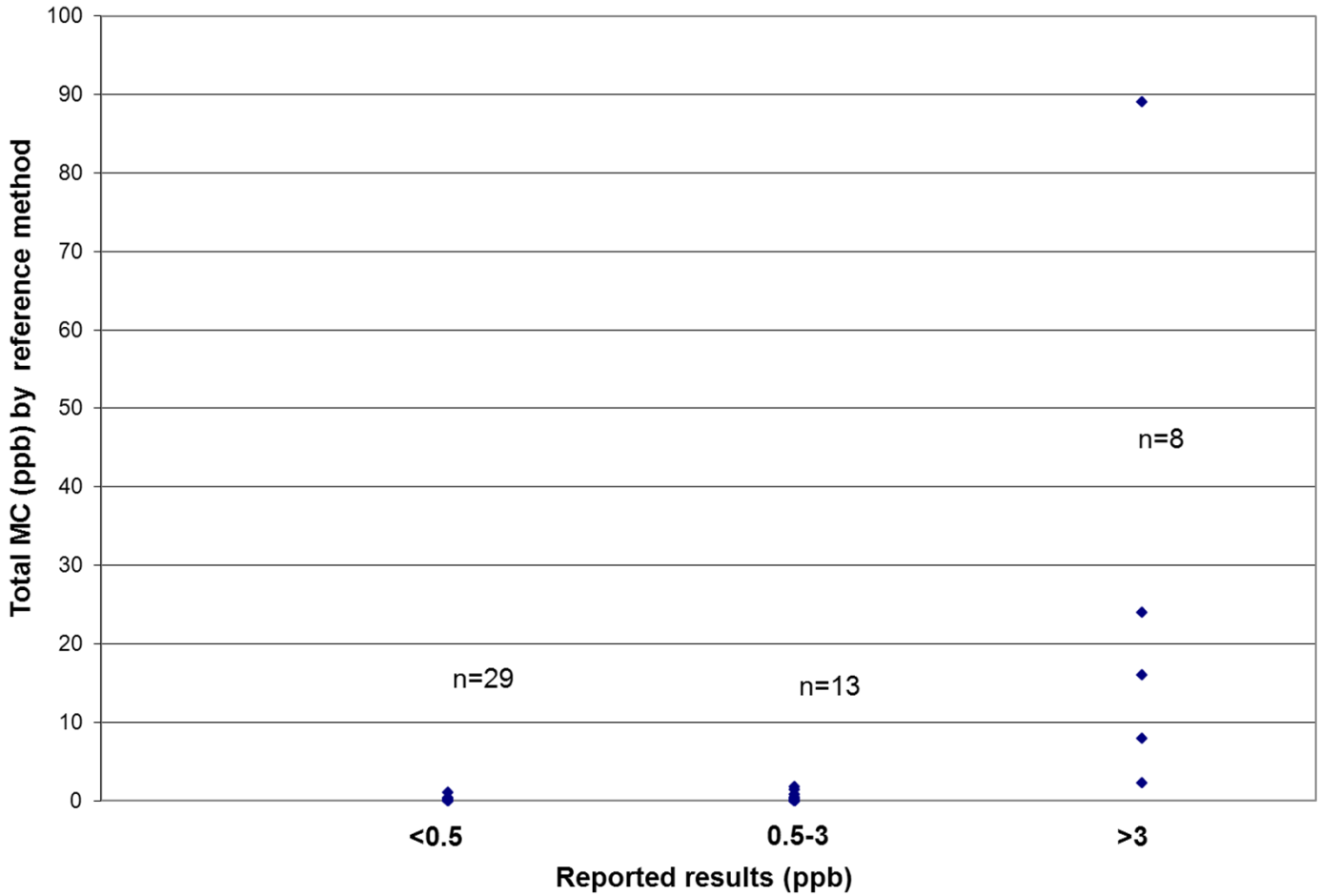
www.abraxiskits.com

Envirologix Qualitube - ET 022
Product specification

- Range: 0.5-3 µg/L (standards included: 0.5 and 3 µg/L)
- Limit of detection 0.3 µg/L (using spectrophotometer)
- http://www.envirologix.com/library/M38.pdf

- No lysing agent available with the kit, therefore the test provides results for free, not total, microcystins.

Envirologix Qualitube



Evaluation of the Qualitube

		Total MC determined by reference method				
		≥3 µg/L	0.5-3 µg/L	<0.5 µg/L	Total	
Total MC determined by Envirologix tube test	≥3 µg/L	**A** **True positive** **(7)**	B False positive (1)	C False positive (0)	8	PPR 0.88
	0.5-3 µg/L	D False negative (0)	E **True positive** **(4)**	F False positive (9)	13	PPR 0.31
	<0.5 µg/L	G False negative (0)	H False negative (1)	I **True negative** **(28)**	29	NPR 0.97
	Total	7	6	37		
		Sensitivity 1	Sensitivity 0.67	Specificity 0.76		

Envirologix QualiTube

- Challenges in the interpretation of the test in the range of 0.5-3 were observed.

a) PPR was very low 0.31 (False positive rate 69%), suggesting visual comparison between standards and sample was not clear

b) Sensitivity of 0.67 confirmed challenges with the interpretation

- Impact is more economical due to unnecessary analysis
- This kit is not suitable at the threshold value of 1 µg/L unless this standard is added to the kit

Other products from Envirologix

QuantiTube Kit for Microcystins - ET 039 (special order)

- Limit of detection 0.18 µg/L
- Calibration range 0.4-2.5 µg/L which includes 1 µg/L MC-LR calibrator

- No lysing agent

Zeu-Inmunotec

- Spanish company
- Product is based on PPA 1 and 2A
- Microcystest Tubo (drinking water) and Microcytest kit (blooms)
- Detection limit: 0.3 µg/L, Calibration range: 0.5-2.5 µg/L
- Time to complete analysis: 30 min
- Portable spectrometer, detection at 405 nm
- Protocol to lyse the cells
- Lysing agent from Abraxis interfered with the test, therefore the kit could not be evaluated with our protocol

Conclusions

- It is important to understand the principle of the kit to understand the data produced: Immunological-antibody vs. PPAs.
- It is important to understand the scope of each kit (free vs bound), semi-quantitative vs qualitative, concentration range of the kit, concentration at which a response is observable.
- More importantly, interpretation of the kit by the end-user.
- What is the impact of false positives vs. false negatives.
- It is recommended to have a training program in place as part of the monitoring policies.
- Overall the kits are providing a presence/absence response with a certain confidence within the performance specification of each kit.
- In cases where false positives are observed, the interpretation still protects the public as samples would be sent to an accredited laboratory for confirmation. However, it will may have an economical impact.

Acknowledgment

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- All the participants
- Zhiyun Jin

More information

- Environmental technology verification report available for
  - Zeu Inmunotec: http://www.epa.gov/nrmrl/pubs/600r11064/600r11064vr.pdf
  - Abraxis: http://www.epa.gov/etv/pubs/600r10158/600r10158.pdf

- Publications related to field test kits
  - Lawton et al. Rapid detection of microcystin in cell and water. Toxicon, volume 55, issue 5, May 2010. Pages 973-978
  - Humpage et al. Evaluation of the Abraxis strip test for microcystins for use with wastewater effluent and reservoir water. Water research 46, 2012 Pages 1556-1565
  - EBD manuscript, under review