Ohio EPA Consensus Microcystin Abraxis ELISA-ADDA Analytical Methodology (August 13, 2014)

INTRODUCTION

The following is a consensus analytical procedure for microcystin in raw and finished drinking water samples using the Abraxis microcystin ELISA immunoassay method. Ohio EPA has developed this consensus analytical procedure in conjunction with U.S. EPA, public water system laboratories, Ohio State University-Stone Laboratory, Abraxis and other national experts. Ohio EPA will continue to work with these groups to evaluate and revise the method as additional information becomes available.

1. SCOPE AND APPLICATION:

This method is used for the determination of microcystins in surface water, ground water and finished drinking water.

Working Range: 0.3 to 5.0 µg/L
Reporting Limit: 0.3 µg/L

2. SUMMARY OF METHOD:

The Abraxis microcystin ELISA is an immunoassay for the detection of microcystins in water samples. This test is an indirect competitive ELISA that allows the congener-independent detection of microcystins and nodularins. It is based on the recognition of microcystins, nodularins and their congeners by specific antibodies. Microcystins, nodularins and their congeners when present in a sample and a microcystin-protein analogue immobilized on the plate compete for the binding sites of antibodies in solution. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of the microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using a microplate reader at 450nm.

3. SAMPLE HANDLING AND PRESERVATION:

3.1 **Finished drinking water:** A 100 mL sample should be collected in a glass container. Sample must be quenched with 10 mg sodium thiosulfate added to 100 ml of sample during collection.

3.2 **Raw water, surface water and ground water:** A 100 mL sample should be collected in a glass container with no preservative.

3.3 All samples must be protected from sunlight and cooled on ice at 0-4°C immediately after collection and maintained at 0-4º C until analysis. Sample must be analyzed within 36 hours from the time of collection.

NOTE: For raw water, surface water and ground water samples, the holding time can be increased by freezing the raw water sample after collection. When freezing, allow adequate volume for expansion and place the sample container on its side to prevent breakage.

4. INTERFERENCES:

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds

that might be found in water samples, test interferences caused by matrix effects cannot be completely ruled out.

5. SAFETY:

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

6. APPARATUS:

6.1 Class 'A' Volumetric Flask: 500 mL

6.2 Log books

6.3 Micropipette with disposable plastic tips: 10 to 100 µL

6.4 Multi-channel pipette: 50-300 µL

6.5 Stepping pipette: 100-500 µL, using 5.0 mL syringe tips

6.6 Pipette tips: 1-200 µL

6.7 Disposable Reagent Reservoir: 55 mL capacity

6.8 Microplate reader capable of analyzing at 450nm

6.9 Beakers: 200 mL and 500 mL

6.10 Amber glass vials with Teflon coated lids: 5 mL

6.11 Amber glass vials with Teflon coated lids: 40 mL

6.12 Syringe filters: 0.45 µm pore size (Teflon)

6.13 Glass Gastight Luer-lock syringes: 5 mL

6.14 ELISA sealing Teflon tape

6.15 Freezer

6.16 Refrigerator capable of 4.0 $\pm$ 2ºC

6.17 Chlorine meter

7. REAGENTS:

7.1 Liquid disinfectant

7.2 Abraxis Kit: Store sealed in refrigerator at 2-8°C, adjust to room temperature (20-25°C) prior to use. Reagents may be used until the expiration date on the box.

7.2.1 Microtiter Plate: coated with an analog of microcystins conjugated to a protein (any unused strips should be resealed in zip-lock bag and placed back in refrigerator)

7.2.2 Stock Wash Solution: Dilute 100 mL of wash solution with 400 mL of reagent water

7.2.3 Standards: 1 (0.0 ppb), 2 (0.15 ppb), 3 (0.40 ppb), 4 (1.0 ppb), 5 (2.0 ppb) and 6 (5.0 ppb)

7.2.4 Positive Control Standard at 0.75 $\pm$ 0.185

7.2.5 Antibody Solution

7.2.6 Anti-sheep-HRP Conjugate Solution

7.2.7 Color Solution: Contains tetramethylbenzidine (TMB)

7.2.8 Stop Solution: Contains diluted H_2SO_4

7.2.9 Diluent

7.2.10 Saturated sodium chloride solution (optional): May be used in the freeze/thaw process: Dissolve 300g of sodium chloride in 900ml reagent water, apply gentle heat and stir to speed up the process.

NOTE: The standard, control, antibody solution, anti-sheep-HRP conjugate, color solution and stop solutions are ready to use and do not require any further dilutions.

7.3 DPD free chlorine reagent

7.4 Dechlorination agent – sodium thiosulfate.

7.5 Reagent Water: Laboratory available water meeting Standard Methods criteria for bacteria analysis.

8. PROCEDURE:

Extreme caution must be taken to avoid human errors such as: Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C), direct sunlight. The assay procedure should be performed away from direct sun light.

NOTE: Neither raw, surface water and ground water nor finished drinking water samples should be subjected to sonication.

8.1 Disinfect the work bench.

8.2 **Finished drinking water samples:** Check the finished drinking water sample for residual chlorine. If residual chlorine is greater than 0.1mg/L, add 10mg of sodium thiosulfate quenching agent and qualify the results (See Section 10.7). **NOTE: Finished drinking water samples must not be frozen prior to analysis.** Go to step 8.4.

8.3 **Raw water, surface water and ground water samples:** Gently shake the sample and pour 20 mL each in two separate 40 mL vials to begin the three freeze/thaw lysing cycles.

8.3.1 Place vials in in the freezer until completely frozen (to speed up the freezing process saturated sodium chloride bath may be used).

8.3.2. Once sample is completely frozen, remove from freezer and thaw it by placing it in a 35ºC water bath until it is completely thawed.

8.3.3 Repeat steps 8.3.1 and 8.3.2 two more times.

8.3.4 Transfer 2 mL through a 0.45 µm Teflon filter to two 5 mL vials.

Samples are ready for immediate analysis.

Freeze remainder of raw water, surface water or ground water samples on their side, to be analyzed at a later date.

8.4 Bring samples and standards to room temperature prior to analysis. Check kits of prepared standards for volume and expiration date. If volumes are too low or have exceeded holding time, a fresh kit should be opened.

8.5 Turn on the power to the microplate reader, computer, and printer and follow proper startup protocol.

8.6 Load the wells with 50 µL of standards, control standard and samples (everything is analyzed in two replicates and then averaged). Add 50 µL of antibody per well and cover tray with ELISA sealing tape. Agitate on counter with swirl motion for 30 seconds and let sit for 90 minutes at room temperature.

8.7 After 90 minutes, discard the well contents down the drain and pat dry by tapping inverted tray with force onto paper towels. Wash wells three separate times with 250 µL of diluted wash solution or flood wells with diluted wash solution using a squeeze bottle. Pat dry wells by tapping inverted tray with force onto paper towels.

8.8 Add 100 µL of the enzyme conjugate solution to each well, recover tray and agitate again for 30 seconds and incubate for 30 minutes at room temperature.

8.9 Discard well contents down the drain and wash three times with 250 µL of diluted wash solution for each well and each washing step. Repeat pat procedure from step 8.8

8.10 Add 100 µL of the color solution, agitate on counter for 30 seconds then incubate at room temperature for 20 minutes (time range of 20-30 minutes).

NOTE: DO **NOT** WASH AT THE END OF STEP 8.10!

8.11 Immediately add 50 µL of the stop solution.

8.12 Read the absorbance at 450 nm using a microplate reader within 15 minutes after the addition of the stopping solution.

8.13 Discard used well strips and disinfect the work area.

8.14 Samples showing a higher concentration than standard 6 (5.0 ppb) must be diluted to bring within the calibration range and reanalyzed to obtain accurate results.

9. DATA TREATMENT:

9.1 Save and print a copy of the calibration curve and sample results as part of the laboratory's record maintenance protocol.

9.2 If sample is diluted, the final values must be calculated by multiplying by the proper dilution factor.

10. QUALITY CONTROL:

10.1 Analyze all calibration standards, positive control standards and samples in two replicates.

10.2 The two replicate results must have a relative percent difference (RPD) <40%, if replicates have a RPD >40% then reanalyze or qualify the results with the appropriate qualifier.

10.3 Positive control standard must be analyzed following the calibration. Results obtained must be 0.75 ± 0.185 µg/L.

10.4 Diluent supplied with the kit as a blank must be analyzed with each batch of samples. Values that exceed the reporting limit indicate laboratory or reagent contamination and require corrective action by the analyst before continuing analysis.

10.5 If control standard fails**, %**CV fails <u>and</u> the R^2 is less than 0.995 then the analyst is to take corrective action, recalibrate and reanalyze. However, if the %CV and positive control are within acceptable range the R^2 may be as low as 0.990.

10.6 Samples not analyzed within the required holding time should be recollected. Results of samples analyzed after the required holding time must be qualified indicating the result is the minimum estimated value detected and should be noted in the final report.

10.7 Samples received with residual chlorine greater than acceptable limit indicate ineffective dechlorination and should be recollected. Results must be qualified indicating the result is the minimum estimated value detected and should be noted in the final report.

11. REFERENCES:

11.1 Abraxis, *Microcystins-ADDA ELISA (Microtiter Plate); Enzyme-Linked Immunosorbent Assay for the Congener Independent* Determination of Microcysitins and Nodularins in Water Samples*, 2007.