Ohio EPA Quick Reference Guide for Laboratories

Performing Raw and Finished Drinking Water Analysis
Using ELISA-ADDA for Microcystin
(August 13, 2014)

Ohio EPA is providing the following quick reference tips for laboratories conducting ELISA analysis of raw and finished drinking water samples for Microcystin. Ohio EPA has developed a 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.

SCOPE AND APPLICATION: This method is used for the determination of microcystins in Raw and Finished Drinking Water.

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

SAMPLE HANDLING AND PRESERVATION:

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.

Raw water (Samples collected prior to filter effluent), surface water and ground water: A 100 mL sample should be collected in a glass container with no preservative.

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

NOTE: Finished drinking water samples must not be frozen prior to analysis.

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

PROCEDURAL TIPS:

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.

- 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.

- Samples showing a higher concentration than standard 6 (5.0 ppb) must be diluted to obtain accurate results. Make sure proper dilution factor is used when reporting final results.

Recommended quality control:

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

- 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.

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

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

- 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 normal control are within acceptable range the R^2 may be as low as 0.990.

- 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.

- 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.

REFERENCES:

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

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