

CYANOTOXIN ELISA TESTING IN DELAWARE: PROCESS DEVELOPMENT

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ABSTRACT

With increased awareness of blue-green algal blooms in recreational freshwaters and the adverse impacts these can have on both human and animal health, the State needed the capability to determine cyanotoxin presence and concentration within hours of bloom notification. This knowledge allows for 1) effective management decisions that directly impact recreational users and (2) a more thorough evaluation of potential causative factors present at the time of fish and waterfowl die-offs in our lakes. The availability of commercial ELISA test kits for Microcystins and Cylindrospermopsin, the chemical-biological expertise of scientists within the State laboratory, and the need to obtain cyanotoxin results within hours of water sample collection motivated the State laboratory to develop the capability to test for Microcystins/Nodularins. The capability to test for Cylindrospermopsin, a related but unique cyanotoxin, has also been developed and is currently under internal review.

Development of the ELISA test process included establishing (1) protocols for sample collection, transport, and storage; (2) protocols for sample preparation including lysing cells, (3) quality control acceptance criteria for Laboratory Blanks, standard curve goodness-of-fit, and control sample accuracy/precision; (4) criteria for evaluating sample result precision using duplicate absorbance measurements; (5) acceptance criteria for the value of sample BO/BO% ratio relative to the range of this ratio in the calibration standards; (6) criteria for evaluating temporal stability of manufacturer-supplied calibration standards and controls; (7) a detailed laboratory standard operating procedure including preparation of environmental sample dilutions and spiking solutions, and operation of a Bio-Tek FL600 Fluorescence/Absorbance Plate Reader in absorbance mode; (8) a minimum quantitative reporting level for environmental samples; and (9) data management protocols.

Quality assurance / quality control measures beyond those recommended by the kit manufacturer were put in place in order to obtain maximum confidence in the ELISA test results. Test performance measures (positive and negative controls) will be monitored as part of normal quality assurance. It became obvious during the development process that tight controls were necessary for laboratory temperature as well as for the temperature of reagents and samples. The same was necessary for pipetting techniques used during sample preparation and execution of the ELISA test.

KEYWORDS

Cyanobacteria, Cyanotoxins, Cylindrospermopsin, Delaware, ELISA, Microcystins, Nodularins